0031-6997/99/5102-0213$03.00/0
PHARMACOLOGICAL REVIEWS
Vol. 51, No. 2
Printed in U.S.A.

Pharmacology of the Eosinophil

MARK A. GIEMBYCZ[1] AND MARK A. LINDSAY

Thoracic Medicine, Imperial College School of Medicine at the National Heart & Lung Institute, London, United Kingdom

This paper is available online at http://www.pharmrev.org

[1] Address for correspondence: Thoracic Medicine, Imperial College School of Medicine at the National Heart & Lung Institute, Dovehouse Street, London SW3 6LY, United Kingdom. E-mail: m.giembycz@ic.ac.uk

I. Introduction and Historical Perspective

Using a compound microscope and the blood of several animals (including the elephant!), the English anatomist, T. W. Jones, discovered, in 1846, that some white blood cells contained granules that became visible when immersed in hypotonic solutions (Jones, 1846). Although it has been claimed that Jones had discovered the eosinophil (Archer, 1963), it is more likely that he visualized the more abundant neutrophil (Spry, 1988). It was Brown (1898) who probably first detected eosinophils in the blood and bone marrow of patients with eosinophilic leukemia in the latter decades of the 19th century, although the lack of appropriate dyes and staining techniques at that time prevented formal identification. Full credit for the discovery of the "eosinophile" is thus given to Paul Ehrlich (1879) who first noticed that a certain population of white blood cells was stained with a negatively charged, brominated fluorescein compound, eosin, and was so named for that property.

Despite the discovery of eosinophils almost 120 years ago, still relatively little is known of their biochemistry and pharmacology when compared to their highly studied sister cell, the neutrophil. This is perhaps surprising given the critical role of these cells both in host defense

(Butterworth and Townley, 1993; Allen and Davis, 1994) and, under certain circumstances, in a variety of diseases, including many, if not all, of those indicated in Table 1. However, a persuasive argument (and one that, through experience, is vigorously championed by the authors of this review!) for the lack of investigation almost certainly reflects the difficulty in obtaining eosinophils in sufficient numbers and of a purity required for detailed studies to be performed and from which unambiguous conclusions can be drawn. Moreover, the process of purification and the effect of previous drug therapy on the ex vivo behavior of human eosinophils invariably leads to alterations in cell function and can make interpretation of results difficult. With the refinement of separation and purification techniques, in particular the use of "negative selection" to remove unwanted leukocytes (Hansel et al., 1989, 1990, 1991b), has come a marked increase in the number of articles published relating to eosinophil biology. Indeed, according to PubMed records, more than 3500 articles have been published since 1990 with a noticeable increase in pharmacological and biochemical content. It thus seems timely to attempt a comprehensive treatise of the pharmacology of the eosinophil, and the authors make no apology for omitting much of the immunology and parasitology which has been elegantly reviewed elsewhere (Capron, 1991, 1992; Weller, 1991; McEwen, 1992; Butterfield and Leiferman, 1993; Butterworth and Thorne, 1993; Wardlaw et al., 1995).

II. Gross Morphology and Ultrastructure

Eosinophils are actively motile, terminally differentiated leukocytes derived from the bone marrow, and have been identified in many mammalian and nonmammalian species (Table 2). Human eosinophils are approximately 8 μm in diameter, have a volume of 275 fl and, in addition to their avidity for eosin, exhibit several distinct characteristics that distinguish them from other granulocytes (Sokol et al., 1988; Dvorak, 1991). Generally, normal healthy eosinophils have a bi-lobed nucleus that is filled with partially condensed chromatin (Figs. 1 and 2). In some diseases, however, the number of lobes is increased to more than four (Sokol et al., 1987). A prominent feature of the eosinophil is the presence of many spherical or ovoid granules (Figs. 1 and 2) that occupy approximately one-fifth of the cytoplasm. Four distinct populations of granule (secondary granules, small granules, primary granules, lipid bodies) have been recognized that house a plethora of proteins, many with enzymatic activity (Fig. 2; Table 3). The first morphological marker of the eosinophil is the appearance of granules that are visible at the promyelocyte stage (Zucker Franklin, 1980). Several proteins are found within these structures, including eosinophil peroxidase (EPO),[2] acid phosphatase, and arylsulphatase B. Despite earlier descriptions to the contrary (Bainton and Farquhar, 1970), these granules are probably precursors of the specific, or

[2] Abbreviations: A1, murine-related Bcl protein; AA, arachidonic acid; ACh, acetylcholine; AH 13205, *trans*-2-(4-[1hydroxyhexyl]phenyl)-5-oxocyclopentaneheptanoate; AMT, 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine; APC, antigen presenting cell; AP-1, activator protein-1; BAL, bronchoalveolar lavage; Bcl-2, B-cell leukemia oncogene-2; Bcl-x_L, Bcl-2-regulated factor $x_{(L)}$; Mcl-1, Bcl-2 homology protein; Bax, Bcl-2-binding protein; BLT, leukotriene B_4 receptor; BN 52021, 3-[1,1-dimethylethyl]hexahydro-1,4,7b-trihydroxy-8-methyl-9H-1,7a(epoxy methanol)-1H,6aH-cyclopenta-[c]furo[2,3-b]furo [3′,2′:3,4] cyclopenta[1,2-d] furan-5,9,12[4H]-trione; BN 50730, [tetrahydro-4,7,8,10-methyl-1(chloro-2 phenyl)-6-(methoxy-4 phenylcarbamoyl)-9-pyrido [4′,3′-4,5]thieno[3,2-f]triazolo-1,2,4[4,3-a]diazepine-1,4]; BQ-123, cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu); bp, base pair; BQ 788, *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyl tryptophanyl-D-norleucine; BRL 35135, (R*,R*)-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl) ethyl amino]propyl]-phenoxyacetate hydrobromide; BRL 37344, sodium 4-{2-[2-hydroxy-2-(3-chlorophenyl)-ethylamino]propyl}phenoxyacetate sesquihydrate (RR.SS diastereoisomer); BW 245C, 5-(6-carboxyhexyl)-1-(cyclohexyl-3-hydroxypropylamino) hydantoin; C3a/C4a/C5a, complement 3a/4a/5a anaphylatoxin; $[Ca^{2+}]_i$, intracellular-free Ca^{2+} concentration; CAT, chloromphenical acetyl transferase; CBP, CREB-binding protein; CDP840, R-(+)-4-[2-(3-cyclopentoxy-4-methoxyphenyl)-2-phenylethyl]pyridine; C/EBP, CCAAT-enhancer binding protein; CGRP, calcitonin gene-related peptide; CHO, Chinese hamster ovary; CI-IB-MECA, 2-chloro-N^6-(3-iodobenzyl)adenosine-5′-methyl uronamide; CP 105,696, (+)-1-(3*S*,4*R*)-[3-(4-phenylbenzyl)-4-hydroxychroman-7-yl] cyclopentane carboxylic acid; CP 80,633, (2′*S*)5-[3-(2′-exobicyclo[2.2.1]-heptyloxy)4-methoxyphenyl] tetrahydro-2(1H)-primidone; CR, complement receptor; CREB, cyclic AMP response element binding protein; CTX, cholera toxin; CV 6209, 2-[*N*-acetyl-*N*-(2-methoxy-3-octadecylcarbamoyloxypropoxy carbonyl)aminomethyl]-1-ethyl pyridinium chloride; D-22888, 1-ethyl-8-methoxy-3-methyl-5-propyl imidazol[1,5a]-pyrdio[3,2-e] pyrazinone; DAG, diacylglycerol; DIDS, 4,4′-diisothiocyanato-stilbene-2,2′-disulphonic acid; diHEPE, dihydroxeicosapentaenoic acid; diHETE, dihydroxyeicosatetraenoic acid; D-NAME, *N*-nitro-D-arginine methyl ester; DSE, diad symmetry element; E-6123, *S*-(+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4′,3′:4,5] thieno [3,2-f]-1-[1,2,4]triazolo]4,3-a][1,4]diazepine; ECF-A, eosinophil chemotactic factor of anaphylaxis; ɛBP, epsilon binding protein; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EGF, epidermal growth factor; EPO, eosinophil peroxidase; EPX, eosinophil protein X; ERK, extracellular-regulated kinase; ETE, eicosatetraenoic acid; FK888, (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-prolyl]-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide); FLAP, 5-lipoxygenase activating protein; fMLP, N-formyl-methionyl-leucyl-phenylalanine; Gsα, α subunit of the stimulatory guanine nucleotide-binding protein; GATA, guanine-adenine-thymine-adenine; GCP, granulocyte chemotactic protein; GF 109203X, 2-[1-(3-dimethylaminopropyl)-1H-indol-3-yl]-3-(1H-indol-3-yl)-maleimide; GlyCAM-1, glycosylation-dependent, cell adhesion molecule; G-CSF; granulocyte colony-stimulating factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; GPI, glycosyl phosphatidylinositol; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GRK, G protein receptor-coupled kinase; GTPγS, guanosine 5′-(3-thio)triphosphate; HB-EGF, heparin-binding, epidermal growth factor-like growth factor; HEPE, hydroxyeicosapentanoic acid; HETE, hydroxyeicosatetraenoic acid; HLA, human leukocyte antigen; HODE, hydroxy-linoleic acid; HPETE, hydroperoxyeicosa tetraenoic acid; HUVECs, human umbilical vein endothelial cells; IBMX, 3-isobutyl-1-methyl-xanthine; ICAM, intercellular adhesion molecule; ICI 118,551, (±)-1-(2,3-[dihydro-7-methyl-1H-inden-4-yl]oxy)-3-([1-methylethyl]-amino-2-butanol; IFN, interferon; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; Ins(1,4,5)P_3, inositol(1,4,5)trisphosphate; JAK, Janus kinase; JNK, c-*jun* N-terminal kinase, L-659,989, (±)-*trans*-2-(3′-methoxy-5′-methylsulphonyl-4′-propoxy phenyl)5-(3″,4″,5″-trimethoxyphenyl)tetrahydrofuran; LFA, leukocyte function-associated antigen; LIF, leukemia inhibitory factor; LPS, lipopolysaccharide; L-NAME, N^{ω}-nitro-L-arginine methyl ester; L-NIL, L-N^6-(1-iminoethyl) lysine; L-NMMA, N^G-mono-

secondary, granules that are first seen at the myelocyte stage of maturation (Hardin and Spicer, 1970; Gleich and Loegering, 1984). In eosinophils harvested from humans (Zucker Franklin, 1980; Tavassoli, 1981; Cohen and Ottesen, 1983) and from many other species, including the dog, mouse, rat, goat, guinea pig and rhesus monkey (Jain, 1986), the specific granules feature a prominent crystalloid core containing major basic protein (MBP). Specific granules, containing multiple cores, also have been visualized (Newman et al., 1996) but their occurance is relatively rare (Fig. 3). In addition, other highly charged cationic proteins typified by eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and EPO (Egesten et al., 1986) are located within the noncrystalloid matrix along with a number of cytokines (Fig. 2; Table 3). Differences in the gross morphology of the secondary granules are apparent between species. Thus, in cats, the core is lamellar rather than crystalloid, whereas in cattle, horses, mink, and gorillas the granules lack a central core and appear to be homogeneous when visualized under the electron microscope (Henderson et al., 1983; Jain, 1986; McEwen, 1992).

A population of small granules also has been identified in human tissue eosinophils that are not seen within circulating cells or those in the bone marrow (Parmley and Spicer, 1974). These structures stain intensely for arylsulphatase B and acid phosphatase (Parmley and Spicer, 1974; Dvorak, 1991) and may also contain catalase (Iozzo et al., 1982).

The third type of storage organelle that has secretory properties is the primary granule, which accounts for approximately 5% of all eosinophil granules (Fig. 2). Morphologically, they are roughly spherical, of variable size, and contain no discernible core. In resting eosinophils, primary granules provide the sole location for Charcot-Leyden crystals (Dvorak et al., 1988), which are colorless, and have a characteristic hexagonal, bipyramidal structure with intrinsic lysophospholipase activity (Ackerman et al., 1980; Weller et al., 1980). In activated cells, trace amounts of Charcot-Leyden crystals have been identified within the nucleus and cytoplasm, implying that this protein can be released intracellularly.

The final population of granules is known as lipid bodies and these structures are not membrane-bound (Fig. 2). Approximately five lipid bodies are found per resting eosinophil, although the number can increase when the cell is activated. Lipid bodies are spherical (0.5–2 μm in diameter), electron-dense organelles, and, as the name implies, provide a principle store for arachidonic acid (AA) that is esterified into glycerophospholipids (Weller and Dvorak, 1985; Weller et al., 1991a).

methyl-L-arginine; LPR, late phase response; LT, leukotriene; LY 293111, 2-[2-propyl-3-[3-[2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenoxy]propoxy] phenoxy]benzoic acid; LY 83583, 6-anilino-5,8-quinoline quinone; Mac-1, macrophage-1 antigen; MAP kinase, mitogen-activated protein kinase; MBP, major basic protein; MCP, monocyte chemotactic protein; MEK, MAP kinase kinase; MHC, major histocompatibility complex; MIF, macrophage migration inhibitory factor; MIP, macrophage inflammatory protein; MK-571, (3-(3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl)((3-dimethylamino-3-oxopropyl)thio)methyl)thio) propanoic acid; MPO, myeloperoxidase; NERDS, nodules, eosinophilia, rheumatism, dermatitis, and swelling; NF-AT, nuclear factor of activated T-cells; NFκB, nuclear factor κB; NGF, nerve growth factor; NK, neurokinin; NPPB, 5-nitro-2-(3-phenyl propylamino)-benzoic acid; NOS, nitric oxide synthase; NPC 567, D-Arg-[Hyp3,D-Phe-7]-bradykinin; NPC 16731, D-Arg-[Hyp-3,Thi5d-Tic7,Tic8]-bradykinin; ODQ, 1H-[1,2,4]- oxidiazolo[4,3-a] quinoxalin-1-one; Org 20241, *N*-hydroxy-4-(3,4-dimethoxyphenyl)-thiazole-2-carboximidamide, PAF, platelet-activating factor; PCA 4248, 2-(phenylthio)ethyl-5-methoxycarbonyl-2,4,6-trimethyl-1,4-dihydropyridine-3-carboxylate; PD 098059, 2′-amino-3′-methoxyflavone; PDE, phosphodiesterase; PDGF, platelet-derived growth factor; PG, prostaglandin; PKB, protein kinase B; PKC, protein kinase C; PLA_2, phospholipase A_2, PLC, phospholipase C; PLD, phospholipase D; PMA, phorbol 12-myristate 13-acetate; PSGL-1, P-selectin glycoprotein ligand-1; PDGF, platelet-derived growth factor; PtdIns-3 kinase, phosphatidylinositol 3-kinase; PTX, pertussis toxin; RANTES, regulated on activation, normal T-expressed and secreted; Ro 20–1724, 4-[(butoxy-4-methoxy phenyl)methyl]-2-imidazolidine; Ro 31–8220, 3-[1-[3-amidinothio)propyl-1H-indol-3-yl]-3-(1-methyl-1H-indol-3-yl)maleimide] methane sulphonate; RP-HPLC, reverse phase high-performance liquid chromatography; RP 73401, 3-cyclopentyloxy-*N*-(3,5-dichloro-4-pyridyl-3-methoxy benzamide; Rp-8-Br-cAMPS, Rp-8-bromo adenosine-3′,5′-cyclic monophosphorothioate; RP 59227, N-(3-benzoylphenyl)-3-(pyridyl)-1H,3H-pyrrolo[1,2-c] thiazole carboxamide; RT, reverse transcription; RV16, rhinovirus 16; SB 202190, 4-(4-fluorophenyl)-2-(4-hydroxphenyl)-5-(4-pyridyl)1H-imidazole; SB 203580, 4-(4-fluorophenyl)-2-(4-methylsulphinylphenyl)-5-(4-pyridyl)1H imidazole; SC, secretory component; SB 209670, (1*RS*-2*SR*,3*RS*)-3-(2-carboxymethoxy-4-methoxyphenyl)-5-(prop-1-yloxy) indane-2-carboxylic acid; SB 207499, c-4-cyano-4-(3-cyclopentyloxy-4-methoxyphenyl)-r-l-cyclohexane carboxylic acid; SCF, stem cell factor; SDZ 64–412, 2,3-dihydro-5-[4-[2-(3,4,5-trimethoxyphenyl)ethyl] phenyl]imidazol[2,1-α] isoquinoline HCl; SHPTP-2, src homology-2, protein tyrosine phosphatase; SK&F 104353, 2*S*-hydroxy-3*R*-(2-carboxyethylthio)-3-(2-[8-phenyloctyl]phenyl)-propanoate; SK&F 88046, *N,N*′-bis [7-(3-chlorobenzene aminosulphonyl)-1,2,3,4-tetrahydroisoquinolyl] disulphonylimide; SH2, Src homology 2; SH3, Src homology-3; SM-10661, (±)-*cis*-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one HCl; SNAP, *S*-nitroso-*N*-acetyl penicillamine; SNP, sodium nitroprusside; SOZ, serum-opsonized zymosan; SP, Substance P; SR 27417A, *N*-(2-dimethyl aminoethyl)-*N*-(3-pyridinylmethyl)(4-[2,4,6-triisopropylphenyl] thioazol-2-yl) amine; STAT, signal transducers and activators of transcription; TGF, transforming growth factor; TNF, tumour necrosis factor; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TRE, TPA responsive element; TRIM, 1-(2-trifluoromethylphenyl) imidazole; TX, thromboxane; U-75412E, 21-[4-(3-ethylamino-2-pyridinyl)-1-piperazinyl]-16α-methylpregna-1,4,9]-(11)-triene-3,20-dione; U-78517F, (2-[4-[2,6-di-(1-pyrrolidinyl)-4-pyridinyl)-1-piperazinyl]methyl]-3,4-dihydro-2,5,7,8-tetramethyl-2H-1-benzopyran-6-ol dihydrochloride), UK 74,505, 4-(2-chlorophenyl)-6-methyl-2-[4-(2-methylimidazol[4,5-c]pyrid-1-yl)phenyl-5-(2-pyridyl carbamoyl)-1,4-dihydro pyridine-3-carboxylic acid methyl ester; VEGF, vascular endothelial growth factor; VCAM, vascular cell adhesion molecule; VIP, vasoactive intestinal peptide; VLA, very late antigen; WEB 2086 (apafant), 3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]-thriazolo-[4,3a][1,4]-(diazepin-2-yl)-1-(4-morpholinyl)-1-propanone; WEB 2170 (bepafant), 6-(2-chlorophenyl)-8,9-dihydro-1-methyl-8-(4-morpholinylcarbonyl)-4H,7H-cyclopenta[4,5]thieno[3,2-f][1,2,4]triazolo [4,3-a]diazepine; Y-24180, (±)-4-(2-chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6H-thieno3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine; YM 264, 1-(3-methyl-3-phenylbutyl)-4-[2-(3-pyridyl) thiazolidine-4-carbonyl]-piperazine fumarate.

TABLE 1
Diseases in which eosinophils are believed to play a pathogenic role

Disease
Allergic disorders
Extrinsic bronchial asthma
Allergic rhinitis
Onchocercal dermatitis
Atopic dermatitis
Drug reactions
Nodules, eosinophilia, rheumatism, dermatitis, and swelling (NERDS)
Vasculitic granulomatous diseases
Temporal vasculitis
Churg-Strauss syndrome
Polyarteritis
Wegener's granulomatosis
Eosinophilic granulomatous prostatitis (?)
Immunological disorders
Autoimmune reactions (e.g. multiple sclerosis)
Graft rejection
Intrinsic bronchial asthma
Interstitial and other pulmonary diseases
Eosinophilic pleural effusions
Transient pulmonary eosinophilic infiltrates (Löffler)
Histiocytosis
Chronic eosinophilic pneumonia
Hypersensitivity pneumonitis
Allergic bronchopulmonary aspergillosis
Sarcoidosis
Idiopathic pulmonary fibrosis
Topical eosinophilia
Infectious parasitic diseases
Toxocariasis
Filariasis
Schistosomiasis
Trichinosis
Strongyloides
Ascariasis
Echinococcosis/cysticercosis
Other infectious diseases
Acute coccidioidomycosis
Cat scratch disease
Afebrile tuberculosis
Chlamydial pneumonia at infancy
Neoplastic and myeloproliferative diseases
Bronchogenic carcinoma
Hypereosinophilic syndrome
T cell lymphomas and Hodgkin's disease

Modified from Kroegel et al. (1994b). Further details on eosinophil-related diseases can be found in the following articles: Zucker Franklin (1974, 1978); Tavassoli (1981); Davis et al. (1984); Kay (1985); Nutman et al. (1989a, b); Liu et al. (1992); Butterfield and Leiferman (1993); Butterfield et al. (1993); Hall and Walport (1993); Allen and Davis (1994); Rothenberg (1998).

TABLE 2
Some nonhuman species in which eosinophils have been identified

Species		
Mammals	Amphibia, Birds, and Fish	Reptiles
Buffalo	Bass	American Alligator
Camel[a]		
Cat (domestic, lion, tiger)	Carp	Lizard
Chinese hamster	Chicken	Turtle
Cow	Duck	
Dog	Frog	
Guinea pig		
Horse	Loach	
Mongolian gerbil	Nurse shark	
Mouse	Pigeon	
Opossum	Tench	
Primates	Torpedo	
Rabbit		
Rat		
Wild fallow deer		
Yak		

Data compiled from Spry (1988) and references therein.
[a] Johnson et al. (1999).

Further description of the morphology of eosinophils in health and disease is beyond the scope of this review, but interested readers should consult articles by Dvorak (1991) and Sokol et al. (1987) which provide a comprehensive treatise of the subject.

III. Life Cycle, Maturation, and Tissue Distribution

Eosinophil turnover, or eosinopoeisis, occurs almost exclusively in the bone marrow although ancillary sites of production can include the spleen, thymus, and lymph nodes (Till and McCulloch, 1961; Jain, 1986; McEwen, 1992). The bone marrow from normal individuals contains about 3% eosinophils, of which 37% are mature, nondividing granulocytes, and the remainder are promyelocytes/myelocytes (37%) and metamyelocytes (26%) that exist in "storage" compartments (Spry, 1988, 1993).

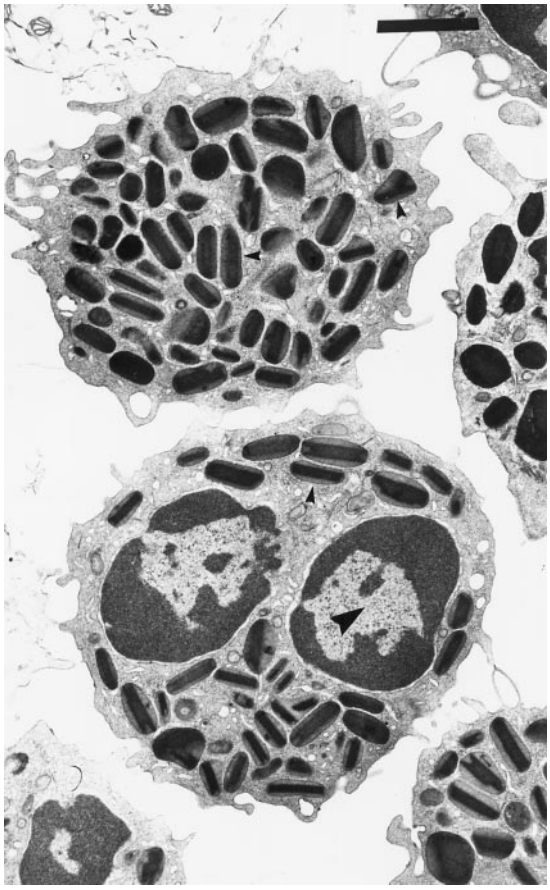

FIG. 1. Electron micrograph of untreated eosinophils purified from the peritoneal cavity of guinea pigs. A bi-lobed nucleus containing condensed chromatin is shown (large arrow) along with cytoplasm packed with many large, membrane-enclosed, dense crystalloid-containing ovoid granules (smaller arrows). Cells were conventionally fixed (glutaraldehyde/osmium tetroxide). Bar, 1 μm. Original magnification, 18,000×. See *II* for further details

At any one time, it has been estimated that about 16% of myelocytes are undergoing DNA synthesis (i.e., are in the S phase of the cell cycle), which lasts about 13 h, and that the time taken from the last mitosis until they

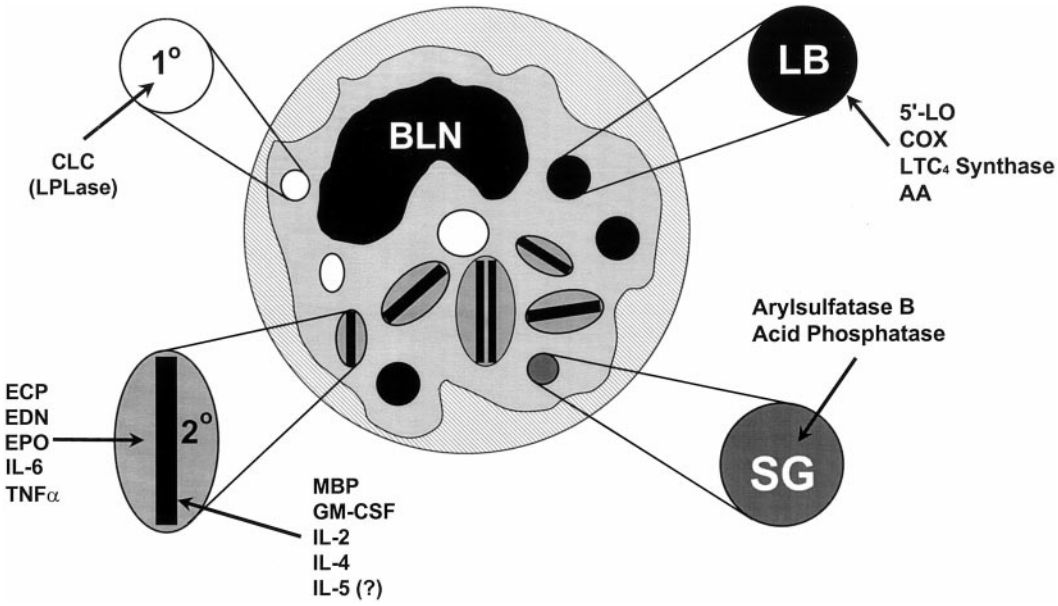

FIG. 2. Cardinal structures of a human eosinophil. Shown are the typical bi-lobed nucleus (BLN) and the four main granules. The primary (1°) granule is the principle site of Charcot-Leyden crystals, whereas MBP, ECP, EDN, and EPO reside within the classically crystalloid secondary (2°) granule along with a number of cytokines and a host of other proteins many with enzymatic activity. Lipid bodies (LB), which represent a site of lipid mediator biosynthesis, also are found in resting and activated eosinophil where their number is increased along with small granules (SG) that store proteins such as arylsulfase B and acid phosphatase. See *II* for further details. COX, cyclooxygenase; 5-LO, 5-lipoxygenase; LPLase, lysophospholipase.

appear in the blood as mature cells (the emergence time) is approximately 2.5 days (Spry, 1988). The migration of eosinophils from the bone marrow to the blood takes about 3.5 days (Parwaresch et al., 1976). Using [^{3}H]thymidine flash-labeled peripheral blood eosinophils, Walle and Parwaresch (1979) performed studies in three hematologically normal men to estimate the eosinophil reserve capacity in the postmitotic granulocyte compartment in the bone marrow and the effective eosinopoeisis. The results of those experiments demonstrated that mean turnover of eosinophils is approximately 2.2×10^8 cells/kg/day and that the bone marrow provides the largest postmitotic eosinophil reserve capacity ($9–14 \times 10^8$ cells/kg).

There is compelling evidence that eosinophils are derived from small populations of self-regenerating, hematopoietic stem cells that also are capable of differentiation into the individual lymphomyeloid lineages. The ultimate commitment of stem cells to unipotential progenitors, and their subsequent survival and expansion into mature eosinophils, has been studied extensively, although a complete understanding of the factors and processes by which this occurs still is lacking. It has been suggested that the fate of a hematopoietic stem cell to regenerate or to commit to a multipotential progenitor is purely stochastic (Till et al., 1964; Nakahata et al., 1982; Nakahata and Ogawa, 1982). In the latter scenario, a host of cytokines and other factors are required including interleukin (IL)-6, IL-11, IL-12, granulocyte colony-stimulating factor (G-CSF), stem cell factor (SCF; CD117; formerly known as *c-kit* ligand and Steel factor), and leukemia inhibitory factor (LIF) (Ploemacher et al., 1993; Ogawa, 1994). Further development of multipotential cells into eosinophil progenitors is under the influence of SCF, IL-3, IL-4, granulocyte/macrophage (GM)-CSF, and eotaxin (Kobayashi, 1993, Peled et al., 1998). Interleukin-5 and possibly eotaxin then provide the major driving force for the terminal stages of maturation and release into the blood stream (Clutterbuck et al., 1989; Sanderson, 1993; Palframan et al., 1998a).

In the guinea pig, IL-5 releases eosinophils from the bone marrow by a mechanism that is blocked by the phosphatidylinositol 3-kinase (PtdIns 3-kinase) inhibitors wortmannin and LY 294002, although the downstream substrates involved in this process are currently undefined (Palframan et al., 1998b). Moreover, the emigration of eosinophils from the marrow precipitated by IL-5 is associated with adhesive interactions involving α_4 and β_2 integrins that act in an opposing manner. In vivo the expression of β_2 integrins is reduced in response to IL-5, whereas the α_4 integrin level remains unchanged. The observation that a β_2 integrin-blocking antibody suppresses IL-5-driven eosinophil mobilization suggests that these adhesion molecules are necessary for effective migration. In contrast, an α_4 integrin-blocking antibody enhances the release of eosinophils from the marrow in response to IL-5, and it has been speculated that this prevents their normal attachment to the bone marrow sinus endothelium (Palframan et al., 1998b). Thus, the egress of mature eosinophils from the marrow involves a number of discrete steps.

Once in the circulation, eosinophils have a half-life of approximately 18 h and a mean blood transit time (26 h) similar to neutrophils (Steinbach et al., 1979). However,

TABLE 3
Contents of eosinophil granules

Granule Protein	Reference(s)
Secondary (specific) granules	
Major basic protein (core)	Egesten et al. (1986); Peters et al. (1986)
Major basic protein (matrix)	Torpier et al. (1988)
Eosinophil cationic protein (matrix)	Egesten et al. (1986); Peters et al. (1986); Torpier et al. (1988)
Eosinophil-derived neurotoxin (matrix)	Peters et al. (1986)
Eosinophil peroxidase (matrix)	Okuda et al. (1981); Egesten et al. (1986); Enomoto and Kitani (1986); Torpier et al. (1988)
Lysozyme (matrix)	Stirling (1989)
Acid phosphatase (matrix)	Ghidoni and Goldberg (1966); Bass et al. (1981)
Arylsulphatase B	Parmley and Spicer (1974)
Catalase (core and matrix)	Iozzo et al. (1982); Yokota et al. (1984)
Enoyl-CoA hydratase (core and matrix)	Yokota et al. (1983)
3-Ketoacyl-CoA thiolase (core and matrix)	Yokota et al. (1983)
β-glucuronidase (core and matrix)	Yokota et al. (1984)
Cathepsin D (core and matrix)	Yokota et al. (1984)
Elastase	Lungarella et al. (1992)
Granulocyte/macrophage colony-stimulating factor (core)	Levi Schaffer et al. (1995)
Interleukin-2 (core)	Levi Schaffer et al. (1996)
Interleukin-4 (core)	Moqbel et al. (1995)
Interleukin-5 (core)	Dubucquoi et al. (1994)
Interleukin-6 (matrix)	Hamid et al. (1992); Melani et al. (1993); Lacy et al. (1998)
Tumor necrosis factor α (matrix)	Beil et al. (1993); Costa et al. (1993)
RANTES	Ying et al. (1996)
Type II phospholipase A_2	Blom et al. (1998)
Bactericidal/permeability-increasing protein	Calafat et al. (1998)
Small granules	
Acid phosphatase	Parmley and Spicer (1974)
Arylsulphatase B	Parmley and Spicer (1974)
Catalase	Iozzo et al. (1982)
Cytochrome b_{558}	Ginsel et al. (1990)
Elastase	Lungarella et al. (1992)
Eosinophil cationic protein (matrix)	Egesten et al. (1986)
Primary granules	
Charcot-Leyden crystal protein	Dvorak et al. (1990, 1991)
Lipid bodies	
Cyclooxygenase	Dvorak et al. (1992b, 1994); Bozza et al. (1997a)
5-Lipoxygenase	Weller (1994); Bozza et al. (1997a)
15-Lipoxygenase	Bozza et al. (1998)
Leukotriene C_4 synthase	Bozza et al. (1997a)
Eosinophil peroxidase	Zabucchi et al. (1991); Dvorak et al. (1992a)
Esterase	Monahan et al. (1981)

the half-life of eosinophils is prolonged when an eosinophilia is precipitated which might be due to an increase in the concentration of certain circulating cytokines that enhance survival (see XII. H) and/or to the saturation of sites through which eosinophils migrate into tissue.

In humans and many domestic animals, eosinophils comprise 2 to 10% of the peripheral leukocytes, but in cows the average titer is approximately 20% (Duncan and Prasse, 1986; McEwen, 1992). The circulating eosinophil count exhibits diurnal variation in some species; thus, in normal human subjects the highest and lowest levels are seen in the evening and the morning, respectively (Horn et al., 1975), whereas the opposite occurs in horses (McEwen, 1992). Eosinophils are predominantly tissue cells and do not reenter the circulation. The gastrointestinal tract, lung, and skin and, in rats, the uterus during dioestrus or oestrogen treatment (see *XIV.F*) are the principle sites of accumulation (Dembele Duchesne et al., 1991), and histological studies with human tissues have identified columnar epithelial surfaces as particularly rich in eosinophil infiltrates. Large numbers of eosinophils can be found in tissues even when the peripheral blood count is low, which suggests that their longevity is enhanced once they leave the circulation. It has been estimated that the number of eosinophils in the bone marrow and tissues of rats is 300 times higher than the circulating count (Rytomaa, 1960). The tissue distribution of eosinophils in subjects with disease has not been systematically quantified, although it is curious that pathogen-free animals have no eosinophils in their blood and tissue eosinophils are difficult to find. This strongly suggests that an increase in the circulating eosinophil count and retention of eosinophils in tissues is disease-related (Spry, 1993), although this might not apply to the gut (see *V.E.1*).

IV. Transcription Factors and Eosinophilia

Gene transcription is regulated in a highly coordinated and complex fashion by a diverse family of DNA-binding proteins known collectively as transcription factors. In diseases such as those associated with peripheral blood eosinophilia, transcription factors may play a key role in inducing or repressing critical genes that control eosinopoiesis. Perhaps the most universal and ubiquitously distributed transcription factors are activator protein 1 (AP-1) and nuclear factor κB (NF-

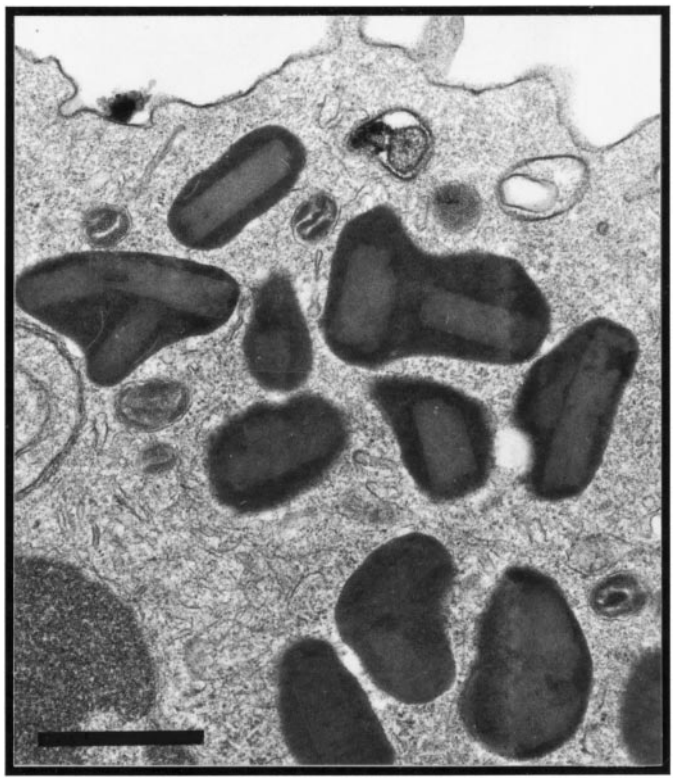

FIG. 3. Identification of single and multiple crystalloid cores in specific granules from streptolysin O-permeabilized guinea pig peritoneal eosinophils stimulated with GTPγS and Ca^{2+}. Bar, 500 nm. Original magnification, 55,000×. See *II* for further details.

κB), which are involved in the regulation and coregulation of many genes. In contrast, other transcription factors have a more cell-specific distribution and regulate the expression of a restricted number of genes. For example, the transcription factors nuclear factor of activated T cells (NFAT) (Rao et al., 1997), guanine-adenine-thymine-adenine (GATA-3) (D. H. Zhang et al., 1997) along with NF-κB (Yang et al., 1998) are critically important in controlling the IL-5 and eotaxin genes that are probably essential for the differentiation, maturation, and trafficking of eosinophils (see *III.*). Similarly, in the lungs of mice that are deficient in the p50 subunit of NF-κB, lymphocyte recruitment after allergen provocation is attenuated compared to wild type animals due to a reduction in the secretion of macrophage inflammatory protein (MIP)-1α and MIP-1β (Yang et al., 1998).

Additional genes are also likely to regulate circulating eosinophil number and eosinophilia associated with disease. In individuals with familial eosinophilia, a rare disease encompassed by the generic term hypereosinophilic syndrome that has no allergic or parasitic basis, a locus (or loci) has been identified on region q31–q33 of chromosome 5 which contains the cytokine gene cluster for IL-3, IL-5, and GM-CSF (Rioux et al., 1998). Since no functional polymorphisms were found within the enhancer, promoter, exons, or introns of any of these genes, it has been speculated that a main cause of familial eosinophilia is due to a novel gene that is situated within region q31–q33 (Rioux et al., 1998). Indeed, this idea is supported by the knowledge that greater than 100 anonymous transcripts have been found in that region of human chromosome 5 (Schuler et al., 1996). Martinez et al. (1998) also have identified markers on the same region of chromosome 5 that controls for circulating eosinophil number as a percentage of total white leukocytes.

V. G Protein-Coupled Receptors and Their Ligands

G protein-coupled receptors are characterized by an extracellular amino-terminal sequence followed by seven transmembrane-spanning domains, with three extracellular and three intracellular loops, and an intracellular carboxyl terminus. Conserved cysteine residues within the amino-terminal sequence and in the third extracellular loop are thought to form a disulfide bond which is required for ligand binding, while a second disulfide bond is probably formed between conserved cysteine residues within the first and second extracellular loops. The functional responses that result from ligand binding are transduced by G proteins. These are heterotrimeric proteins consisting of α, β, and γ subunits that each exist in multiple isoforms (20 α, five β, 10 γ) in mammalian cells. Several G proteins and/or subunits thereof have been identified in human and guinea pig eosinophils including $G_{\alpha s}$, $G_{\alpha i3}$, $G_{\alpha 0}$, $G_{\alpha q/11}$, and G_{β} (Agrawal et al., 1992; Lacy et al., 1995).

In excess of 17 G protein-coupled receptors have thus far been identified on eosinophils (Table 4). These receptors can couple to a vast array of effector proteins that ultimately produce a host of functional responses resulting both in stimulation and suppression of eosinophil activity. These are identified and discussed in detail below.

A. Platelet-Activating Factor

1. Receptors and Signaling. The ether lipid, platelet-activating factor (PAF), evokes its biological effects by interacting with a classical seven transmembrane-spanning receptor that is composed of 342 amino acids and has a molecular mass of approximately 39 kDa (Honda et al., 1991; Nakamura et al., 1991). Radioligand-binding experiments have identified PAF receptors on many cells, including eosinophils. However, until the early 1990s ^{3}H-labeled PAF was the only radioligand available for this purpose and proved to be unsatisfactory for several reasons. Notably, it causes activation of cells and, with prolonged exposure, receptor down-regulation. Furthermore, the lipophilicity of PAF gives rise to high levels of nonspecific binding, "specific" nonreceptor binding, and the labeling of intracellular or internalized receptors, factors that hamper its utility for accurate determination of cell surface receptor density and ligand affinity (Dent et al., 1989). Nevertheless, estimates of K_d (2.3 nM) and B_{max} (104 fmol/10^6 cells) have been made

TABLE 4
Eosinophil-derived mediators and receptor expression

Mediators			Receptors			
Lipids	Cytokines and Chemokines	Growth Factors	G protein-Coupled	Immunoglobulins	Hematopoietins, IFN, and TNF Families	Miscellaneous
PAF	IL-1α	TNFα	PAF Receptor	FcαRIa.1-5	IL-3R	IL-1R
LTB_4[a]	IL-2	TNFβ	BLT Receptor	FcαRib	IL-5R	IL-2R
LTC_4	IL-3	PDGF	*Cys*-LT Receptor[b]	FcεRI	GM-CSFR	IL-4R
						IL-9R
TXA_2	IL-4	VEGF	fMLP Receptor	FcεRIIa	IFNγR	IL-13R
PGE_2	IL-5	HB, EGF	CCR1	FcεRIIb	IL-10R	TGFβR
5-HETE	IL-6	NGF	CCR3	Mac-2/εBP	TNFαRI	PDGFR
15-HETE	IL-8	Endothelin[c]	CXCR1/2	FcγRI[d]	TNFαRII	*c-Kit*
5,15-diHETE	IL-10		C3aR	FcγRII[d]	CD30	CD4
	IL-11[c]					
8,15-diHETE	IL-12		C5aR	FcγRIII[d]	CD40	CD9
14,15-diHETE	IL-16		β_2-adrenoceptor	FcμR[d]	CD44	CD52
LXA_4	INFγ		NK_1 receptor		CD69	CR1 (cC1qR)
13-HODE	TNFα		Adenosine (A_2 and A_3)		CD95	GC1qR
	GM-CSF		sst[b]		NGFR	CR3
	MIP-1α		Histamine (H_1, H_2, and H_3)			CR4
	RANTES		VIP[b]			SCR
	MIF		EP_2			HLA
			P2Y[c]			

[a] Guinea-pig and bovine eosinophils lack LTC_4 synthetase and produce LTB_4.
[b] Receptor subtype not known.
[c] Preliminary reports: Muro et al. (1999); Chakir et al. (1999); Raible et al. (1999); Dussault et al. (1999).
[d] Receptor can be induced.

for ^{3}H-labeled PAF in human eosinophils (Korth, 1996), and it seems likely that the ligand-labeled sites represent specific receptors because binding was reversed by unlabeled PAF and the PAF antagonist apafant (WEB 2086) (Korth, 1996).

The introduction of metabolically stable, hydrophilic radiolabeled PAF antagonists such as [^{3}H]apafant and [^{3}H]L-659,989 (Hwang et al., 1989), has largely circumvented the problems that have been encountered with ^{3}H-labeled PAF. Thus, [^{3}H]apafant labels a homogeneous population of noninteracting binding sites on guinea pig and human eosinophils with B_{max} values of 35,000 and 64,000 sites/cell, respectively (Ukena et al., 1989, 1990). The observation that the binding of [^{3}H]apafant is concentration-dependent, saturable, reversible, of high affinity (K_d = 15–20 nM), and is prevented by a range of structurally distinct PAF antagonists and by a natural ligand, C_{16}-PAF, indicate that these binding sites represent bona fide receptors (Laduron, 1984). PAF receptor expression determined with [^{3}H]apafant is up-regulated after exposure (6–18 h) of human eosinophils to IL-3, IL-5, and GM-CSF (Kishimoto et al., 1996a,b).

Pharmacological evidence is available for two PAF receptors on guinea pig peritoneal eosinophils (Kroegel et al., 1989a). This is derived from the finding that PAF-induced Ca^{2+} mobilization and degranulation are inhibited by apafant with an affinity in the low nanomolar range, whereas PAF-induced superoxide anion generation is approximately 1000 times less sensitive. Although no corroborating evidence has thus far been published, data are available for PAF receptor heterogeneity between cell types and, moreover, between species (Lambrecht and Parnham, 1986; Hwang, 1988).

Some progress has been made in understanding the cell-signaling pathways activated by PAF in leukocytes. In guinea pig eosinophils loaded with fura-2/AM, the $[Ca^{2+}]_i$ increases rapidly (approximately 4-fold) after the addition of a maximally effective concentration (1 μM) of PAF (Kroegel et al., 1989b). As in many other cells, this effect is transient, antagonized by apafant, and not mimicked by lyso-PAF (Kroegel et al., 1989b,c). Unlike leukotriene B_4 (LTB_4)- and C5a-mediated Ca^{2+} mobilization (see *V.B.1* and *V.F.3*), the Ca^{2+} transient evoked by PAF is not blocked by pertussis toxin (PTX) excluding G_i and G_o in PAF receptor-effector coupling (Teixeira et al., 1997b). There is some controversy surrounding the sources of Ca^{2+} mobilized by PAF in guinea pig eosinophils. Kroegel et al. (1989b) have provided evidence that Ca^{2+} ions are derived primarily from the extracellular compartment through ill-defined ion channels that are blocked by Ni^+ but are resistant to classical 1,4-dihydropyridine Ca^{2+} antagonists such as nimodipine. Conversely, a primary role for intracellular Ca^{2+} stores also has been proposed (Minshall et al., 1990). The reason for this difference is unexplored.

Exposure of human eosinophils to PAF also is associated with Ca^{2+} mobilization that is similarly transient (Koenderman et al., 1990; Kernen et al., 1991; Zoratti et al., 1991; Wymann et al., 1995; Elsner et al., 1996a). However, in contrast to guinea pig cells a number of differences are apparent. In particular, Ca^{2+} are liberated predominately from intracellular stores (Zoratti et al., 1991; Elsner et al., 1995) via a population of PAF receptors that are sensitive to PTX (Kernen et al., 1991; Wymann et al., 1995; Zeck Kapp et al., 1995). More contemporary studies have found that PAF activates the $p21^{ras}$-extracellular-regulated kinase (ERK)-2 and Pt-

dIns 3-kinase-protein kinase B (PKB) pathways (Coffer et al., 1998), and increases the open-state probability of Ca^{2+}-activated K^+-channels (Saito et al., 1997). These latter two effects have been implicated in priming eosinophils for SOZ- and A23187-mediated respiratory burst (Saito et al., 1995; Coffer et al., 1998) and in PAF-induced degranulation (see *XII.B.4* and *XII.G*, respectively). A role for protein kinase C (PKC) in the regulation of the NADPH oxidase and eicosanoid generation also is suggested by the findings that the PKC inhibitors GF109203X and calphostin C suppress PAF-induced H_2O_2 generation and enhance the elaboration of LTC_4 and thromboxane (TX) (Dent et al., 1998). In this respect, multiple species of PKC have been identified in human eosinophils including the α, β_1, β_2, δ, ϵ, μ, ι, and ζ isoforms (Bates et al., 1993; Evans et al., 1999), which presumably subserve distinct, but as yet undefined, functional roles.

If PAF releases a significant proportion of Ca^{2+} from intracellular stores, then what are the biochemical mechanisms that bring this about? Classically, intracellularly stored Ca^{2+} can be released from the endoplasmic reticulum by inositol(1,4,5)trisphosphate [Ins(1,4,5)P_3] for which several distinct and specific receptors have been defined (for review, see Joseph, 1996). It is now firmly established that Ins(1,4,5)P_3, in combination with diacylglycerol (DAG), is derived from a minor membrane lipid, PtdIns(4,5)P_2 under the influence of a family of enzymes collectively known as phospholipase C (PLC). Evidence for such a mechanism in PAF-stimulated guinea pig eosinophils is provided by the observation that PAF enhances the incorporation of [^{3}H]inositol into membrane phospholipids (Kroegel et al., 1990a) and elicits a rapid, transient, and apafant-sensitive increase in Ins(1,4,5)P_3 mass (Kroegel et al., 1991). Temporally, the time course of Ca^{2+} mobilization is preceded by the increase in Ins(1,4,5)P_3 mass consistent with a causal relationship between these two parameters.

A PLC that readily hydrolyses PtdIns(4,5)P_2 and which may represent the enzyme responsible for agonist-induced Ins(1,4,5)P_3 accumulation in intact eosinophils is present in guinea pig-washed eosinophil membranes (Perkins, 1993). The enzyme is deoxycholate-dependent, sensitive to Ca^{2+} in the high nanomolar range, and exhibits a single pH optimum at pH 7.5. Kinetic studies indicate that PtdIns(4,5)P_2 is the preferred substrate for PLC and that its activity is augmented by guanosine 5′-(3-thio)triphosphate (GTPγS). These findings complement the observation that PAF stimulates GTPase activity in eosinophil membranes in a concentration-dependent and apafant-sensitive manner (Dent and Barnes, 1993).

2. In Vitro Effects. PAF is a potent chemoattractant and selectively promotes the migration of eosinophils over neutrophils. The ability of PAF to promote directional migration is significantly increased in eosinophils taken from asthmatic subjects both in remission and during an attack when compared with healthy volunteers (Shindo et al., 1997), suggesting that they have been primed in vivo. Possible candidate-priming agents include GM-CSF, which enhances PAF-induced pulmonary and cutaneous eosinophilia in guinea pigs (Sanjar et al., 1990a) and mice (Yukawa et al., 1992), and IL-3 and IL-5, which prime murine eosinophils for enhanced chemotactic activity induced by PAF (Yukawa et al., 1992). Other proinflammatory effects of PAF include the generation of a plethora of other bioactive lipids (Table 5) and the release of preformed mediators from both the specific and small granules.

Guinea pig, bovine, and human eosinophils, when challenged with PAF, display a marked increase in oxygen consumption and liberate superoxide anions extracellularly as a consequence of the activation of the NADPH (respiratory burst) oxidase. In guinea pig cells, this effect occurs at concentrations of PAF greater than 100-fold higher than are necessary to promote chemotaxis, TX production, degranulation, Ins(1,4,5)P_3 accumulation, and Ca^{2+} mobilization (Kroegel et al., 1989a, 1991). However, the finding that oxidant production was antagonized by apafant in those studies indicates that this response also is PAF receptor-mediated. Furthermore, those data also imply that the PAF receptors on eosinophils either are heterogeneous and mediate different functional responses, or that PAF can recruit diverse signaling pathways that have different sensitivities to activation (Kroegel et al., 1989a). In addition to increasing directly oxidative metabolism, low concentrations of PAF that produce little, if any, superoxide anions per se, prime the eosinophil NADPH oxidase to activation by *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) (Zoratti et al., 1992) and serum-oponized zymosan (SOZ) (Coffer et al., 1998). Likewise, the ability of human eosinophils to form stable aggregates (i.e., undergo homotypic aggregation) in response to SOZ also is primed by PAF (Koenderman et al., 1991; Blom et al., 1992). Mechanistically, the latter effect apparently involves a structural change in the complement receptor CR3 (see *IX.B.2*) rather than an increase in receptor density (Koenderman et al., 1991; Blom et al., 1992).

In addition to the aforementioned effects, PAF elicits a multitude of less well defined responses including chemokine generation, aggregation, adhesion, and adhesion molecule expression (see Table 5 for additional details).

3. In Vivo Effects. In guinea pigs, rabbits, and primates, aerosol and systemic administration of PAF results in the extravascular infiltration of eosinophils into the lungs which resembles, both in amplitude and duration, that seen in response to allergen in sensitized animals (Denjean et al., 1983; Arnoux et al., 1988; Lellouch Tubiana et al., 1988; Sanjar et al., 1990b; Gundel et al., 1991; Herd et al., 1992; Wegner et al., 1992). Comparable observations have been made with rats given PAF directly into the pleural cavity (Silva et al., 1989) and in atopic individuals where intradermal administration of

TABLE 5
Functional effects evoked by platelet-activating factor in eosinophils

Function	Species	Reference(s)
Activates NADPH oxidase	Human	Bruynzeel et al. (1986); Bruijnzeel et al. (1987); Kroegel et al. (1989c); Dri et al. (1991); Zoratti et al. (1991); Tool et al. (1992); Horie and Kita (1994); Elsner et al. (1995); Wymann et al. (1995); Dent et al. (1998a)
Activates NADPH oxidase	Cow	Freiburghaus et al. (1991)
Activates NADPH oxidase	Guinea pig	Kroegel et al. (1989a); Shute et al. (1990)
Primes NADPH oxidase to fMLP and SOZ	Human	Zoratti et al. (1992); Nagata et al. (1995b); Coffer et al. (1998)
Promotes chemotaxis and actin polymerization	Human	Wardlaw et al. (1986); Sigal et al. (1987); Tamura et al. (1987); Czarnetzki and Csato (1989); Kurihara et al. (1989); Little and Casale (1991); Sun et al. (1991); Fukuda et al. (1992); Miyagawa et al. (1992); Numao and Agrawal (1992); Townley et al. (1994); Elsner et al. (1996a); Erger and Casale (1996); Schweizer et al. (1996)
Promotes chemotaxis	Horse	Foster et al. (1992)
Promotes chemotaxis (weak)	Guinea pig	Sun et al. (1991)
Promotes chemotaxis	Monkey	Sun et al. (1991)
Promotes chemotaxis	Rat	Martins et al. (1989)
Promotes degranulation	Human	Kroegel et al. (1988, 1989c); Kernen et al. (1991); Horie and Kita (1994)
Promotes degranulation	Guinea pig	Kroegel et al. (1989a, 1991)
Promotes PGD_2, PGE_2, $PGF_{2\alpha}$ and TX formation	Human	Foegh et al. (1986); Kroegel and Matthys (1993); Dent et al. (1998a)
Promotes PGE_2, 6-keto-$PGF_{2\alpha}$, and TX formation	Guinea pig	Hirata et al. (1989); Giembycz et al. (1990)
Promotes a hypodense phenotype	Human	Kloprogge et al. (1989a); Yukawa et al. (1989a)
Promotes IL-8 release from GM-CSF-primed eosinophils	Human	Simon et al. (1995a)
Primes for IL-5-induced adherence	Horse	Foster et al. (1997)
Promotes LTC_4 formation	Human	Weller et al. (1983); Kajita et al. (1985); Bruynzeel et al. (1986, 1987); Tamura et al. (1988); Miyagawa et al. (1992); Dent et al. (1998a)
Promotes LTB_4 formation	Guinea pig	Sun et al. (1989); Hirata et al. (1990)
Reduces L-selectin expression	Human	Smith et al. (1992); Neeley et al. (1993)
Promotes homotypic aggregation	Guinea pig	Teixeira et al. (1995c)
Primes for SOZ-induced homotypic aggregation	Human	Koenderman et al. (1991); Blom et al. (1992)
Increases CD23 expression and IgE binding	Human	Walsh et al. (1989, 1990c); Moqbel et al. (1990b); Kawabe et al. (1991)
Increases expression of ICAM-1	Eos-1 cell line	Seguchi and Nakajima (1995)
Promotes lipid body formation	Human	Bozza et al. (1997a)
Increases CD11b/CD18 expression	Human	Walsh et al. (1991a); Zoratti et al. (1992); Neeley et al. (1993); Tsai et al. (1993)
Promotes adherence to HUVECs and BSA-coated plastic	Human	Kimani et al. (1988); Lamas et al. (1988); Walsh et al. (1990b)

PAF produces a cellular infiltrate rich in eosinophils that is reminiscent of the eosinophilia seen in the same subjects after antigen provocation (Henocq and Vargaftig, 1988). Similarly, in individuals with seasonal allergic rhinitis PAF, given intranasally and outside the pollen season, evokes a marked increase in the number of eosinophils (Klementsson and Andersson, 1992) and in the concentration of ECP (Tedeschi et al., 1994) in the nasal lavage fluid.

The ability of PAF to mimic many of the effects associated with allergen challenge led to the hypothesis, in the late 1980s, that PAF might play a central pathogenic role in allergic inflammatory diseases (Barnes et al., 1988; Page, 1988). That possibility prompted numerous preclinical and clinical studies designed to evaluate the potential anti-inflammatory activity of PAF antagonists. In animal models of inflammation, a bewildering number of structurally dissimilar PAF antagonists have been studied for their ability to suppress tissue eosinophil accumulation in response to a variety of stimuli [e.g., lipopolysaccharide (LPS), bradykinin, IL-1β] and following allergen provocation in sensitized animals. Table 6 identifies a cross-section of the current literature and illustrates an equivocal role for PAF in allergic inflammation. Of the 29 articles cited, 14 of them describe the failure of PAF antagonists to reduce allergic eosinophilia whereas the remainder report efficacy. In the clinical situation, the PAF antagonists apafant (Freitag et al., 1993; Spence et al., 1994), UK 74,505 (Kuitert et al., 1993), modipafant (Kuitert et al., 1995), and BN 52021 (Hsieh, 1991) do not affect allergen-induced airway responses, implying that PAF is not a mediator of allergic airway inflammation. However, PAF might merit "revisiting" since in 1997, Evans et al. reported that a highly potent PAF antagonist, foropafant (SR 27417A), reproducibly attenuated the late-phase response (LPR) in 12 male subjects with mild atopic asthma. Although measurements of pulmonary granulocyte titers were not made, the authors speculated that PAF may play a minor role in the genesis of the LPR by attracting eosinophils and other proinflammatory cells to the lung (Evans et al., 1997). Taken together, the results of the aforementioned studies are reminiscent of the early clinical experience with cysteinyl-leukotriene (LT) receptor antagonists and could indicate that complete antagonism of the effects of PAF needs to be achieved before clinical

TABLE 6
In vivo studies in laboratory animals in which platelet-activating factor antagonists were shown to be active and inactive in attenuating induced tissue eosinophil recruitment

PAF Antagonist	Route of Administration	Species	Comment(s)	Reference(s)
Active				
Y-24180	p.o.	Guinea pig	Attenuated recovery of eosinophils from the BAL fluid in response to antigen	Kagoshima et al. (1997)
Y-24180	p.o.	Guinea pig	Attenuated antigen-induced LPRs and recovery of eosinophils from BAL fluid	Inoue et al. (1992)
YM 264	p.o.	Guinea pig	Attenuated antigen-induced LPRs and infiltration of eosinophils into tracheal mucosa	Arima et al. (1995)
UK 74,505	i.v.	Rat	Attenuated IL-1β-induced cutaneous eosinophilia	Sanz et al. (1995)
UK 74,505	i.v.	Guinea pig	Attenuated cutaneous eosinophilia following passive cutaneous anaphylaxis	Sanz et al. (1994)
BN 52021	p.o.	Rabbit	Attenuated corneal allograft eosinophilia	Cohen et al. (1994)
CV 6209	i.p.	Mouse	Attenuated antigen-induced cutaneous eosinophilia	Iwamoto et al. (1993b)
PCA 4248	p.o.	Rat	Attenuated antigen-induced pleural eosinophilia	Martins et al. (1993)
SM 10661	p.o.	Guinea pig	Attenuated antigen-induced early and LPRs and recovery of eosinophils from BAL fluid	Sugasawa et al. (1991)
Apafant*	p.o.	Guinea pig	Attenuated antigen-induced bronchial eosinophilia	Chand et al. (1992b)
Bepafant*	i.p.	Guinea pig	Attenuated antigen-induced airway hyper-responsiveness and pulmonary eosinophilia	Seeds et al. (1991)
Yangambin	i.p.	Rat	Attenuated recovery of eosinophils from the pleural cavity in response to antigen	Serra et al. (1997)
E-6123	p.o.	Guinea pig	Attenuated recovery of eosinophils from BAL fluid in response to antigen	Tsunoda et al. (1991)
E-6123	p.o.	Guinea pig	Attenuated antigen-induced pulmonary eosinophilia	Sakuma et al. (1990)
BN-52021	Local	Rat	Attenuated antigen-induced peritoneal eosinophilia	Etienne et al. (1989a)
BN-52021	p.o.	Guinea pig	Attenuated antigen-induced airway hyper-responsiveness and pulmonary eosinophilia	Coyle et al. (1988)
Inactive				
CV 6209	i.p.	Mouse	Failed to affect SP-induced cutaneous eosinophilia	Iwamoto et al. (1993b)
L-659,989	i.p.	Guinea pig	Failed to affect ozone-induced eosinophil infiltration into airway mucosa	Tan and Bethel (1992)
RP 59227	p.o.	Guinea pig	Failed to affect eosinophil accumulation in BAL fluid in response to antigen	Underwood et al. (1992)
Apafant[a]	p.o.	Guinea pig	Failed to affect eosinophil accumulation in BAL fluid in response to antigen	Underwood et al. (1992)
Apafant[a]	i.v.	Horse	Failed to affect antigen-induced cutaneous eosinophilia	Foster et al. (1995)
Apafant[a]	i.d.	Guinea pig	Failed to affect cutaneous eosinophilia following passive cutaneous anaphylaxis	Weg et al. (1994)
Apafant[a]	p.o.	Guinea pig	Failed to affect accumulation of eosinophils in BAL fluid in response to antigen	Havill et al. (1990)
Apafant[a]	i.p.	Rat	Failed to affect accumulation of eosinophils in BAL fluid in response to compound 48/80	Martins et al. (1990)
BN 50730	p.o.	Rat	Failed to affect LTB_4, bradykinin, and LPS-induced pleural eosinophilia	Pires et al. (1994)
BN 52021	Local	Rat	Failed to affect bradykinin-induced pleural eosinophilia	Pasquale et al. (1991)
SDZ 64-412	p.o.	Guinea pig	Failed to affect antigen-induced pulmonary eosinophilia	Ishida et al. (1990)
SDZ 64-412	p.o.	Guinea pig	Failed to affect accumulation of eosinophils in BAL fluid in response to antigen	Havill et al. (1990)
SR 27417	s.c.	Mouse	Failed to affect antigen-induced peritoneal eosinophilia	Zuany-Amorim et al. (1993)
UK 74,505	i.v.	Guinea pig	Failed to affect SP-induced cutaneous eosinophilia	Walsh et al. (1995a)

[a] Apafant WEB 2086; Bepafant WEB 2170.

benefit is seen. Alternatively, PAF simply might play only a minor part in human asthma despite its prominent role in many animal models of the disease.

B. Leukotriene B_4

1. Receptors and Signaling. The BLT, or LTB_4, receptor, which is expressed on guinea pig, mouse, and probably human eosinophils, was cloned in 1997 from retinoic acid-differentiated HL-60 cells. This human receptor is composed of 352 amino acids and is a member of the seven transmembrane-spanning family of G protein-coupled receptors (Yokomizo et al., 1997). A cDNA that encodes a 351-amino acid murine glycoprotein that is 78% identical with the human BLT receptor has also been identified and expressed in Chinese hamster ovary (CHO) cells (Huang et al., 1998). An analysis of [^{3}H]LTB_4 binding to membrane fractions prepared from CHO cells, and retinoic acid-differentiated HL-60 and COS-7 cells transfected with the cDNA for the human and murine LTB_4 receptor show similar binding characteristics, with K_d values of 0.1, 0.14, and 0.15 nM, respectively (Yokomizo et al., 1997; Huang et al., 1998). Binding studies also have identified and partially characterized the BLT receptor on murine and guinea pig eosinophils (Maghni et al., 1991; Ng et al., 1991; Sehmi et al., 1992a; Huang et al., 1998) also using [^{3}H]LTB_4 as the radioligand. However, notable differences are apparent between these studies. Using intact peritoneal eosinophils from guinea pigs, Ng et al. (1991) reported that [^{3}H]LTB_4 interacts with an apparently homogeneous

population of binding sites with a B_{max} of 40,000 sites per cell and a K_d of 2.8 nM, which is approximately 10-fold lower than that reported in the transfection experiments described by Yokomizo et al. (1997). Similar results were reported for the murine receptor (Huang et al., 1998). The sites labeled on eosinophils probably represent functional receptors since various compounds related structurally to LTB_4 compete with the radioligand with affinities that correlate closely with their ability to induce chemotaxis and to evoke the formation of superoxide anions (Ng et al., 1991). Intriguingly, the rank order of potency for the displacement of [^{3}H]LTB_4 from intact peritoneal eosinophils [LTB_4 > 20-hydroxy-LTB_4 > 12*R*-hydroxyeicosatetranoic acid (HETE) > 12*S*-HETE > 20-carboxy-LTB_4 > 5*S*,12*S*-dihydroxyeicosapentanoic acid (diHEPE) (Ng et al., 1991)] differs from the rank order obtained using membranes from COS-7 cells transfected with the LTB_4 receptor [LTB_4 > 20-hydroxy-LTB_4 > 20-carboxy-LTB_4 > 5*S*,12*S*-diHEPE > 12*R*-HETE > 12*S*-HETE (Yokomizo et al., 1997)] which might indicate species difference, LTB_4 receptor heterogeneity (see below), and/or the existence of different conformations of a single LTB_4 receptor. With respect to the two latter possibilities, Maghni et al. (1991) reported that [^{3}H]LTB_4 interacts with a heterogeneous population of binding sites on guinea pig alveolar eosinophils; approximately 1000 sites/cell are labeled with high affinity (K_d = 1 nM), whereas 5500 sites/cell are labeled with low affinity (K_d = 63 nM). Identical results have been obtained with guinea pig peritoneal eosinophils (Sehmi et al., 1992a). Thus, a small population (B_{max} = 900 sites/cell) of receptors for which LTB_4 has high affinity (K_d = 0.3 nM) were identified by radioligand binding along with a large number of sites (60,000/cell) at which LTB_4 has relatively low affinity (K_d = 140 nM). Again, the finding that various metabolites of LTB_4 competed with [^{3}H]LTB_4 for binding to alveolar eosinophils with a rank order of potency in good agreement with their ability to induce chemotaxis (Maghni et al., 1991) supports the belief that the high-affinity sites represent bona fide receptors. Of considerable interest is the role of the two populations of receptor expressed by these cells. Maghni et al. (1991) have considered the hypothesis, posed originally by Goldman and Goetzl (1984), that they mediate different functional responses: the receptor for which LTB_4 has high affinity subserving chemokinesis and chemotaxis, the receptor for which LTB_4 has low-affinity mediating respiratory burst and prostanoid generation. Support for this idea derives from affinity estimates of the LTB_4 antagonist U-75302, which differs significantly (~17-fold) between the two populations of receptor (Maghni et al., 1991). Collectively, the available data suggest that peritoneal eosinophils express the same LTB_4 receptor that is labeled with high affinity by [^{3}H]LTB_4 on guinea pig alveolar eosinophils [albeit at a much higher (~ 40-fold) density]. A reason for the inability of Ng et al. (1991) to identify receptors on guinea pig peritoneal eosinophils for which LTB_4 has low-affinity may relate to the fact that in those studies [^{3}H]LTB_4 was not used at concentrations that would detect the low-affinity sites.

LTB_4 exerts a number of effects on eosinophils (Table 7) and progress has been made in understanding the second messenger pathways underlying LTB_4 receptor signal transduction (Fig. 4). In guinea pig eosinophils, which can be obtained in large numbers and of high purity, LTB_4 induces a rapid and transient accumulation of Ins(1,4,5)P_3 and elevates $[Ca^{2+}]_i$ via a PTX-sensitive pathway (Subramanian, 1992; Perkins et al., 1995; Teixeira et al., 1997b; Lindsay et al., 1998c; Huang et al., 1998). However, Ca^{2+} mobilization (EC_{50} = 0.6 nM) occurs without a detectable increment in Ins(1,4,5)P_3 mass (EC_{50} = 200 nM), which suggests that they are unrelated events. Indeed, the Ca^{2+} ions mobilized by LTB_4 are extracellular in origin and enter the cell through a PTX-sensitive, receptor-operated Ca^{2+} channel (Subramanian, 1992; Perkins et al., 1995; Lindsay et al., 1998a,c). In addition to coupling to PLC, LTB_4 also promotes the extracellular release of [^{3}H]AA (Lindsay et al., 1998a,b,c). This effect is due to the direct

TABLE 7
Functional effects evoked by LTB_4 in eosinophils

Function	Species	Reference(s)
Activates NADPH oxidase	Human	Palmblad et al. (1984)
Activates NADPH oxidase	Guinea pig	Maghni et al. (1991); Ng et al. (1991); Rabe et al. (1992); Subramanian (1992); Perkins et al. (1995); Lindsay et al. (1998c)
Promotes chemotaxis	Human	Czarnetzki and Mertensmeir (1985); Czarnetzki and Rosenbach (1986); Czarnetzki and Csato (1989); Numao and Agrawal (1992); Kim et al. (1994)
Promotes chemotaxis	Horse	McEwen et al. (1990)
Promotes chemotaxis	Guinea pig	Taylor et al. (1989, 1991); Coeffier et al. (1991a); Ng et al. (1991); Sun et al. (1991)
Promotes chemotaxis	Mouse	Huang et al. (1998)
Promotes homotypic aggregation	Guinea pig	Teixeira et al. (1995c)
Promotes histaminase release	Guinea pig	Popper et al. (1989)
Promotes peroxidase release	Guinea pig	Popper et al. (1989)
Promotes chemokinesis	Human	Spada et al. (1994)
Promotes PLA_2 activity/AA release	Guinea pig	Perkins (1993); Lindsay et al. (1998a,b,c)
Promotes prostanoid formation	Guinea pig	Maghni et al. (1991); Souness et al. (1994)
Increases CR1 expression	Human	Nagy et al. (1982)
Increases FcεRII (CD23) expression and binding of IgE	Human	Walsh et al. (1989); Moqbel et al. (1990b); Walsh et al. (1990c)

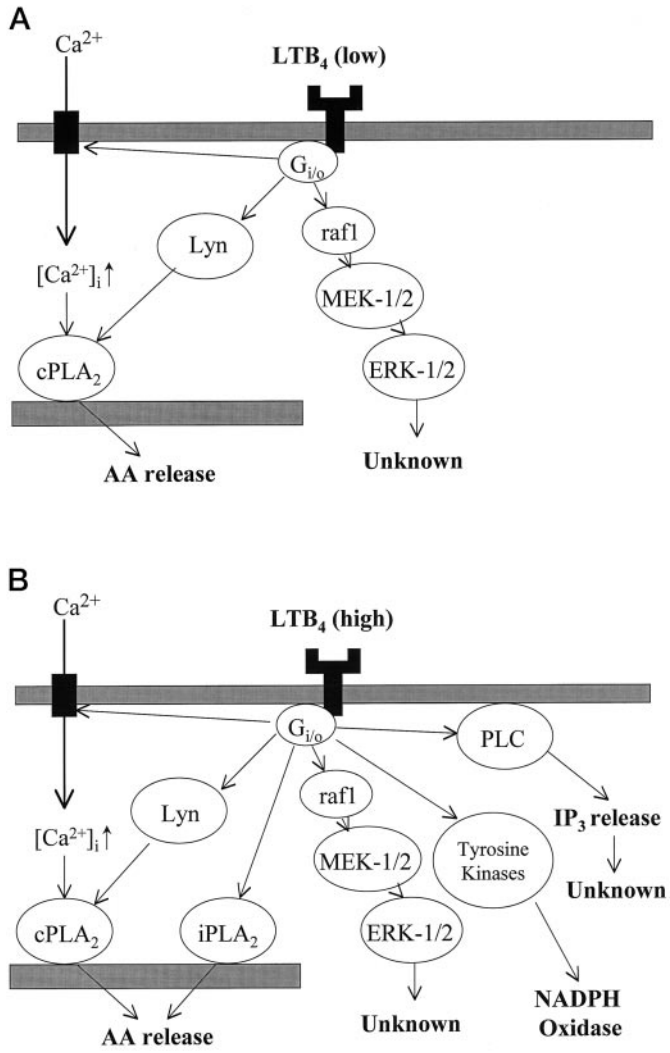

FIG. 4. LTB_4-induced signaling in guinea pig peritoneal eosinophils. Scheme A, low concentrations (1 pM to 10 nM) of LTB_4 induce a PTX-sensitive increase in the $[Ca^{2+}]_I$ and activate the *src*-related tyrosine kinase *lyn* and the raf-1/MEK-1/2/ERK-1/2 protein kinase cascade. The increase in $[Ca^{2+}]_I$ is due exclusively to influx of extracellular Ca^{2+}, whereas the activation of *lyn*, is thought to mediate the activation of a Ca^{2+}-dependent PLA_2 (possibly $cPLA_2$) and the subsequent release of AA. Scheme B, higher concentrations (100 nM to 1 μM) of LTB_4 activate PLC, with attendant generation of Ins(1,4,5)P_3, and a Ca^{2+}-independent PLA_2 (possibly $iPLA_2$) with a further liberation of AA. LTB_4 also is thought to stimulate a tyrosine kinase-dependent pathway that is implicated in the activation of the NADPH oxidase. See *V.B* for additional details.

coupling of the BLT receptor to phospholipase A_2 (PLA_2) since it is preserved under conditions where signaling through PLC is prevented (Lindsay et al., 1998c). Moreover, the elaboration of [^{3}H]AA is biphasic (Perkins, 1993) and involves the activation of two PTX-sensitive PLA_2s in these cells; one of these is Ca^{2+}-dependent and is activated by low concentrations of LTB_4 whereas the other apparently does not require Ca^{2+} for activity and is activated by high concentrations of LTB_4 (Lindsay et al., 1998a,c). Exposure of guinea pig eosinophils to LTB_4 also causes a rapid activation of ERK-1, ERK-2 (Araki et al., 1995; Lindsay et al., 1998b), and the *src*-related tyrosine kinases, $p53^{lyn}$, and $p56^{lyn}$ (Lindsay et al., 1998a); it does not activate phospholipase D (PLD) (Perkins et al., 1995).

A comparison of the concentration-response relationships which describe a number of biochemical responses evoked by LTB_4 implies that the increase in $[Ca^{2+}]_i$ and the subsequent activation of the Ca^{2+}-dependent PLA_2 and ERK is mediated via the BLT receptor for which LTB_4 has high affinity. In contrast, Ins(1,4,5)P_3 accumulation (index of PLC activity) and the activation of Ca^{2+}-independent PLA_2 is mediated by the BLT receptor that is recognized by LTB_4 with low affinity. In agreement with Maghni et al. (1991), those data support the idea that the two populations of the LTB_4 receptor mediate chemotaxis and activation of NADPH oxidase, respectively.

2. In Vivo Effects. A number of in vivo animal models have been developed to establish the potential pathogenic role of LTB_4 in allergic eosinophil inflammatory disorders and autoimmune diseases such as multiple sclerosis and asthma. In 1996, Gladue et al. reported that the LTB_4 antagonist CP 105,696 abolished the ability of encephalogenic T lymphocytes, injected into naïve mice, to evoke two cardinal features of experimental allergic encephalomyelitis (multiple sclerosis): paralysis and weight loss. Moreover, the protection was associated with a 97% reduction in the accumulation of eosinophils to the lower spinal cord as determined by light and electron microscopy, and by the level of EPO (Gladue et al., 1996). Those findings have important implications since they show that agonism of LTB_4 receptors results in eosinophil recruitment *and* that they play a hitherto unrecognized role in the pathogenesis of experimental allergic encephalomyelitis. Clearly, the possible utility of LTB_4 antagonists in the treatment of human multiple sclerosis, and the part eosinophils play in that disease, merits evaluation.

With respect to asthmatic inflammation, LTB_4 given by the inhaled route promotes eosinophil influx into the airways of guinea pigs and Brown Norway rats (Silbaugh et al., 1987; Richards et al., 1991b), which is entirely consistent with its chemotactic activity in vitro. Moreover, pulmonary eosinophilia after allergen provocation of sensitized animals is attenuated by LTB_4 antagonists (Richards et al., 1989, 1991b), implicating immunologically released LTB_4 as an important chemoattractant. In this respect, it is noteworthy that allergen challenge of sensitized mice is associated with an increase in BLT receptor mRNA levels in lung tissue with a time course that parallel eosinophil influx (Huang et al., 1998). Despite the aforementioned data, the mechanisms responsible for allergen-induced eosinophil recruitment in humans in vivo is little investigated. However, the possibility that LTB_4 is an important chemotaxin has been proposed following the observation that eosinophils harvested from the airways of ragweed-sensitive, allergic volunteers and subjected to segmental antigen challenge were significantly less sensitive to LTB_4-driven chemotaxis studied ex vivo when com-

pared with peripheral blood eosinophils purified from the same individuals (Kim et al., 1994). The additional finding that PAF- and fMLP-induced chemotactic responses in the two populations of cell were identical led Kim et al. (1994) to conclude that eosinophils had been exposed to LTB_4 in vivo, and that this provided evidence that allergen-induced pulmonary eosinophilia is partly driven by immunologically generated LTB_4. However, a subsequent clinical study with the LTB_4 antagonist LY 293111 failed to implicate LTB_4 in allergen-induced early and LPRs, and did not reduce eosinophil numbers recovered in bronchoaveolar lavage (BAL) fluid (Evans et al., 1996).

C. Cysteinyl Leukotrienes

Two receptors (*Cys*-LT_1 and *Cys*-LT_2) for the cysteinyl LTs, which include LTC_4, LTD_4, and LTE_4, have been classified pharmacologically but supporting molecular evidence is still awaited. Both receptors couple predominantly through the $G_{q/11}$ class of heterotrimeric GTP-binding proteins, and it is highly likely that they are members of the seven transmembrane-spanning family of receptors (see Coleman et al., 1995 for additional details). Currently, selective antagonists are available only for the *Cys*-LT_1 receptor and these have been used to identify those receptors on eosinophils. However, antagonist affinities have not been calculated and the assignment of eosinophil leukotriene receptors as *Cys*-LT_1 is equivocal.

1. In Vitro Effects. Relatively little is known of the pharmacological actions of cysteinyl-leukotrienes on eosinophil function compared to those of LTB_4. Although early studies failed to demonstrate that LTD_4 possessed chemoattractant activity (Nagy et al., 1982; Camp et al., 1983), convincing evidence is now available to the contrary. Using a novel in vitro method, which allows the quantification of migration distance and vectorial orientation, it has been shown that LTD_4 is a potent chemoattractant for human eosinophils, with activity in the subnanomolar range. Moreover, LTD_4-induced chemotaxis is antagonized by SK&F 104353, suggesting that *Cys*-LT_1 receptors are involved (Spada et al., 1994, 1997). In contrast, LTD_4 does not increase the chemokinetic response of eosinophils above spontaneous migratory activity (Spada et al., 1994).

2. In Vivo Effects. In laboratory animals, LTD_4 and LTE_4 given locally and systemmically can stimulate the accumulation of eosinophils into various sites including the skin, eye, and lungs (Spada et al., 1986, 1988; Chan et al., 1990; Foster and Chan, 1991; Woodward et al., 1991; Wegner et al., 1993; Underwood et al., 1996). For example, in one study guinea pig eosinophils were labeled with [^{111}In]oxime and injected (i.v.) into recipient animals (naïve and sensitized), and the effect of LTD_4 and allergen on their emigration into the conjunctiva was monitored. Using that model, it was consistently found that LTD_4 and allergen significantly enhanced conjunctival radioactivity by a mechanism that was abolished (LTD_4) and reduced by 50% (allergen) by the *Cys*-LT_1 receptor antagonist MK-571 (Chan et al., 1990). Significantly, LTD_4 neither promotes the infiltration of eosinophils into the skin of guinea pigs following intradermal administration nor is it active in other ocular anterior segment structures such as the iris, cornea, and ciliary body after topical or intracameral administration (Woodward et al., 1991). Thus, it appears that LTD_4 regulates eosinophilia in a tissue-dependent manner.

With respect to pulmonary eosinophilia, Underwood et al. (1996) reported that administration of LTD_4 by aerosol to conscious guinea pigs increased the number of eosinophils in the BAL fluid and in the bronchi and subepithelium by a mechanism sensitive to the *Cys*-LT_1 receptor antagonist pranlukast. Intriguingly, LTD_4 evoked a sustained eosinophilia for up to 4 weeks after single exposure, although it was not established whether this was due to continued trafficking of eosinophils to and away from the lung, or to the enhanced survival of the same population of invading cells. This nonbronchoconstrictor activity of LTD_4 was mediated indirectly through the liberation of IL-5 (Underwood et al., 1996). Although the target cells at which LTD_4 acts to produce this effect have not been determined, the limited number of cells that secrete IL-5 suggests that resident T lymphocytes are prime candidates.

In asthmatic subjects, inhalation of LTD_4 and LTE_4, the most stable cysteinyl-LT, promotes pulmonary eosinophilia in the sputum (Diamant et al., 1997) and airway biopses (Laitinen et al., 1993), respectively. These findings are consistent with studies performed in animal models of asthma including the rat (Asano et al., 1992; Harris et al., 1997), rabbit (Herd et al., 1992), guinea pig (Gulbenkian et al., 1990; Nakagawa et al., 1993; Yeadon et al., 1993; Chabot-Fletcher et al., 1995; Seeds et al., 1995; Tohda et al., 1997), and mouse (Henderson et al., 1996) where *Cys*-LT_1 antagonists and inhibitors of 5-lipoxygenase and 5-lipoxygenase-activating protein (FLAP) reduce allergen-stimulated pulmonary eosinophilia. Although similar results have yet to be convincingly confirmed in clinical asthma, preliminary data are available on the effect of the *Cys*-LT_1 antagonist montelukast on eosinophil numbers and ECP content of sputum taken before and after allergen challenge (Grootendorst et al., 1997; Leff et al., 1997). In agreement with results obtained from studies examining the effect of a single dose of inhaled glucocorticosteroids (Pizzichini et al., 1995), a short course of treatment (10 mg administered 36 h and 12 h before and 12 h after allergen) with oral montelukast failed to reduce sputum eosinophilia and ECP content despite protecting against allergen-induced airway responses (Grootendorst et al., 1997). In contrast, treatment of asthmatic subjects with montelukast for 4 weeks (10 mg daily) significantly reduced sputum eosinophil numbers compared to those of placebo (Leff et al., 1997), indicating that prolonged administration of asthmatic individuals with *Cys*-LT_1

receptor antagonists could be required before an anti-inflammatory effect is seen. Clinical studies with zafirlukast (Calhoun et al., 1998) and montelukast (Reiss et al., 1996) have demonstrated a reduction in inflammatory cell number that appears in the BAL fluid of asthmatic subjects after segmental allergen challenge and a decrease in the titer of circulating blood eosinophils, respectively. A reduction in circulating blood eosinophils also has been reported in patients with nocturnal asthma following treatment with the 5-lipoxygenase inhibitor zileuton, which was associated with clinical improvement (Wenzel et al., 1995). Collectively, the implications of these data are clear: *Cys*-LT_1 antagonists, in addition to acting as bronchodilators, possess additional "anti-inflammatory" properties that might contribute to their therapeutic utility in diseases such as asthma.

D. N-Formyl-Methionyl-Leucyl-Phenylalanine

Two variants of the human fMLP receptor have been isolated from a CDM8 expression library prepared from mRNA extracted from dibutyryl cyclic AMP-differentiated HL-60 cells (Boulay et al., 1990a). Both recombinant forms of the receptor are composed of 350 amino acids, have a predicted molecular mass of 38 kDa, but differ from each other by two residue changes at positions 101 and 346; significant differences also are apparent at the 3′- and 5′-untranslated regions (Boulay et al., 1990a). Expression of these proteins in COS-7 cells results in the appearance of two populations of a highly glycosylated receptor for which the hydrophilic ligand *N*-fMLP-lysine has high affinity with K_d values of 0.5 to 1 nM and 5 to 10 nM (Boulay et al., 1990b). Moreover, several transcripts have been identified by Northern blot analysis, suggesting that the fMLP receptor is a family of closely related proteins.

At present, there are no molecular data concerning the nature of the fMLP receptor(s) expressed by eosinophils of any species. However, functionally, fMLP elicits a variety of effects in isolated cells, some of which are listed in Table 8. Less is published on the in vivo effects of formylated peptides, although in guinea pigs fMLP promotes lumenal chemotaxis of eosinophils as assessed by histology and differential cell counts (Munoz et al., 1997a). Intriguingly, that effect is attenuated by the LTB_4 antagonist LTB_4 dimethyl amide, by zileuton, a 5-lipoxygenase inhibitor, and by zafirlukast, a *Cys*-LT_1 antagonist, indicating that the ability of fMLP to facilitate the migration of eosinophils from the lamina propria to the airway lumen of guinea pigs is indirect and requires the liberation of LTB_4 and LTD_4 (Munoz et al., 1997a).

Compared to other G protein-coupled receptors, relatively little is known of the signaling pathways recruited following ligation of the fMLP receptor on eosinophils. It is established that fMLP promotes a rapid and transient increase in $[Ca^{2+}]_i$ in both human (Yazdanbakhsh et al., 1987b; Wymann et al., 1995) and guinea pig eosinophils (Kroegel et al., 1990b) that is believed to be important for the generation of oxygen-derived free radicals (Kernen et al., 1991). Furthermore, many of the functional effects elicited by fMLP including degranulation (Kita et al., 1991a), activation of the NADPH oxidase (Kernen et al., 1991), the release of IL-8 (Miyamasu et al., 1995), as well as intracellular markers of activation (stimulation of PLC, Ca^{2+} mobilization) are mediated by a PTX-sensitive mechanism(s), indicating the involvement of one of more members of the G_i or G_o family of heterotrimeric GTP-binding proteins.

E. Chemokines

Chemokines are an expanding superfamily of proteins with molecular masses of between 8 and 10 kDa (for reviews, see Horuk, 1994; Power and Wells, 1996; Rapport et al., 1996a). Characteristically, human chemokines

TABLE 8
Functional effects evoked by N-formyl methionyl leucyl phenylalanine in eosinophils

Function	Species	Reference(s)
Activates the NADPH oxidase	Human	Palmblad et al. (1984); Sedgwick et al. (1985); Yazdanbakhsh et al. (1987a); Koenderman and Bruijnzeel (1989); Sedgwick et al. (1990b); Dri et al. (1991); Kernen et al. (1991); White et al. (1993); Wymann et al. (1995)
Weak chemoattractant	Human	Ogawa et al. (1981b); Morita et al. (1989a,b)
Releases β-glucuronidase (weak effect)	Human	Morita et al. (1989b)
Releases EPO	Human	Kernen et al. (1991); White et al. (1993)
Releases EDN	Human	Kita et al. (1991a)
Promotes LTC_4 generation	Human	Fitzharris et al. (1986); Owen et al. (1987, 1991); Takafuji et al. (1991, 1992); White et al. (1993)
Increases CR3 expression	Human	Neeley et al. (1993)
Reduces L-selectin expression	Human	Neeley et al. (1993)
Promotes adherence to HUVECs and serum-coated plastic	Human	Kimani et al. (1988)
Promotes PAF generation	Human	Lee et al. (1984); Triggiani et al. (1992)
Promotes MCP-1 release	Human	Izumi et al. (1997)
Promotes IL-8 release	Human	Miyamasu et al. (1995)
Activates the NADPH oxidase	Guinea pig	Kroegel et al. (1990b)
Promotes EPO release	Guinea pig	Kroegel et al. (1990b)
Promotes thromboxane generation	Guinea pig	Hirata et al. (1989); Giembycz et al. (1990)
Promotes prostacyclin generation	Guinea pig	Hirata et al. (1989)
Chemoattractant	Horse	McEwen et al. (1990)

contain four distinct and conserved cysteine residues that have provided the basis of their classification either as CXC or α chemokines, where the first two cysteine residues are separated by an amino acid, or CC or β chemokines, where the first two cysteines are adjacent. Two other chemokine families have been described: C (or γ) chemokines that contain a single cysteine residue and include lymphotactin, and the CX_3C chemokine family (also known as δ chemokines) where three amino acids separate the two cysteine residues, of which fractalkine and neurotactin are examples. The CXC chemokines generally are involved in the recruitment of neutrophils and have been implicated in acute inflammatory responses. In contrast, CC chemokines exert their actions upon other leukocyte populations, including eosinophils, monocytes, and T lymphocytes, and are believed to be involved in the pathogenesis of chronic inflammation. Four CXC and eight CC chemokine receptors have been cloned thus far that are recognized by a selective range of chemokines with characteristic rank orders of potency (see Gerard and Gerard, 1994; Murphy, 1994; Ben Baruch et al., 1995; Combadiere et al., 1995, Gao and Murphy, 1995; Power et al., 1995; Hoogewerf et al., 1996; Ponath et al., 1996a,b; Power and Wells, 1996; Raport et al., 1996b; Samson et al., 1996; Heath et al., 1997). It is this diversity of chemokine receptor expression and the selective release of chemokines that provide a mechanism for the recruitment of different leukocyte populations to inflammatory sites. Moreover, in the context of asthma, chemokines activate distinct cellular and biochemical pathways that act in a coordinated fashion to elicit complex pathophysiological changes such as eosinophilia and airways hyperreactivity (Gonzalo et al., 1998).

Of the multitude of chemokine receptors thus far identified, human eosinophils express CCR1, CCR3, and possibly a receptor for IL-8 that is either CXCR1 or CXCR2 (Table 9). The pharmacological properties of these receptors and the functional responses they subserve are discussed below.

1. CC Chemokines. The eotaxin receptor, or CCR3, is selectively expressed upon eosinophils, basophils, and $CD4^+$ T lymphocytes (Ponath et al., 1996a), and is a major binding site for CC chemokines (Daugherty et al., 1996; Gao et al., 1996; Kitaura et al., 1996; Ponath et al., 1996b; Forssmann et al., 1997; Heath et al., 1997). CCR3 has been cloned from guinea pig (Sabroe et al., 1998), murine (Gao et al., 1996), and human eosinophils (Ponath et al., 1996a), and the latter has been transfected into AML14.3D10 (Daugherty et al., 1996) and murine pre-B lymphoma cell lines (Ponath et al., 1996a) where it binds eotaxin, regulated on activation, normal T-expressed and secreted (RANTES), and monocyte chemotactic protein (MCP) 3 at levels that are indistinguishable from those achieved in binding studies with primary eosinophils. Furthermore, a study using an antagonistic monoclonal antibody demonstrated that >95% of the eosinophil's response evoked by eotaxin, RANTES, MCP-2, MCP-3, and MCP-4 was mediated through CCR3 (Heath et al., 1997). Eosinophils also express low levels of the chemokine receptor CCR1, which appears to mediate the effects of MIP-1α (Daugherty et al., 1996). The expression of CCR1 and CCR3 is up-regulated during the maturation of eosinophilic HL-60 cells, although the kinetics of these effects is different with CCR1 levels rising first (Tiffany et al., 1998). Significantly, increased CC chemokine receptor expression correlates with the development of specific binding sites for MIP-1α and eotaxin, and the accompanying ability of the cells to generate Ca^{2+} and chemotactic responses (Tiffany et al., 1998). CCR3 expression also is increased on eosinophilic HL-60 cells by IL-5, suggesting that the chemokine receptors represent a marker of late eosinophilic differentiation (Tiffany et al., 1998).

Cloning and sequencing studies have established that the human CCR3 is composed of 355 amino acids with an approximate molecular mass of 41 kDa (Daugherty et al., 1996; Ponath et al., 1996a,b). The receptor contains four cysteine residues at amino acids 24, 106, 183, and 273, and a DRYLAIVHA motif between residues 130 and 138, that is characteristic of all chemokine receptors. In addition, the receptor contains two PKC phosphorylation sites, one in the third intracellular loop at amino acid 231 and the second in the cytoplasmic tail at position 333. Eight serine/threonine residues also are present within the cytoplasmic tail, providing additional possibilities for post-translational modifications (Ponath et al., 1996a,b). Binding studies have demonstrated that eotaxin, RANTES, and MCP-3 bind to a single population of noninteracting sites (B_{max} = 40,000 per cell) with affinities of 0.1, 2.7, and 3.1 nM, respectively (Daugherty et al., 1996). The guinea pig CCR3 is a 358-amino acid protein that shares 67 and 69% primary sequence identity to its human and murine homologs, respectively (Sabroe et al., 1998).

CC chemokines induce eosinophil chemotaxis and increase the intracellular free Ca^{2+} concentration and ac-

TABLE 9
Human chemokine receptors expressed by eosinophils and their endogenous ligands

Chemokine Subfamily	Nomenclature	Endogenous Ligand(s)
CXC chemokines	*CXCR1*	IL-8, GCP-2
	CXCR2	IL-8, GCP-2, GROα, GROβ, GROδ, NAP-2, ENA-78
CC chemokines	*CCR1*	MIP1α, RANTES, MCP-2, MCP-3, MCP-5, leukotactin-1
	CCR3	Eotaxin, eotaxin-2, leukotactin-1, MCP-3, MCP-4, RANTES

tin polymerization that is associated with this response. These include eotaxin-1 (Jose et al., 1994b; Elsner et al., 1996b; Garcia Zepeda et al., 1996a), eotaxin-2 (Forssmann et al., 1997), RANTES, (Kameyoshi et al., 1992, 1994; Rot et al., 1992; Alam et al., 1993; Kameyoshi et al., 1992, 1994; Schweizer et al., 1994; Elsner et al., 1996b), MCP-2 (Noso et al., 1994; Weber and Dahinden, 1995), MCP-3 (Dahinden et al., 1994; Noso et al., 1994; Elsner et al., 1996b), and MCP-4 (Garcia Zepeda et al., 1996b; Stellato et al., 1997). In contrast, the related chemokines MCP-5 (Sarafi et al., 1997) and MIP-1α (Rot et al., 1992; Dahinden et al., 1994) are relatively weak chemoattractants.

In addition, CC chemokines induce a range of additional cellular responses in eosinophils and display a similar spectrum of activities. Thus, RANTES, eotaxin, eotaxin-2, MIP-1α, and MCP-4 activate the NADPH oxidase (Rot et al., 1992; Chihara et al., 1994; Kapp et al., 1994; Elsner et al., 1995, 1996b; Tenscher et al., 1996; Elsner et al., 1998; Petering et al., 1998), transiently promote CR3- and VLA-4-dependent adherence to fibronectin and vascular cell adhesion molecule (VCAM) 1 (Weber et al., 1996), enhance the expression of CD11b (Alam et et., 1993; Tenscher et al., 1996), and stimulate the release of IL-8 from eosinophils primed with GM-CSF (Simon et al., 1995b). In addition, activation of CCR3 enhances firm adhesion of eosinophils to human umbilical vein endothelial cells (HUVECs) through α_4 and β_2 intergrins even in shear flow (Kitayama et al., 1998). Many of the aforementioned eosinophil responses are inhibited by PTX, indicating that CCR1 and CCR3 can couple to their effectors through G_i and/or G_o.

In vivo, eotaxin and eotaxin-2 selectively promote cutaneous eosinophilia in humans (Forssmann et al., 1997). Moreover, in allergic reactions, these chemokines are believed to cooperate with IL-5 in the mobilization and subsequent "homing" of eosinophils to specific tissues (see *XII.A.3*). Similarly, RANTES when injected into the skin of dogs promotes a local eosinophilia (Meurer et al., 1993). In asthmatic subjects, allergen provocation is associated with an increase in mRNA transcripts and protein for eotaxin that appears before the development of the LPR and infiltration of eosinophil into the airways (Brown et al., 1998). Significantly, at late time points, when eotaxin expression declined, the number of eosinophils recovered from the BAL fluid continued to rise, suggesting that eotaxin contributes only to the early phase of eosinophilia and that other mediators regulate the persistent eosinophilia (Brown et al., 1998).

Although eotaxin expression usually is associated with inflammation, it also is expressed basally and is involved in the fundamental baseline trafficking of eosinophils from the circulation to tissues in health (Matthews et al., 1998). However, this appears to be restricted to the gut where appreciable degranulation is also common (Kato et al., 1998b).

RANTES also promotes degranulation of eosinophils with the release of ECP and EDN (Alam et al., 1993; Horie et al., 1996) by a mechanism that might involve the opening of high conductance Ca^{2+}-activated K^+ channels (Saito et al., 1996). Indeed, patch-clamping studies have shown that RANTES increases the open-state probability of these channels in EoL-1 cells with a unit conductance of 14 pS. Moreover, channel activation is blocked by PTX and mimicked by the intracellular application of GTPγS (in inside-out patches) and by Ca^{2+} consistent with the interaction of RANTES with the G_i-coupled receptor CCR3 (Saito et al., 1996). RANTES also activates PtdIns 3-kinase and promotes subsequently the phosphorylation of PKB in human eosinophils (Coffer et al., 1998). Although the functional consequences of these biochemical effects are largely unexplored they might represent upstream effectors of the NADPH oxidase (Coffer et al., 1998).

2. CXC Chemokines. Only one CXC chemokine, IL-8, is known to activate eosinophils. However, whether it mediates its effects through CXCR1 (IL-8_A-R) or CXCR2 (IL-8_B-R) is unexplored. Several inconsistencies exist in the literature with respect to the sensitivity of eosinophils to IL-8. Erger and Casale (1995) found that IL-8 promoted eosinophil migration across micropore filters and through monolayers of HUVECs and A549 cells. However, those findings contradict the results obtained in a previous study (Ebisawa et al., 1994). This discrepancy may have resulted from cell priming during the isolation of eosinophils (Rozell et al., 1996). Indeed, this explanation would be in agreement with a number of in vitro studies where IL-8-induced chemotaxis is observed only after preincubation of eosinophils with IL-5 or GM-CSF (Warringa et al., 1991, 1992b; Schweizer et al., 1994; Heath et al., 1997). Interleukin-8-induced chemotaxis is associated with an increase in the $[Ca^{2+}]_i$ (Collins et al., 1993) and actin polymerization (Schweizer et al., 1994). In vivo studies have established that IL-8 elicits eosinophil migration into the BAL fluid (Lagente et al., 1995) and skin of guinea pigs (Collins et al., 1993), although it is uncertain whether this is a direct or indirect response.

F. Complement

Complement is a collective term that refers to a group of at least 30 proteins including proteolytic proenzymes, nonenzymatic components from which active enzymes are derived, and receptors that together form part of an intricate enzyme system found in plasma (Ember and Hugli, 1997). Triggering of these systems sets in motion an amplification cascade that ultimately results in the formation of the, so-called, terminal attack sequence that promotes cell lysis and is central to protecting the host from invading parasites and microbes. In excess of nine receptors for complement fragments have been identified and characterized to some extent (see Ross, 1989; Krych et al., 1992; Wetsel, 1995; Ember and Hugli,

1997). The 74- to 77-amino acid anaphylatoxins (C3a, C4a, C5a) are known to signal through G protein-coupled receptors and these are described below. The remaining complement receptors relevant to eosinophil biology are discussed in *XI. M.*

1. Complement 3a Anaphylatoxin. The most abundant and important complement component is C3, which is split by a convertase into the anaphylatoxin C3a and a larger fragment, C3b. Through combination with factor B and in the presence of a normal plasma enzyme, factor D, C3b forms C3bBb that can act in a positive feedback loop to further degrade C3.

The ability of C3a to bind to the surface of human eosinophils was first demonstrated in 1979 (Glovsky et al., 1979), and specific saturable binding sites for this anaphylatoxin were subsequently identified (Goers et al., 1984; Martin et al., 1997). In 1996, a 482-amino acid C3a receptor was cloned from a LPS-activated human neutrophil cDNA library that had 37% nucleotide identity with the human C5a receptor throughout the coding region (Ames et al., 1996). The C3a receptor is a member of the G protein-coupled family of seven transmembrane-spanning receptors but has an uncharacteristically large extracellular loop of over 160 amino acids between transmembrane domains four and five, and features two *N*-linked glycosylation sites. Stable transfection of the rat basophilic leukemia cell line RBL-2H3, with expression plasmids encoding the C3a receptor, showed that agonist ligation with the C3a carboxyl-terminal analog WWGKKYRASKLGLAR resulted in robust Ca^{2+} mobilization under conditions where C5a was inactive.

The murine C3a receptor also has been isolated using a probe derived from the large extracellular loop found in the human homolog to screen a mouse brain cDNA library (Tornetta et al., 1997). The receptor is 65% identical with the 482 amino acids comprising the coding region of the human C3a receptor and features four extracellular *N*-linked glycosylation sites. Consistent with the results of Ames et al. (1996), stable transfection of RBL-2H3 cells with expression plasmids encoding the murine C3a receptor confers sensitivity to C3a but not to C5a (Tornetta et al., 1997).

C3a is a selective chemoattractant for eosinophils but not neutrophils (Daffern et al., 1995), activates the NADPH oxidase (Bach et al., 1990; Elsner et al., 1994), and promotes the release of EPO, EDN, and ECP from cytochalasin B-treated cells (Bach et al., 1990; Takafuji et al., 1994), which can be enhanced by IL-3 and IL-5 (Takafuji et al., 1996). The mechanism of eosinophil activation by C3a is poorly understood, although it is known to elevate the $[Ca^{2+}]_i$ and to promote the production of reactive oxygen intermediates by a PTX-sensitive mechanism (Elsner et al., 1994). Those preliminary data are consistent with findings in other cells expressing the C3a receptor. Thus, in U937 cells, C3a increases $[Ca^{2+}]_i$ by a PTX-sensitive mechanism and elevates Ins(1,4,5)P_3 mass, indicating that the C3a receptor couples to a PLC-β isoform via G_i or G_o (Klos et al., 1992). Identical results have been obtained with blood- and skin-derived macrophages and monocytes (Zwirner et al., 1997).

2. Complement 4a Anaphylatoxin. The second component of the classical complement pathway, C4, is split by C1 into the anaphylatoxin C4a and the larger C4b. Controversy surrounds the mechanism by which C4a anaphylatoxin elicits its functional effects. Indeed, evidence is available that C4a interacts with a structurally distinct C4a receptor (Murakami et al., 1993; Ames et al., 1997) and that it shares the same receptor as C3a (Hugli, 1984; Gerard and Gerard, 1994). To the authors' knowledge, nothing is known of the effect of C4a on eosinophil function. However, a C3a receptor-mediated effect of C4a seems unlikely given that it fails to promote Ca^{2+} mobilization in RBL-2H3 cells stably transfected with an expression plasmid encoding the murine C3a receptor (Tornetta et al., 1997).

3. Complement 5a Anaphylatoxin. Activation of the complement cascade can result in enzymatic cleavage of complement C5 and the release of a small polypeptide, C5a, from the remainder of the molecule, C5b, which remains loosely attached to the catalyst C5 convertase. One possible source of C5 degradation is provided by the eosinophil itself when exposed to immune complexes or SOZ (Ogawa et al., 1981a). Under those conditions, eosinophils can secrete a neutral protease that cleaves C5 to yield an eosinophil chemotactic activity that may well be C5a (Ogawa et al., 1981a). In 1977, Klebanoff et al. reported that eosinophils taken from the peritoneum of a child with eosinophilic gastroenteritis were activated by C5a, suggesting that receptors for this anaphylatoxin were expressed. Subsequent studies confirmed the expression of C5a-binding sites on human eosinophils using [^{125}I]C5a as a ligand and identified two apparently distinct populations of saturable sites (Gerard et al., 1989). One of these is present in relatively low abundance (B_{max} = 15,000–20,000 sites/cell) for which C5a has high affinity (K_d = 31 pM). The other constitutes the majority (>90%) of the total binding capacity (B_{max} = 375,000 sites/cell) although the affinity (K_d = 100 nM) of C5a is considerably (>300-fold) lower.

Autoradiography of eosinophils cross-linked to [^{125}I]C5a and run on SDS-polyacrylamide gels identified a dominant 60- to 65-kDa receptor complex (Gerard et al., 1989). However, using the same technique, the C5a receptors on human neutrophils are of a lower mass (50–52 kDa), suggesting possible C5a receptor heterogeneity (Gerard et al., 1989). The apparent difference between the eosinophil and neutrophil C5a receptor is supported by expression studies. A 2.3-kilobase (kb) cDNA, isolated from a library prepared from the mRNA of dibutyryl cAMP-differentiated HL60 cells, and expressed in COS cells encoded a 50- to 52-kDa C5a receptor consistent with the neutrophil variant (Boulay et al., 1991). It is noteworthy, that binding studies with that

cloned receptor revealed sites for which C5a had high (K_d = 1.7 nM) and low (K_d = 20–25 nM) affinity (Boulay et al., 1991) which might point to different conformations of the same protein.

The amino acid sequence of the C5a receptors cloned from U937 and HL-60 cells indicates that they adopt a seven transmembrane-spanning architecture and feature the necessary motifs for interaction with heterotrimeric GTP-binding proteins (Boulay et al., 1991; Gerard and Gerard, 1991). Concordant with those data is the finding that in human and guinea pig eosinophils, C5a evokes a rapid and transient, PTX-sensitive increase in $[Ca^{2+}]_i$ (Elsner et al., 1995) that is derived almost exclusively from intracellular storage organelles (Elsner et al., 1994, 1995; Takafuji et al., 1994; Wymann et al., 1995; Teixeira et al., 1997b). C5a also has been shown to activate PLD in human normodense eosinophils (Minnicozzi et al., 1990) as well as PKB and PtdIns 3-kinase (Coffer et al., 1998), but the functional consequences of these effects have not been investigated further.

Ligation of the C5a receptor on eosinophils evokes a number of functional responses, many of which are shared with other agonists that act through G protein-coupled receptors (Table 10). Perhaps the most effective activity of C5a is its ability to act as a chemoattractant, although it has been reported to generate lipid mediators, oxygen-derived free radicals, and certain cytokines, promote degranulation, chemotaxis, and adherence, and to modulate the expression of certain receptors and adhesion molecules (see Table 10). With respect to the aforementioned functional effects, Ca^{2+} ions are apparently required for the activation of the NADPH oxidase complex (Elsner et al., 1994, 1995;Wymann et al., 1995) and for promoting chemotaxis and actin polymerization (Elsner et al., 1996a).

In vivo, C5a is an effective eosinophil chemoattractant, although in some species its effects are partially indirect through the generation of secondary factors such as LTB_4 (Faccioli et al., 1991; Pettipher et al., 1994).

G. 5-Oxoeicosatetraenoic Acid (ETE), Hydroxyeicosatetraenoic Acids (HETEs), and Dihydroxyeicosatetraenoic Acids (diHETEs)

The lipids 5-oxo-ETE (Powell et al., 1995; Schwenk and Schroder, 1995; O'Flaherty et al., 1996a; Czech et al., 1997), 5-oxo-15-HETE (Schwenk et al., 1992; Powell et al., 1995; O'Flaherty et al., 1996a; Czech et al., 1997), 5-HETE (O'Flaherty et al., 1996b), and 8,15-diHETE (Morita et al., 1990a; Sehmi et al., 1991) are powerful eosinophil chemoattractants. In addition 5-oxo-ETE, the most potent of these novel lipid mediators (Powell et al., 1995; O'Flaherty et al., 1996a), induces degranulation of GM-CSF-treated eosinophils and enhances, by up to 10,000-fold, the ability of C5a, LTB_4, PAF, and fMLP to effect secretion of stored proteins (O'Flaherty et al., 1996a). Similarly, 5-oxo-ETE, at substimulatory concentrations, potentiates the chemotactic activity of PAF (Powell et al., 1995). Interestingly, 5-oxo-ETE, but not 5-HETE or 15-HETE, is approximately 100 times more potent as an eosinophil stimulant than its activity on neutrophils, suggesting that this compound may act selectively to induce eosinophil margination and activation (O'Flaherty et al., 1996a).

The cell surface receptor(s) on human eosinophils at which 5-oxo-ETE and 5-HETE act are not defined but their ligation results in rapid actin polymerization, intracellular Ca^{2+} mobilization, and the generation of oxygen radicals via a PTX-sensitive mechanism (Czech et al., 1997). Thus, the receptor for 5-oxo-ETE is likely to be G_i protein-coupled. 5-Oxo-ETE also enhances the ex-

TABLE 10
Functional effects evoked by C5a anaphylatoxin in eosinophils

Function	Species	Reference(s)
Induces chemotaxis	Human	Kay et al. (1973); Klebanoff et al. (1977); Ogawa et al. (1981a); Fischer and Czarnetzki (1982); Morita et al. (1989b); Rot et al. (1992); Elsner et al. (1996a,b)
Mobilizes and activates CR3	Human	Lundahl et al. (1993)
Activates VLA-4	Human	Weber et al. (1996)
Activates the NADPH oxidase	Human	De Simone et al. (1986b); Elsner et al. (1995); Wymann et al. (1995); Zeck Kapp et al. (1995); Elsner et al. (1996a,b)
Generates PAF	Human	Lee et al. (1984)
Generates LTC_4 in IL-3 and IL-5-primed cells	Human	Takafuji et al. (1991)
Releases β-glucuronidase	Human	Morita et al. (1989b)
Releases EPO	Human	De Simone et al. (1986b); Kernen et al. (1991); Takafuji et al. (1994); Zeck Kapp et al. (1995)
Releases ECP in cytochalasin-treated cells	Human	Zeck Kapp et al. (1995)
Releases MBP	Human	Koyanagi et al. (1995)
Releases IL-8 in cytochalasin-treated cells	Human	Takafuji et al. (1991)
Activates the hexose monophosphate shunt	Human	Klebanoff et al. (1977)
Promotes binding of estrogen	Human	Klebanoff et al. (1977)
Promotes degradation of thyroid hormone	Human	Klebanoff et al. (1977)
Releases MIF	Human	Rossi et al. (1998)
Generates TXB_2	Guinea pig	Giembycz et al. (1990)
Promotes CD18/lectin-dependent homotypic aggregation	Guinea pig	Teixeira et al. (1996c)
Promotes adherence to fibronectin (weak)	Horse	Foster et al. (1997)
Promotes adherence to bronchial epithelial cells	Human	Burke-Gaffney and Hellewell (1998)

pression of CD11b and the shedding of L-selectin by a mechanism that is insensitive to PD 098059, wortmannin, and staurosporine (Powell et al., 1999). Some 5-oxo-ETE-elicited responses might be attributable to its ability to promote the phosphorylation of ERK-1 and ERK-2 (O'Flaherty et al., 1996b).

In vivo, 5-oxo ETE, given by the intratracheal route to Brown Norway rats, produces a drammatic (5- to 8-fold) increase in the number of eosinophils around the airway wall that is not blocked by LTB_4 or PAF antagonists but is attenuated (~ 75%) by monoclonal antibodies directed against the adhesion molecules very late antigen (VLA) 4 and CD11a (Stamatiou et al., 1998). The magnitide of this effect is significantly greater than that effected by LTB_4.

H. Sensory Neuropeptides

Sensory neuropeptides represent a host of biologically active mediators, many of which have a variety of effects on eosinophil function. The most studied of these peptides include SP, NKA, NKB, calcitonin gene-related peptide (CGRP), gastrin-releasing peptide, peptide histidine isoleucine, secretin, helodermin, secretoneurin, cholecystokinin octapeptide, and vasoactive intestinal peptide (Goetzl and Sreedharan, 1992), and some of these are discussed below.

1. Substance P. Substance P (SP), NKA, and NKB comprise the tachykinins and exert many (if not all) of their effects by acting through at least three structurally distinct, seven transmembrane-spanning receptors denoted neurokinin (NK) $_1$, NK_2, and NK_3. In humans, the NK_1, NK_2, and NK_3 receptors are composed of 407, 398, and 468 amino acids, respectively, represent distinct gene products and couple primarily through the $G_{q/11}$ family of GTP-binding proteins. Heterogeneity of tachykinin receptors also is seen in cells and tissues from mice and rats. See Regoli et al. (1994) for additional details.

SP is a undecapeptide which is localized to sensory nerves that innervate various organs, in particular the gut and respiratory tract. In addition, eosinophils have the capacity to synthesize, store, and release large quantities of peptides, including SP and CGRP (Aliakbari et al., 1987; Weinstock et al., 1988; Weinstock and Blum, 1989,1990b; Weinstock, 1991; Metwali et al., 1994), that may act in an autocrine fashion. High concentrations (>1 μM) of SP effectively degranulate eosinophils (measured as secreted EPO and ECP) but, unlike melittin (see *XI.N*), do not promote the biosynthesis of TX, indicating a selective effect on the exocytotic response (Kroegel et al., 1990b; Iwamoto et al., 1993a). SP-induced EPO release is thought to be mediated by the amino terminus of the molecule as evinced from the ability of the truncated analog SP_{1-4}, but not SP_{4-11}, to promote secretion (Kroegel et al., 1990b). Paradoxically, the release of ECP by SP is mediated by the carboxyl terminus of the peptide since SP_{1-9} failed to promote degranulation, unlike the truncated peptides SP_{4-11} and SP_{6-11}, although the degree of ECP release (less than 10% of total stored) was modest (Iwamoto et al., 1993a). The significance of these discrepant findings is unclear. The ability of SP to effect eosinophil degranulation is not mimicked by the related peptide NKA, which could indicate a non-NK receptor-mediated process. Indeed, it has been proposed that the effects of SP and other amphiphilic peptides are due to physicochemical properties of these molecules, possibly involving the direct interaction and activation of G proteins (Mousli et al., 1990). This is a plausible explanation and particularly relevant to SP-induced EPO release given that a novel G protein, G_E, is believed to regulate the terminal stages of exocytosis (Gomperts, 1990; Nusse et al., 1990; Cromwell et al., 1991; Gomperts and Cromwell, 1991).

SP also promotes the migration of human eosinophils at extremely low concentrations (EC_{50} = 1–10 pM) (Wiedermann et al., 1993; Dunzendorfer et al., 1998a), possibly through a PtdIns 3-kinase-dependent mechanism, and potentiates the chemotactic activity of IL-5, LTB_4, and PAF (Numao and Agrawal, 1992; Elshazly et al., 1996a; Dunzendorfer et al., 1998a,b). Although there is some discrepancy over whether priming occurs in eosinophils purified from the blood of nonallergic subjects (see Numao and Agrawal, 1992; Elshazly et al., 1996a), this action is, again, directed by the carboxyl terminus of the peptide (Numao and Agrawal, 1992; Wiedermann et al., 1993). However, in contrast to its ability to promote directional migration, SP-induced priming is probably mediated through NK_1 receptors since it is antagonized by FK888 (Elshazly et al., 1996a). Similar data have been obtained with NKA and cholecystokinin octapeptide (Numao and Agrawal, 1992).

Submicromolar concentrations of SP have been reported to up-regulate the expression of receptors for Fcϵ and Fcγ on human eosinophils and to augment antibody-dependent eosinophil-mediated cytotoxicity toward erythrocytes (De Simone et al., 1987). With respect to the NADPH oxidase, human eosinophils in suspension are insensitive to SP (Dri et al., 1991). However, when they adhere to polystyrene-based enzyme-linked immunosorbent assay plastic, SP can evoke a respiratory burst provided very high concentrations (in the high micromolar range) are used (Dri et al., 1991). Whether NK receptors are involved in either of these functional effects is unclear.

In vivo, SP generally promotes tissue eosinophilia. This has been observed in both guinea pigs, where intradermal administration elicits cutaneous eosinophil accumulation by a NK_1-independent mechanism that relies on the generation of 5-lipoxygenase products (D. T. Walsh et al., 1995), and in BALB/c mice which is dependent upon the secondary formation of LTB_4 (Iwamoto et al., 1993b). Similar data have been reported in human studies. Fajac et al. (1995) found that SP, nebulized into each nostril of seven patients with seasonal allergic rhinitis 24 h after nasal provocation,

markedly (>10-fold) enhanced the already numerous number of eosinophils present in the nasal lavage fluid. That effect was associated with increased nasal obstruction and leakage of plasma proteins from the vasculature. Since SP is released after nasal allergen challenge, it is possible that this peptide plays an important role in chronic eosinophilic inflammation of the nasal mucosa in symptomatic allergic rhinitis. However, the ability of SP to activate eosinophils in the lung, gut, or nose may have little physiological or pathophysiological significance given that the concentration released from sensory nerves and proinflammatory cells would have to be in the micromolar range for most functional effects to be manifest.

2. CGRP. Four distinct receptors for the CGRP family of proteins (which include amylin, calcitonin, and adrenomedullin) have been partially classified based on rank orders of agonist potencies and molecular cloning. Each receptor is G protein-coupled, probably through G_s, to adenylyl cyclase, although this is probably not the only effector.

Controversy exists regarding the effect of CGRP on eosinophil function. Numao and Agrawal (1992) reported that CGRP primed human eosinophils to chemotactic agents but had no direct effect itself. However, most other investigators have provided results to the contrary. Thus, in vitro CGRP has been shown to be an extremely potent chemotactic agent for human eosinophils with an EC_{50} of approximately 1 pM (Dunzendorfer et al. 1998a). Similarly, rat CGRP is chemotactic for guinea pig eosinophils (Manley and Haynes 1989). Intriguingly, the amino acid sequence VGSE, which represents ratCGRP$^{32–35}$, is identical with ECF-A reported by Goetzl and Austen (1975) and is more effective than CGRP in the chemotaxis assay (Manley and Haynes, 1989). Since CGRP is a substrate for endopeptidase 24.11, it could be converted into ECF-A in vivo. Thus, a novel function of endopeptidase 24.11 may be to enhance rather than terminate the biological activity of CGRP (Davies et al., 1992).

The chemotactic activity of CGRP is somewhat surprising given that receptors for GCRP and related family members are believed to couple primarily to adenylyl cyclase via G_s. However, the possibility exists that CGRP could promote chemotaxis by activating an alternative G_s-regulated protein such as an ion channel. Alternatively, coupling of CGRP receptors through other G proteins could be inferred from the report that human eosinophils chemotaxis is abolished by wortmannin at a concentration that selectively inhibits PtdIns 3-kinase (Dunzendorfer et al., 1998a). These possibilities are supported by the general observation that cAMP-elevating agents suppress eosinophil chemotaxis (see *XIV.A.5*, *XIV.C,* and *XIV.D* for details).

Little is known of the effects of CGRP on leukocyte accumulation in vivo. Bellibas (1996) reported that rats given nebulized CGRP developed a pulmonary eosinophilia. Similarly, injection of CGRP into human skin causes a long-lasting flare associated with eosinophil infiltration (Piotrowski and Foreman, 1986). Whether CGRP acts directly or indirectly has not been explored.

3. Secretoneurin. Dunzendorfer et al. (1998a,b) have reported that secretoneurin, a novel 33-amino acid peptide derived from secretogranin II (Kirchmair et al., 1993) that is released from sensory afferent C-fibers by capsaicin (Kirchmair et al., 1994), is an effective chemoattractant for human eosinophils with a potency 10 to 50 times less than SP, RANTES, and IL-8. Preliminary studies designed to evaluate the signaling pathway(s) utilized by secretoneurin established that chemotaxis was abolished by the PtdIns 3-kinase inhibitor wortmannin, but not by tryphostin-23, whereas the same response evoked by SP was inhibited by both pharmacological agents (Dunzendorfer et al., 1998a,b). Thus, it would appear that secretoneurin-induced human eosinophil chemotaxis is mediated, in part, by mechanisms distinct from those recruited by SP. Studies with the phosphodiesterase (PDE) inhibitor 3-isobutyl-1-methylxanthine (IBMX), which significantly attenuated secretoneurin-, but not SP-, induced chemotaxis support this idea (Dunzendorfer et al., 1998a,b).

4. Vasoactive Intestinal Peptide. In humans and rats, three receptors (PAC, $VPAC_1$, and $VPAC_2$) unequivocally have been defined at which vasoactive intestinal peptide (VIP) is an agonist. Molecular genetics has established that each receptor is encoded by a distinct gene that activates effector elements by coupling exclusively through G_s. See Harmar et al. (1998) for additional details.

Eosinophils have the capacity to synthesize, store, and release large quantities of a variety of peptides including VIP (Aliakbari et al., 1987; Weinstock and Blum, 1990a; Weinstock, 1991; Metwali et al., 1994) that may act in an autocrine fashion to modulate cell function. A preliminary report has described the specific binding of ^{125}I-labeled VIP to intact eosinophils harvested from the peritoneal cavity of guinea pigs (Sakakibara et al., 1990). This effect is rapid, time-dependent, and saturable and can be inhibited by unlabeled VIP and the related peptide helodermin (Sakakibara et al., 1990). Scatchard analyses of ^{125}I-labeled VIP-binding isotherms indicates a single class of low-affinity (K_d = 140 nM), high-capacity (744,000) sites/cell (Sakakibara et al., 1990). In the presence of the nonselective PDE inhibitor IBMX, neither VIP nor helodermin increased measurably the cAMP content of guinea pig eosinophils (Sakakibara et al., 1990), suggesting that if the VIP-binding sites represent bona fide receptors they are uncoupled from, or do not couple positively to, adenylyl cyclase. Moreover, exogenous VIP does not inhibit the production of superoxide anions from phorbol ester-stimulated eosinophils (Sakakibara et al., 1990). However, that negative result is not entirely unexpected because cyclic nucleotide-elevating drugs generally do not inhibit func-

tional responses in eosinophils effected by phorbol diesters or calcium ionophores (Dent, 1991). The knowledge that cAMP suppresses receptor-mediated respiratory burst activity in eosinophils implies that the substrate(s) phosphorylated by PKA is upstream of PKC. At the present time, therefore, the nature of the specific binding sites labeled by ^{125}I-labeled VIP in guinea pig eosinophils is unclear.

In contrast, VIP is a potent chemokinetic agent for human isolated eosinophils with activity in the femtomolar range. This effect appears to be receptor mediated for it is abolished by [L^{17}-G^{29},K^{30}]VIP, a VIP antagonist. Moreover, secretin mimicks the effect of VIP, whereas helodermin is relatively inactive, suggesting that $VPAC_1$ receptors mediate chemokinesis (Dunzendorfer et al., 1998a). Interestingly, the nonselective PDE inhibitor IBMX, *prevented* VIP- and secretin-induced chemokinesis (consistent with the inhibitory effect of cAMP on eosinophil locomotion) which tempts speculation that the receptor through which VIP acts does not couple through G_s (Dunzendorfer et al., 1998a). In this respect, a low concentration (10 nM) of wortmannin abolished VIP- and secretin-induced chemokinesis, implicating PtdIns 3-kinase in eosinophil locomotion (Dunzendorfer et al., 1998a).

I. Bradykinin

Two subtypes (B_1 and B_2) of the bradykinin receptor have been defined by pharmacological and molecular techniques and additional evidence for a B_3 receptor has been provided from antagonist studies (Farmer, 1995). In humans, the B_1 and B_2 receptors are composed of 353 and 364 amino acids, respectively, represent distinct gene products and couple primarily through the $G_{q/11}$ family of GTP-binding proteins. See Hall (1997) for additional details.

Bradykinin has no known direct effect on eosinophil function although, in vivo, it promotes localized eosinophilia in several species, including the guinea pig (Fechter et al., 1986; Farmer et al., 1992) and rat (Pasquale et al., 1991; Bowden et al., 1994; Pires et al., 1994; Ferreira et al., 1996). Bradykinin B_2 receptors are implicated in the cavine model since eosinophil accumulation is suppressed by the B_2-selective antagonists NPC 567 and NPC 16731. In rats, bradykinin acts, in large part, by effecting the generation of lipoxygenase products (Pasquale et al., 1991).

J. Endothelin

Two receptors (ET_A and ET_B) for the endothelins have been classified in a number of species that couple to intracellular effectors through the G_q/G_{11} family of GTP-binding proteins. In humans, the ET_A and ET_B receptors are composed of 427 and 442 amino acids, respectively, and are distinct gene products. Pharmacological evidence is available for ET_B receptor heterogeneity but this is yet to be confirmed at the molecular level. See Masaki et al. (1994) for additional details.

Fujitani et al. (1997) have reported that the appearance of eosinophils in the BAL fluid of allergen-challenged, sensitized BALB/c mice was significantly suppressed by BQ 123 (ET_A antagonist), SB 209670 (ET_A/ET_B antagonists), and a neutralizing anti-endothelin antibody. The additional finding that the ET_B antagonist BQ-788 was inactive suggests that endogenously released endothelin promotes pulmonary eosinophilia through an action at ET_A receptors. It is not known whether eosinophils express functional endothelin receptors but the mechanism of action of BQ 123 and SB 209670 in the murine model probably resides in their ability to release interferon γ (IFNγ) from Th_1 T lymphocytes (Fujitani et al., 1997). Indeed, the i.v. administration of endothelin to guinea pigs does not cause pulmonary leukocyte accumulation (Macquin-Mavier et al., 1989).

K. Adenosine

Currently, four receptors for adenosine have been unequivocally defined in human tissues and are denoted A_1, A_{2A}, A_{2B}, and A_3. Each, so-called, purinoceptor is a member of the seven transmembrane-spanning family of receptors and couples to multiple effectors through G_i, G_o, and G_s. Thus, adenosine can act as an excitatory and inhibitory ligand depending upon the receptor subtype expressed by the cell or tissue of interest. In humans, the A_1, A_{2A}, A_{2B}, and A_3 purinoceptor are composed of 326, 412, 332, and 318 amino acids, respectively, and represent distinct gene products. Adenosine receptor multiplicity also is found in cells and tissues from mice and rats. See Fredholm et al. (1994) for detailed description of classification.

Human and guinea pig eosinophils generate and release adenosine spontaneously in biologically active quantities. This phenomenon is seen in cells pretreated with adenosine deaminase and the adenosine receptor antagonist 8-phenyltheophylline (which does not inhibit PDE). Both of these pharmacological interventions augment the generation of superoxide anions in response to SOZ, indicating that adenosine acts in an autocrine manner to suppress, tonically, the activity of the NADPH oxidase (Yukawa et al., 1989a). Pharmacological experiments designed to determine the adenosine receptor coupled to the inhibition of the NADPH oxidase implicate the A_2 subtype since 5′-*N*-ethyl-carboximide adenosine has a greater inhibitory effect than *R*-*N*-phenyl-isopropyl adenosine (Yukawa et al., 1989a). Furthermore, adenosine has been shown to increase the $[Ca^{2+}]_i$ in fura-2/AM-loaded guinea pig eosinophils and to significantly enhance PAF-induced superoxide anion generation and Ca^{2+} mobilization (Walker, 1996). The receptor that mediates these latter effects is not defined but is unlikely to be either of the A_2 purinoceptor subtypes since they couple predominantly through G_s.

In situ hybridization and polymerase chain reaction (PCR) studies have localized transcripts of the adenosine A_3 receptor to human eosinophils from normal and atopic donors (Kohno et al., 1996; Walker et al., 1997). More detailed experiments have established that eosinophil membranes express a homogeneous population of noninteracting, high-affinity (K_d = 3.2 nM) binding sites for ^{25}I-labeled N^6-(4-aminobenzyl)-adenosine-5′-*N*-methyluronamide, an adenosine A_3 receptor agonist, with a B_{max} of 1.3 pmol/mg protein (Kohno et al., 1996). Intriguingly, the density of adenosine A_3 receptor transcripts is higher in lung tissue taken from subjects with airway inflammation than from normal donors (Walker et al., 1997), although whether this is associated with an increase (or decrease) in functional receptors is currently unknown. In addition to suppressing the activation of the NADPH oxidase via A_2 receptors (see above), adenosine exerts effects in eosinophils through agonism of the adenosine A_3 receptor that are considered to be both proinflammatory and anti-inflammatory. Thus, PAF-, RANTES-, and LTB_4-induced chemotaxis of human eosinophils is prevented by 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propyxanthine, a selective antagonist at A_3 receptors (Knight et al., 1997; Walker et al., 1997). In contrast, the highly potent and selective A_3 agonist CI-IB-MECA mobilizes Ca^{2+} from both intracellular stores and from the extracellular compartment, suggesting that the A_3 purinoceptor can couple to a PLC (Kohno et al., 1996).

L. Histamine

Histamine (2-(4-imidazole)ethylamine) can act at three distinct receptors denoted H_1, H_2, and H_3. Classical pharmacology, allied with molecular techniques, has identified the H_1 and H_2 receptor in humans, mice, and rats and has established that they belong to the seven transmembrane-spanning family of receptors. The human H_1 and H_2 receptors are composed of 487 and 359 amino acids, respectively, and are the products of different genes. Although the H_1 receptor couples primarily through a PTX-insensitive G protein, probably of the $G_{q/11}$ class, the H_2 receptor is linked to effector enzymes via G_s. Thus, like adenosine, histamine can activate or inhibit depending upon the receptor subtype expressed by the cell or tissue of interest. The H_3 receptor has not yet been cloned but has pharmacology distinct from the other histamine receptors. The effector molecules involved in H_3 receptor signaling are unknown, although radioligand-binding experiments imply a possible link to a G protein. See Hill et al. (1997) for detailed review.

Pharmacological evidence points to the expression of H_1, H_2, and H_3 histamine receptors on human eosinophils and much of this is derived from experiments assessing locomotion in vitro. Although many studies have examined the chemotactic potential of histamine in several species (Clark et al., 1975, 1977; Bryant et al., 1977; Jones and Kay, 1977; Wadee et al., 1980; McEwen et al., 1990; Foster and Cunningham, 1998), much of those data are contradictory with respect to the receptor subtype(s) involved. Thus, in the late 1970s, the chemotactic activity of histamine on guinea pig eosinophils was attributable to an interaction at receptors of the H_2 subtype (Jones and Kay, 1977). In contrast, similar experiments performed at the same time with human cells failed to corroborate that finding and, instead, proposed the existence of a novel receptor based on the finding that histamine-induced chemotaxis was not blocked by H_1 or H_2 antagonists (Clark et al., 1975, 1977). Further discrepancy is provided by the results of additional studies where histamine was shown to augment human eosinophil chemokinesis (random migration), effected by endotoxin-activated serum, through a pyrilamine (H_1)-sensitive receptor (Clark et al., 1977; Wadee et al., 1980), whereas, in the same experimental setup, directional motility (chemokinesis) was mediated through H_2 receptors (Clark et al., 1977; Wadee et al., 1980). In equine eosinophils, histamine promotes migration and adherence to serum- and fibronectin-coated plastic solely through the histamine H_1 subtype (Foster and Cunningham, 1998). Thus, although species differences should not be discounted, the histamine receptor subtype(s) that promotes eosinophil locomotion still is equivoval.

Histamine H_3 receptors were identified on human eosinophils by Raible et al. in the early 1990s. Using Ca^{2+} mobilization as an index of activation, the affinity of the selective H_3 antagonists burimamide, thioperamide, and impromidine were similar to those calculated for the H_3 receptors in the central nervous system (Raible et al., 1992, 1994). However, *R*-α-methylhistamine and *N*-α methylhistamine (H_3-selective agonists) were less active than histamine itself which led Raible et al. (1994) to suggest that the eosinophil H_3 receptor is different from those expressed in other tissues. However, low receptor expression or poor receptor-effector-coupling efficiency equally could explain this apparently anomalous result.

With the possible exception of motility, the functional effects of histamine in eosinophils are surprisingly little studied. Reports that histamine evokes superoxide anion generation from guinea pig and human eosinophils (Pincus et al., 1982) and enhances C3b rosette formation (Anwar and Kay, 1980) by a H_1 receptor-mediated mechanism have been suggested but not corroborated.

One of the first investigations to address the in vivo effects of histamine was published by Vegad and Lancaster (1972) who reported that local application produced cutaneous eosinophilia in sheep. That finding has since been confirmed in guinea pigs (Woodward et al., 1985) and in humans, where the chemoattraction was greater in atopic subjects when compared to normal individuals (Bryant and Kay, 1977). A role for histamine in eosinophil recruitment is not restricted to the skin. Histamine promotes the emigration of eosinophils to the conjunctiva of guinea pigs (Woodward et al., 1986;

Spada et al., 1986) and also is implicated in allergen-induced pulmonary eosinophilia in sensitized dogs (Johnson et al., 1992). In a guinea pig model of cutaneous and conjunctival eosinophilia, pyrilamine and cimetidine administered concurrently is necessary to significantly blunt eosinophil infiltration, indicating that histamine H_1 and H_2 receptors are involved (Woodward et al., 1985, 1986). However, eosinophil trafficking was not abolished by that treatment, tempting speculation that H_3 receptors also play a role (Woodward et al., 1986). Paradoxically, local application of histamine to unroofed heat-suction blisters of ragweed-sensitive subjects inhibited allergen-induced cutaneous eosinophilia (Ting et al., 1981). An important role for inhibitory H_2 receptors is, therefore, proposed.

M. Prostanoids

Elegant studies performed since the mid-1970s have provided pharmacological evidence for five main classes of receptor for the naturally occurring prostanoid agonists (reviewed in Coleman et al., 1994). These receptors have been given the prefix DP-, EP-, FP-, IP-, and TP- and belong to the G protein-coupled receptor superfamily. Because of the lack of selective antagonists, this taxonomy was formulated predominantly from rank orders of agonist potencies obtained in various pharmacological preparations where each prostanoid is at least one order of magnitude more potent than the others at a specific prostanoid receptor. Molecular biological techniques have recently confirmed this pharmacological classification with the cloning and expression of cDNAs for representatives of the five prostanoid receptors in a number of species including humans (Hirata et al., 1991; Abramovitz et al., 1994; Boie et al., 1994,1995; Kunapuli et al., 1994; Regan et al., 1994a,b; Yang et al., 1994).

In vitro studies suggest that eosinophils might express excitatory DP receptors based on the finding that prostaglandin (PG) D_2 (but not $PGF_{2\alpha}$ or TX mimetics) enhances zymosan-activated serum-induced eosinophil migration (Butchers and Vardey, 1990). This possibility is supported by an earlier description of the chemokinetic activity of PGD_2 (Goetzl et al., 1979) and its ability to promote Ca^{2+} mobilization in fura-2-loaded human eosinophils (Raible et al., 1992). In vivo, PGD_2 promotes eosinopenia and the accumulation of eosinophils in the airways of dogs (Marsden et al., 1984; Emery et al., 1989) in a manner that is attenuated by the nonselective prostanoid receptor antagonist SK&F 88046. Thus, it seems likely that the chemokinetic action of PGD_2 results from a direct action on the eosinophil (Emery et al., 1989). Furthermore, PGD_2 (acting through TP receptors on the airways smooth muscle) evokes potent bronchoconstriction in humans (Beasley et al., 1989; Johnston et al., 1992). This effect raises important clinical considerations given that PGD_2 is present in the BAL fluid of mild asthmatic subjects and is released into the lungs following acute allergen provocation (Murray et al., 1986; Liu et al., 1990).

Evidence derived from pharmacological studies suggests that eosinophils express a population of prostanoid receptors that, when activated, suppress several indices of activation. Butchers and Vardey (1990) reported that fMLP-induced ECP release from a mixed population of human granulocytes was suppressed by PGD_2, PGE_2, and $PGF_{2\alpha}$ with a rank order of potency in good agreement with that found with other cells and tissues that express DP receptors such as human platelets (Keery and Lumley, 1988). Similarly, the synthetic PGD_2 agonist BW 245C was more potent than the natural ligand at blocking degranulation (Butchers and Vardey, 1990). In complete agreement with those data, Sturton and Norman (1991) noted that PGD_2 was the most effective natural prostaglandin at preventing fMLP-induced respiratory burst (assessed as luminol-enhanced chemiluminescence) in human eosinophils. Thus, it appears that DP receptors can mediate both excitatory and inhibitory effects in human eosinophils that might reflect DP receptor heterogeneity (see Fernandes and Crankshaw, 1995).

PGE_2 inhibits, albeit modestly (20–30%), PAF-induced CD11b expression and the shedding of L-selectin on human eosinophils (Berends et al., 1997), implying that their interaction with the appropriate counter ligands on vascular endothelial cells would be reduced. A similar result was documented for PGE_1 which attenuated the up-regulation by PAF and C5a of the β_2 integrin CD18 in guinea pig eosinophils (Teixeira et al., 1996a). This action would temper the directional migration of eosinophils in response to chemoattractants and might attenuate eosinophil-driven inflammatory responses. The identity of the prostanoid receptor subtype at which E-series prostaglandins suppress adhesion molecule expression has not been determined, although it is curious that the PDE4 inhibitor rolipram is inactive, which tempts speculation that EP receptors coupled positively to adenylyl cyclase are not involved. Nevertheless, eosinophils may express inhibitory prostanoid receptors of the EP_2 subtype (Butchers and Vardey, 1990; Teixeira et al., 1997a). In human cells, this is suggested by the finding that PGE_2 increases the cAMP content (indicative of agonism at EP_2 or EP_4 receptors (Coleman et al., 1994)), and that misoprostol (EP_2-/EP_3-selective agonist), but not sulprostone (EP_1-/EP_3-selective agonist), inhibits fMLP-induced ECP release. In guinea pig eosinophils, pharmacological evidence based on the rank order of agonist potency (PGE_2 > PGE_1 > 11-deoxy-PGE_1 > misoprostol > butaprost > AH 13205) also implicates EP_2 receptors in the inhibition of PAF-induced homotypic aggregation (Teixeira et al., 1997a). In those studies, the selective EP_2 agonists butaprost and AH 13205 were uniformly weak, which might question the classification of the inhibitory eosinophil EP receptor as an EP_2 subtype. However, comparable results have been

described in rat neutrophils (Wise and Jones, 1994) and human monocytes (Meja et al., 1997) that express EP_2-like receptors. Thus, given the high selectivity of butaprost for EP_2 receptors, an alternative possibility is that guinea pig eosinophils express a modest number of EP_2-binding sites at which butaprost and AH 13205 have low efficacy. Regardless of their precise identity, the inhibitory EP receptors are apparently coupled positively to adenylyl cyclase since inhibition of PKA reduced the ability of PGE_1, 11-deoxy-PGE_1, and AH 13205 to suppress PAF-induced aggregation (Teixeira et al., 1996a, 1997a).

In vivo, E-series prostaglandins inhibit cutaneous eosinophilia in guinea pigs in response to PAF and compound 48/80 and after passive cutaneous anaphylaxis under conditions where local edema formation is enhanced (Teixeira et al., 1993). Prostaglandins exert several direct effects on eosinophils that could contribute to their ability to reduce eosinophil number to sites of an inflammatory insult (see above). However, the accumulation of neutrophils in the skin of guinea pigs is *enhanced* by PGE_1 and PGE_2, whereas in vitro neutrophil activation is generally attenuated (Teixeira et al., 1996a, 1997a; Berends et al., 1997). Thus, E-series prostaglandins might affect eosinophil emigration indirectly. However, studies with the long-acting β_2 adrenoceptor agonist salmeterol (Teixeira and Hellewell, 1997a) has provided persuasive evidence that agents that elevate cAMP can prevent eosinophil locomotion; thus, the mechanism of action of E-series prostaglandins in vivo remains to be elucidated.

Neither functional nor radioligand-binding experiments have provided any evidence for IP, FP, or TP receptors on human or guinea pig eosinophils (Butchers and Vardey, 1990; Giembycz et al., 1990; Sturton and Norman, 1991). As described in *XII. C.2*, the major cyclooxygenase products generated by PAF- and LTB_4–stimulated eosinophils are TX and PGE_2 (Giembycz et al., 1990; Perkins et al., 1995). However, exposure of guinea pig eosinophils to the cyclooxygenase inhibitor flurbiprofen, at a concentration that abolished PGE_2 generation, did not affect LTB_4- or PAF-induced functional responses (Giembycz et al., 1990; Rabe et al., 1992), indicating that this prostanoid is not generated in an amount sufficient to act in an autocrine manner.

N. α Adrenoceptors

Although formal identification (by radioligand binding or pharmacological means) of cell surface α adrenoceptors is lacking, Masuyama and Ishikawa (1985) suggested that they might be expressed on guinea pig eosinophils based on the finding that noradrenaline (α-selective) inhibited eosinophil phagocytosis and free radical production under conditions where isoprenaline (β-selective) was less active. However, in the absence of data obtained with selective agonists and antagonists, the expression of α_1 or α_2 adrenoceptors (or subtypes thereof) on eosinophils is equivocal.

O. β Adrenoceptors

In the context of asthma, β_2 adrenoceptor agonist are, without exception, the most effective bronchodilators available clinically and can reverse tone by acting on airways smooth muscle directly, irrespective of the causative spasmogen. A far more contentious issue is whether they exert an anti-inflammatory influence in vivo. In the following sections the in vitro and in vivo actions of short- and long-acting β adrenoceptor agonists on eosinophil function are reviewed and their role in the treatment of inflammation discussed.

1. Receptors. Three distinct β adrenoceptor subtypes (β_1, β_2, and β_3) have been unequivocally classified. Each subtype is a member of the seven transmembrane-spanning family of receptors and is the product of a different gene. In humans, the β_1, β_2, and β_3 adrenoceptor are composed of 477, 413, and 408 amino acids, respectively, and interact predominantly, but not exclusively, with G_s-linked effectors (see Bylund et al., 1994 for details). Pharmacological evidence also is available for β_4 adrenoceptors (Molenaar et al., 1997).

Radioligand-binding studies using the β adrenoceptor antagonist ^{125}I-labeled pindolol have identified a homogenous population of very high-affinity ($K_d \sim 25$ pM) binding sites on intact eosinophils harvested from human blood ($B_{max} = 4333$ sites/cell) and from the peritoneal cavity of guinea pigs ($B_{max} = 7166$ sites/cell) that have the characteristics of the β_2 adrenoceptor subtype (Yukawa et al., 1990). mRNA for the β_2 but not β_1 adrenoceptor subtype also has been identified in the same cells (Peters et al., 1993). Those results are concordant with the ability of isoprenaline and salbutamol to elevate the cAMP content and to activate PKA (Kita et al., 1991b; Souness et al., 1991; Dent et al., 1994; Munoz et al., 1995), and confirm that the β adrenoceptors on eosinophils can couple positively to adenylyl cyclase. Compared to isoprenaline, the selective β_2 adrenoceptor agonist salbutamol is less potent and is a partial agonist ($\alpha = 0.8$) at increasing cAMP in eosinophils (Yukawa et al., 1990), whereas the long-acting β_2 agonist salmeterol is inactive (Rabe et al., 1993; Munoz et al., 1995). The finding that the affinity of atenolol and ICI 118,551, antagonists of β_1 and β_2 adrenoceptors, respectively, for inhibiting isoprenaline-induced cAMP accumulation in eosinophils is essentially the same as their K_i values calculated from binding studies (Yukawa et al., 1990) indicates that the sites labeled by ^{125}I-labeled pindolol and the receptors subserving cAMP accumulation are identical (i.e., β_2 adrenoceptors).

In vitro, β_2 adrenoceptor agonists suppress several indices of eosinophil activation (detailed below) provided the preincubation time is not too long (Yukawa et al., 1990). However, prolonged exposure to β adrenoceptor agonists promotes rapidly a state of tolerance and, in

one study, salbutamol, salmeterol, and isoprenaline were reported to enhance eosinophil activation (Nielson and Hadjokas, 1998). Desensitization, noted also in other leukocytes (e.g., Tecoma et al., 1986), is probably due to uncoupling of β_2 adrenoceptors from adenylyl cyclase and/or enhanced metabolism of the cAMP formed following activation of the β adrenoceptor since receptor down-regulation normally is not observed. These biochemical changes are attributable to several mechanisms that are not mutually exclusive including the activation of PKA (Bouvier et al., 1989; Lohse, 1993; Giembycz, 1996), induction of PDE4 (Torphy et al., 1995; Giembycz, 1996; Seybold et al., 1998), and down-regulation of the activity and amount of the 45- and 52-kDa splice variants of Gsα (Finney et al., 1998). Desensitization through the activation of one or more members of the G protein receptor-coupled kinase (GRK) family (Bouvier et al., 1989; Lohse et al., 1990; Chuang et al., 1992; McGraw and Liggett, 1997), particularly GRK2 (formerly β adrenoceptor kinase 1), also is likely. Indeed, high levels of GRK-2 have been identified in the cytosol of human eosinophils (Onorato et al., 1995). The β_2 adrenoceptor also is a substrate for GRKs 1, 3, 5, and 6 (Chuang et al., 1996) which could further compromise signaling.

2. Activation of the NADPH Oxidase. In human and guinea pig eosinophils, β_2 adrenoceptor agonists effectively suppress the activation of the NADPH oxidase (Rabe et al., 1993; Dent et al., 1994; Hadjokas et al., 1995; Ezeamuzie and Al-Hage, 1998). In the latter species this effect may not be mediated by receptors of the β_1 or β_2 subtype since the affinities of propranolol ($pA_2 = 7.2$), atenolol ($pA_2 > 5$), and ICI 118,551 ($pA_2 \sim 7.1$) in antagonizing LTB_4-induced H_2O_2 generation (a reliable measure of the respiratory burst) are considerably less than would be predicted from an interaction with classical β_1 or β_2 adrenoceptors (Rabe et al., 1993). Moreover, the long-acting β_2 adrenoceptor agonist salmeterol is inactive at suppressing oxidant production in response to LTB_4 and actually behaves as an antagonist at this "atypical" receptor subtype with reasonable affinity ($pA_2 = 5.9$) (Rabe et al., 1993). This finding also provides additional evidence for atypical β adrenoceptors on eosinophils. Indeed, logic dictates that if β_2 adrenoceptors were involved, salmeterol should inhibit H_2O_2 generation since it has essentially the same efficacy as salbutamol (Dougall et al., 1991).

The concentration-response curve that describes β adrenoceptor-mediated cAMP accumulation in eosinophils lies one to two orders of magnitude to the left of that which describes the inhibition of H_2O_2 generation (EC_{50} values = 50 nM and 10 μM, respectively) (Yukawa et al., 1990; Rabe et al., 1993). One interpretation of those findings is that the atypical β receptors on guinea pig eosinophils suppress oxidative metabolism by coupling to signal transduction elements distinct from the adenylyl cyclase/cAMP/PKA cascade. The failure of the PDE inhibitors rolipram and zardaverine to potentiate the inhibitory action of salbutamol on SOZ-induced superoxide anion production from human eosinophils is consistent with this proposal (Dent et al., 1994).

In human eosinophils, the acute effects of β_2 adrenoceptor agonists on NADPH oxidase activity differ from what is seen with guinea pig cells (Ezeamuzie and Al-Hage, 1998). Thus, salmeterol, but not salbutamol, effectively inhibits IL-5-induced superoxide anion generation from human eosinophils with an EC_{50} in the low micromolar range. It was suggested that the selective inhibitory effect is related to the nature of the activating stimulus since both salbutamol and salmeterol suppressed oxidant production when the NADPH oxidase was activated by PAF (Ezeamuzie and Al-Hage, 1998). Significantly, ICI 118,551 failed to antagonize the inhibitory effect of salmeterol, which points to a mechanism of action independent of β_2 adrenoceptor activation (Ezeamuzie and Al-Hage, 1998).

Although inhaled β_2 adrenoceptor agonists are the most effective bronchodilators known, they may be associated with an increase in asthma mortality and morbidity when high doses are taken chronically (see Sears and Taylor, 1994). One theory that could explain this paradox is that prolonged use of sympathomimetic bronchodilators compromises the anti-inflammatory effect of glucocorticosteroids (Peters et al., 1995). Evidence to support this proposal derives from studies where chronic exposure of human eosinophils to salbutamol, salmeterol, and isoprenaline, at therapeutically relevant concentrations, prevented the ability of dexamethasone to suppress fMLP-induced superoxide anion generation (Nielson and Hadjokas, 1998). That effect was antagonized by propranolol, indicating that β adrenoceptors were involved, and time-dependent such that a 24-h exposure rendered the steroid inactive (Nielson and Hadjokas, 1998). Of potential significance is that the negative functional interaction between β_2 adrenoceptor agonists and steroids in eosinophils is supported by studies performed at the molecular level in a number of other cells and tissues. In particular, Peters et al. (1995) reported that salbutamol and fenoterol when added concurrently with dexamethasone reduced the binding of the activated glucocorticoid (GR) to DNA without altering receptor number or the affinity of dexamethasone. The activation of the transcription factor cAMP-response element binding protein (CREB) and its associated coactivator, CREB-binding protein (CBP) by cAMP is believed to underlie this effect because forskolin also reduced the binding of the activated GR to DNA (Peters et al., 1995). However, the extent to which this molecular mechanism accounts for the inability of dexamethasone to inhibit the activity of the NADPH oxidase in β adrenoceptor agonist-treated eosinophils is unknown.

3. Degranulation. Another in vitro functional response where β adrenoceptor agonists demonstrate an inhibitory effect is on degranulation. In human normodense

eosinophils, isoprenaline, salbutamol, and eformoterol inhibit (albeit weakly) the secretion of products (ECP, EDN, or EPO) stored within the specific granules in response to fMLP (Munoz et al., 1995; Ezeamuzie and Al-Hage, 1998), PAF (Eda et al., 1993a), and Ig (IgG and secretory IgA)-coated Sepharose beads (Kita et al., 1991b). Curiously, salmeterol is inactive at blocking fMLP-induced EPO release (Munoz et al., 1995; Ezeamuzie and Al-Hage, 1998) and actually blocks the inhibitory effect of salbutamol under the same experimental conditions, although the nature of the antagonism was not elucidated (Munoz et al., 1995). Those findings confirm previous observations with guinea pig eosinophils (Rabe et al., 1993) that salmeterol can act as a competitive β adrenoceptor antagonist.

Intriguingly, IgG-evoked EDN release is significantly more sensitive to β adrenoceptor agonists, and the inhibition produced greater, than the same response elicited by secretory IgA (Kita et al., 1991b). It is possible that functional antagonism explains this discrepancy since secretory IgA is a more effective stimulus of degranulation than is IgG (Kita et al., 1991b). Alternatively, the finding that Fc receptors for IgA and IgG couple to different G proteins might alter the sensitivity of the secretory mechanism to cAMP (see *XII. B.3.a*).

The exocytosis of arylsulphatase from guinea pig eosinophils elicited by opsonised *Candida albicans* is attenuated by isoprenaline, indicating that the mechanism(s) governing the release of contents from the small granules is similarly sensitive to the actions of β_2 adrenoceptor agonists (Masuyama and Ishikawa, 1985).

4. Chemotaxis and Chemokinesis. Salmeterol and formoterol partially inhibit PAF- and fMLP-induced chemotaxis of human eosinophils under experimental conditions where salbutamol is inactive (Koenderman et al., 1992; Eda et al., 1993a; Tool et al., 1996). However, the concentrations required to achieve this effect are very high (1–100 μM) and in excess of those required to increase maximally the cAMP content of eosinophils, inhibit homotypic aggregation (see below), and effect airways smooth muscle relaxation. Thus, the relevance of these findings in relation to the concentration of β_2 adrenoceptor agonist achieved in clinical practice is questionable. Isoprenaline similarly inhibits eosinophil chemotaxis stimulated by endotoxin-activated serum using two indices of migration, the Zigmond-Hirsch assay and a nucleopore filter assay (Clark et al., 1977). It would appear that species or the nature or concentration of the activating stimulus has a profound effect on whether or not β adrenoceptor agonists are active given that isoprenaline does not inhibit chemotaxis of guinea pig eosinophils (Sugasawa and Morooka, 1992).

β Adrenoceptor agonists are similarly effective at suppressing LTB_4-induced eosinophil chemotaxis (Sugasawa and Morooka, 1992) and, consistent with their effect on the NADPH oxidase (Rabe et al., 1993), are believed to act via an apparently atypical β receptor subtype (Sugasawa and Morooka, 1992). Using a novel, nonradioactive chemotaxis assay modified from the method described by Capsoni et al. (1989), Sugasawa and Morooka (1992) reported that although isoprenaline and fenoterol failed to suppress LTB_4-induced chemotaxis at concentrations up to 100 μM, the atypical β adrenoceptor agonist BRL 35135, but not its demethylated derivative BRL 37344 (Arch et al., 1984; Wilson et al., 1984; Arch and Kaumann, 1993), was active with an IC_{50} of 9 μM. Intriguingly, propranolol failed to antagonize the effect of BRL 35135 which is consistent with its low affinity for the atypical β adrenoceptors that predominate on rat adipocytes, guinea pig ileum, and rat colon and for the human cloned β_3 adrenoceptor (see Arch and Kaumann, 1993). In contrast, the nonselective β adrenoceptor-blocking drug alprenolol antagonized the inhibition of chemotaxis elicited by BRL 35135 with an affinity (pA_2 = 5.62) approximately 10-fold lower than predicted for an interaction with the atypical β adrenoceptors expressed on guinea pig ileum (pA_2 = 6.46). Given that isoprenaline, which is a strong agonist at β_3 receptors, was without inhibitory effect in this system, Sugasawa and Morooka (1992) have proposed that guinea pig eosinophils express a novel variant of the β adrenoceptor that is distinct from the β_1, β_2, and β_3 adrenoceptor subtypes currently classified.

In contrast to guinea pig and, to some extent, human eosinophils, the β_2 adrenoceptor agonists salbutamol and salmeterol are inactive at preventing PAF- and LTB_4-induced migration of rat peritoneal eosinophils (Alves et al., 1996).

5. Adhesion and Adhesion Molecule Expression. In anesthetized, pathogen-free F344 rats, the i.v. administration of SP and bradykinin produces an inflammatory response in the airways characterized by the adherence of proinflammatory leukocytes to venular endothelial cells along with plasma extravasation and edema (see *V.H.1* and *V.I*). Bowden et al. (1994) demonstrated that acute administration of rats with eformoterol reduced the number of eosinophils adherent to venules in the airway mucosa in response to both inflammatory stimuli. This effect was mediated by β_2 adrenoceptors since it was abolished by ICI 118,551 (Bowden et al., 1994). A clue to the mechanism of action of eformoterol in that model can be inferred from a study by Berends et al. (1997) in which isoprenaline, at a maximally effective concentration, suppressed the up-regulation of the adhesion of CD11b (by 43%) and the shedding of L-selectin (by 34%) on human eosinophils evoked by PAF.

Salmeterol, but not salbutamol, has been reported to inhibit the adherence of human eosinophils to fibronectin-coated plastic in response to PAF and IL-5 by a mechanism that does not apparently involve agonism of β_2 adrenoceptors (Ezeamuzie and Al-Hage, 1998).

6. Membrane Lipid Metabolism. Few reports have appeared in the literature describing the effect of β_2 adrenoceptor agonists on the liberation of lipid mediators

from eosinophils and the little information available is contradictory. For example, the short-acting β_2 adrenoceptor agonist salbutamol has been reported to inhibit fMLP-, C5a-, and PAF-induced LTC_4 generation from human eosinophils (Munoz et al., 1994; Tenor et al., 1996), whereas salmeterol was inactive under roughly comparable experimental conditions at concentrations that suppressed chemotaxis (Tool et al., 1996). Salmeterol similarly failed to prevent fMLP-induced PAF generation (Tool et al., 1996).

7. Homotypic Aggregation. The ability of guinea pig eosinophils to undergo homotypic aggregation in response to PAF and C5a is effectively antagonized by β adrenoceptor agonists (Teixeira et al., 1996a; Teixeira and Hellewell, 1997a). In fact, salbutamol is significantly more potent at suppressing aggregation than H_2O_2 formation with an EC_{50} similar to that required for cAMP accumulation. Moreover, in contrast to studies on the NADPH oxidase, the PDE4 inhibitor rolipram markedly potentiates the inhibitory effect of salbutamol at a concentration that has no effect on aggregation per se (Teixeira et al., 1996a), suggesting that cAMP-dependent mechanisms regulate this response. It is intriguing that whereas salmeterol fails to inhibit H_2O_2 generation from LTB_4-stimulated eosinophils (Rabe et al., 1993) and actually behaves as a β adrenoceptor antagonist, PAF- and C5a-induced homotypic aggregation are, paradoxically, suppressed in a propranolol-sensitive manner (Teixeira et al., 1996a; Teixeira and Hellewell, 1997a). Several explanations can be advanced for this discrepancy, although no firm conclusion can be drawn at the present time. The first is that guinea pig eosinophils express two populations of inhibitory β adrenoceptor that regulate, independently, the cell-signaling pathways responsible for the activation of the NADPH oxidase and homotypic aggregation. This hypothesis would be consistent with the anomalous pA_2 values that have been calculated for a range of β adrenoceptor antagonists in chemotaxis and respiratory burst assays (Sugasawa and Morooka, 1992; Rabe et al., 1993). Alternatively, the sensitivity of the signal transduction pathway that ultimately promotes homotypic aggregation to the inhibitory action of cAMP might be considerably greater that those mechanisms that govern the activation of the NADPH oxidase. However, it is noteworthy that the failure of PDE4 inhibitors to potentiate the inhibitory effect of salbutamol on H_2O_2 generation is not consistent with a cAMP-dependent mechanism of action. Thus, as in other tissues, β adrenoceptor agonists might recruit multiple and distinct signal transduction cascades that negatively regulate eosinophil activation (Maguire and Erdos, 1980; Barber et al., 1989; Rooney et al., 1991; Vaziri and Downes, 1992; Wu et al., 1995; Xiao et al., 1995) which can theoretically involve signaling via G_α and $G_{\beta\gamma}$ heterodimers (Daaka et al., 1997).

8. In Vivo Effects. The effect of β adrenoceptor agonists on stimulus-induced eosinophil recruitment in vivo is the subject of some debate. When acute studies are performed in laboratory animals, short- and long-acting β_2 adrenoceptor agonists are generally active (but see Banner et al., 1995; Namovic et al., 1996) at preventing pulmonary and cutaneous eosinophilia in response to a variety of stimuli including allergen (Fugner, 1989; Whelan and Johnson, 1990, 1992; Sanjar et al., 1991; Whelan and Johnson, 1990, 1992; Sugiyama et al., 1992; Teixeira et al., 1993, 1995a; Whelan et al., 1993; Howell et al., 1995; Teixeira and Hellewell, 1997a). Similarly, in humans, the systemic administration of isoprenaline can decrease circulating eosinophil number (Ohman et al., 1972) which may be responsible, at least in part, for the ability of β_2 adrenoceptor agonists to abolish cutaneous eosinophilia in sensitized human volunteers (Ting et al., 1983). It is likely that part of the inhibitory effect of β_2 adrenoceptor agonists on eosinophil recruitment is due to a direct effect on the eosinophil (Teixeira and Hellewell, 1997a). This is suggested from a study performed with salmeterol-treated, ^{111}In-labeled guinea pig eosinophils (where the inhibitory effect persists for many hours even after extensive washing) which, when injected into recipient guinea pigs, do not migrate to skin sites exposed to proinflammatory stimuli (Teixeira and Hellewell, 1997a).

Considerable controversy surrounds the effect of β_2 adrenoceptor agonists on various direct and indirect indices of immune and proinflammatory cell activation. In clinical asthma, the demonstration of a LPR is indicative of airway inflammation where eosinophils are believed to play a pathogenic role. Accordingly, the sensitivity of the LPR to β_2 adrenoceptor agonists has been studied in some detail. However, it must be borne in mind that allergen-induced inflammatory responses are acute events contrived to monitor relatively rapid changes in lung function. Thus, the assessment of these parameters is similar to many of the measurement that are made in animal models of "asthma" and must be distinguished from the true pathology which is characterized by a self-perpetuating, chronic inflammation of the airways. It is vital to make this distinction because β_2 adrenoceptor agonists might not affect allergen-induced LPR and the chronic inflammatory response equally.

The administration of a "standard" dose (200 μg) of salbutamol to asthmatic subjects has no effect on the LPR (Cockcroft and Murdock, 1987), yet high doses of short- and long-acting β_2 adrenoceptor agonists (salmeterol and formoterol) are effective at blocking the late bronchoconstriction that is manifest in many asthma sufferers (Twentyman et al., 1990, 1991; Palmqvist et al., 1992; Pedersen et al., 1993). Unfortunately, interpretation of these data is complicated by the possibility that β_2 adrenoceptor agonists prevent the LPR by functional antagonism at the level of the airways smooth muscle (even in the absence of detectable bronchodilatation), rather than by exerting an anti-inflammatory action. This difficulty has necessitated the study of ad-

ditional, more direct and unambiguous measurements of airway inflammation. For example, several investigators have assessed the effect of β_2 adrenoceptor agonists on eosinophil number in the circulation and BAL fluid and on the level of degranulation products in the serum. Dahl and Venge (1978) reported that the acute administration of salbutamol and terbutaline promoted eosinopenia and lowered the serum concentration of ECP in a group of asthmatic subjects, indicating a direct action on circulating eosinophils. In follow-up studies, inhaled salmeterol blocked the increase in serum ECP concentration during the LPR following general or local endobronchial allergen provocation (Dahl et al., 1995; Murray et al., 1995); however, no change in the number of eosinophils present in the BAL fluid was noted (Murray et al., 1995) which is in agreement with the results presented by Calhoun et al. (1995). In contrast, Di Lorenzo and coworkers (1995) were unable to detect any reduction in the serum ECP concentration or in circulating eosinophil number in 20 mild atopic asthmatic subjects given salbutamol. This lack of consistency is compounded by the results of another investigation where salmeterol effectively attenuated the increase in serum ECP and EDN levels evoked by allergen in 12 asthmatic subjects but did not prevent the blood eosinophilia (Pedersen et al., 1993).

Fiber-optic bronchoscopy has allowed an assessment of airway inflammation within the clinical setting and has been used to evaluate the potential anti-inflammatory effect of a variety of drugs including β_2 adrenoceptor agonists. With the exception of one study (Dahl et al., 1995), which found that salmeterol (50 μg b.i.d. for 4 weeks) produced a significant reduction in the levels of ECP in the BAL fluid, no evidence has been provided that chronic administration of either short-acting or long-acting β_2 agonists to asthmatics is efficacious when eosinophil number or secretory products are used as indices of inflammation (Adelroth et al., 1990; Howarth et al., 1992; Jeffery et al., 1992; Laitinen et al., 1992; Davies et al., 1993; Kraft et al., 1995; Manolitsas et al., 1995). In fact, in two investigatations (Davies et al., 1993; Manolitsas et al., 1995), compelling evidence was presented that eosinophils accumulated in the airway mucosa in subjects given salbutamol chronically (200 μg q.i.d; 4 months) when compared to placebo. Moreover, the number of cells that were $EG2^+$ (a marker of the secreted form of ECP) was increased, suggesting that they were activated (Manolitsas et al., 1995). Similarly, regular inhaled salbutamol was shown in 1997 to increase the number of eosinophils and level of ECP in the sputum of 10 mild asthmatic (Gauvreau et al., 1997). Taken together, these findings could have some bearing on the observation that regular treatment of asthmatic subjects with isoprenaline (Van Metre, 1969) and fenoterol (Sears et al., 1990) has been associated with an apparent worsening of the disease and a predisposition to increased risk of a life-threatening attack.

P. Somatostatin

Five distinct somatostatin receptors (denoted sst_1 to sst_5) have been identified in humans and mice and belong to the seven transmembrane-spanning family of receptors. Each sst receptor is the product of a different gene and couples primarily to $G_{o/i}$. See Bruns et al. (1995) for additional details.

Eosinophils have the capacity to synthesize, store, and release (Aliakbari et al., 1987) somatostatin, although it is not known whether they express cognate sst receptors. However, the somatostatin antagonist lanveotide effectively inhibits the peripheral blood and peritoneal eosinophilia precipitated in rats by i.p. administration of Sephadex beads, cyclophosphamide, PAF, or allergen (in sensitized animals) (Etienne et al., 1989a,b). Since somatostatin is known to affect T lymphocyte proliferation, and since T cells are involved in the differentiation of hematopoetic cells to eosinophils, it is possible that somatostatin decreases, indirectly, eosinophil availability and recruitment.

Q. Lipoxins

Structurally, lipoxins are acyclic eicosanoids that contain a conjugated tetraene structure and three alcohol groups (Serhan et al., 1984a,b; Serhan and Samuelsson, 1988; Steinhilber and Roth, 1989; Serhan, 1991). The two major lipoxins in this series of eicosanoids are positional isomers and have been named LXA_4 (5*S*,6*R*,15*S*-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid) and LXB_4 (5*S*,14*R*,15*S*-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid). Other lipoxins also have been identified and are known as LXC_4, LXD_4, and LXE_4 (Steinhilber and Roth, 1989). The human and murine LXA_4 receptors have been cloned, expressed, and their distribution at the mRNA level mapped (Fiore et al. 1994; Serhan et al., 1994; Takano et al., 1997). In the mouse, LXA_4 receptor mRNA transcripts are most abundantly expressed in blood leukocytes followed by the spleen and lung (Takano et al., 1997). Both receptors have a sequence indicative of a seven transmembrane-spanning G protein-coupled receptor and share 73% identity at the amino acid level (Fiore et al. 1994; Serhan et al., 1994; Takano et al., 1997). Binding studies have established that ^{3}H-labeled LXA_4 interacts with LXA_4 receptors with high affinity (K_d = 1–2 nM); LTD_4 effectively competes for this site whereas LXB_4 does not, indicative of lipoxin receptor heterogeneity. In CHO cells transfected with the murine or human LXA_4 receptor, LXA_4 promotes GTP hydrolysis and the release of esterified arachidonic acid by a pertussis toxin-sensitive mechanism (Fiore et al., 1994, Takano et al., 1997). These results are consistent with findings in human neutrophils where LXA_4 evokes functional responses through G_i/G_o-coupled receptors (Fiore et al., 1994). Phylogenetically, the murine and human LXA_4 receptor belongs to the CC chemokine family of G protein-coupled

receptors rather than to the eicosanoids such as the prostanoids (Toh et al., 1995).

Receptors for LXA_4 have not been unequivocally identified on eosinophils but they are probably expressed based on functional studies. Thus, although little is known of the biological activities of the lipoxins, LXA_4 is weakly chemotactic for human eosinophils, evoking responses about 20% of that produced by PAF and fMLP. In addition, LXA_4 inhibits PAF- and fMLP-induced eosinophil migration (Soyombo et al., 1994) but has no effect on ECP release per se or on degranulation effected by fMLP (Soyombo et al., 1994). LXA_4 has been shown to activate PKC with potency greater than DAG (Hansson et al., 1986). However, it displays selectivity for PKCγ (Shearman et al., 1989) which predominates in the central and peripheral nervous systems but is not expressed by human eosinophils (Evans et al., 1999). The biological activities of LXB_4, C_4, D_4, and E_4 equally are obscure.

VI. Interleukin-3, Interleukin-5, and Granulocyte/Macrophage Colony-Stimulating Factor

The hematopoietins, which include IL-3, IL-5, and GM-CSF, are important regulators of eosinophil function and exert both distinct and overlapping effects (Tables 11, 12, and 13 for details and Miyajima et al., 1992a,b). The IL-5 receptor in humans is selectively expressed by eosinophils and basophils but not neutrophils or monocytes (Chihara et al., 1990; Ingley and Young, 1991). This contrasts with cell surface receptors for IL-3 and GM-CSF that have a more ubiquitous distribution (Clutterbuck et al., 1989; Ogawa, 1993).

A. Receptor Expression and Regulation

Radioligand-binding experiments using ^{125}I-labeled IL-5 have demonstrated cross-competition among IL-3, IL-5, and GM-CSF (Lopez et al., 1989, 1991) due to a structural similarity in hematopoeitic cytokine receptors. Thus, all three receptors are composed of two subunits: a 60- to 80-kDa α subunit, that is unique to each receptor, and a common β subunit (β_c), which has a mass between 120 and 140 kDa (Tavernier et al., 1991). Interleukin-3, IL-5, and GM-CSF interact with the α subunit of their respective receptors with low affinity, whereas the additional interaction with the β_c subunit results in the formation of a high-affinity ligand-receptor complex, thereby permiting cell signaling to occur

TABLE 11
Some functional effects evoked by IL-3 in eosinophils

Functional Effect	References
Promotes proliferation and differentiation	Warren (1988); Dvorak et al. (1989); Clutterbuck and Sanderson (1990); Ema et al. (1990)
Increases cell survival	Rothenberg et al. (1988); Tai et al. (1991); Wallen et al. (1991)
Inhibits apoptosis	Tai et al. (1991); Atsuta et al. (1995)
Induces hypodense phenotype	Chihara and Nakajima (1989); Clutterbuck et al. (1989); Rothenberg et al. (1989)
Weak chemoattractant	Warringa et al. (1991); Hakansson and Venge (1994); Yamaguchi et al. (1988a)
Increases phagocytosis of *Candida albicans*	Fabian et al. (1992a, b)
Increases killing of *Staphylococcus aureas* and *Schistosoma mansoni*	Rothenberg et al. (1988); Fabian et al. (1992a)
Increases CR3 expression	Thorne et al. (1990); Walsh et al. (1990b); Walsh et al. (1991a); Hartnell et al. (1992a)
Increases CR3 binding affinity	Blom et al. (1994)
Increases binding to glass	Walsh et al. (1990b)
Increases binding to ICAM-1/VCAM-1	Fattah et al. (1996)
Induces ICAM-1 expression	Czech et al. (1993)
Synergizes with TNFα/β to induce ICAM-1	Hansel et al. (1992); Czech et al. (1993)
Increases FcγRII expression and binding	Hartnell et al. (1992b); Koenderman et al. (1993)
Stimulates GM-CSF release	Alam and Grant (1995)
Induces CD4/CD25 expression	Riedel et al. (1990)
Increases CD23 expression	Mawhorter et al. (1996)
Increases CD69 expression	Hartnell et al. (1993)
Increases CD30L expression	Pinto et al. (1996)
Increases CD81 expression	Mawhorter et al. (1996)
Increases TGFα mRNA and protein expression	Brach et al. (1994); Elovic et al. (1998)
Increases TGFβ_1 mRNA and protein expression	Elovic et al. (1998)
Increases PAF receptor expression	Kishimoto et al. (1996b)
Synergizes with GM-CSF to increase IL-5 binding	Chihara and Nakajima (1989)
Up-regulates PAF receptor expression	Kishimoto et al. (1996a, b)
Enhances C5a-induced IL-8 release	Miyamasu et al. (1997)
Enhances chemotaxis to PAF, LTB_4, fMLP, C5a, IL-8, and opsonised particles	Warringa et al. (1991); Hakansson et al. (1994)
Enhances LTC_4 release to A23187, fMLP, C5a, and PAF	Rothenberg et al. (1988); Takafuji et al. (1991)
Induces EDN degranulation and NADPH oxidase activation from adherent eosinophils	Horie et al. (1996)
Enhances A23187-induced arylsulphatase and β-glucuronidase release	Fabian et al. (1992a)
Enhances ECP and EPX release to C3b-coated Sepharose	Tai and Spry (1990); Carlson et al. (1993)
Enhances C3a- and C5a-induced ECP release	Takafuji et al. (1995, 1996)
Enhances EDN release to IgA- and IgG-Sepharose-coated beads	Fujisawa et al. (1990)
Enhances dexamethasone-induced HLA-DR and HLA-DP expression	Guida et al. (1994)
Synergizes with IFN-γ to induce HLA-DR expression	Hansel et al. (1992)

TABLE 12
Some functional effects evoked by IL-5 in eosinophils

Functional Effect	Reference(s)
Promotes proliferation and differentiation	Enokihara et al. (1988); Jabara et al. (1988); Warren (1988); Clutterbuck et al. (1989); Dvorak et al. (1989); Clutterbuck and Sanderson (1990); Ema et al. (1990)
Increases cell survival	Yamaguchi et al. (1988a); Tai et al. (1991); Kita et al. (1992)
Inhibits apoptosis	Her et al. (1991); Tai et al. (1991); Yamaguchi et al. (1991); Stern et al. (1992)
Induces hypodense phenotype	Rothenberg et al. (1989); Owen et al. (1990); Kita et al. (1992)
Weak chemoattractant	Yamaguchi et al. (1988b); Coeffier et al. (1991b); Sehmi et al. (1992b); Hakansson et al. (1994)
Increases phagocytosis in *Candida albicans*	Fabian et al. (1992a,b)
Increases killing of *Staphylococcus aureas*	Fabian et al. (1992a,b)
Weak inducer of EDN, ECP, EPO, and MBP release	Fujisawa et al. (1990); Kita et al. (1992)
Increases CR3 expression	Thorne et al. (1990); Walsh et al. (1990b); Hartnell et al. (1992a); Lundahl et al. (1993); Neeley et al. (1993); Sedgwick et al. (1995)
Increases CR3 binding affinity	Walsh et al. (1991a); Blom et al. (1994)
Increases binding to glass	Walsh et al. (1990b)
Increases binding to HUVECs	Walsh et al. (1991a); Sedgwick et al. (1995)
Increases binding to HMVECs	Walsh et al. (1990b); Walsh et al. (1991a)
Increases binding to ICAM-1/VCAM-1	Fattah et al. (1996)
Synergizes with TNFα/β to induce ICAM-1	Czech et al. (1993)
Increases FcγRII expression	Koenderman et al. (1993)
Increases CD69 expression	Hartnell et al. (1993)
Increases CD30L expression	Pinto et al. (1996)
Increases TGFα mRNA and protein expression	Brach et al. (1994); Elovic et al. (1998)
Increases TGFβ_1 mRNA and protein expression	Elovic et al. (1998)
Increases PAF receptor expression	Kishimoto et al. (1996a,b)
Enhances chemotaxis to PAF, LTB_4, fMLP, C5a, RANTES, IL-4, and opsonized particles	Sehmi et al. (1992b); Warringa et al. (1992a); Hakansson and Venge (1994); Schweizer et al. (1994)
Enhances LTC_4 release to A23187, fMLP, C5a, and PAF	Takafuji et al. (1991); Laviolette et al. (1995); Takafuji et al. (1995)
Enhances NADPH oxidase response to PMA, fMLP, and opsonized particles	Tagari et al. (1993); van der Bruggen et al. (1993a); Sedgwick et al. (1995)
Induces EDN degranulation from adherent eosinophils	Horie et al. (1996)
Enhances C3b-induced ECP release	Carlson et al. (1993)
Enhances C3a- and C5a-induced ECP release	Takafuji et al. (1995, 1996)
Enhances EDN release to IgA- and IgG-Sepharose-coated beads	Fujisawa et al. (1990); Kita et al. (1991a)
Enhances dexamethasone-induced HLA-DR/DP expression	Guida et al. (1994)
Promotes expression of p35 and p40 IL-12 mRNAs and biologically active protein	Grewe et al. (1998)
Up-regulates α_d integrin	Grayson et al. (1998)
Increases LTB_4 receptor mRNA levels	Huang et al. (1998)
Releases MIF	Rossi et al. (1998)

(Miyajima et al., 1992b; Murata et al., 1992; Koike and Takatsu, 1994). It is possible that the cross-competition between cytokines results from a limited number of β_c subunits that would limit the extent of eosinophil activation. In human eosinophils, a single population of receptors for IL-5 has been identified although the binding constants are variable. Thus, IL-5 has been reported to interact with eosinophils with an affinity of 19 pM (Tagari et al., 1993), 120 pM (Lopez et al., 1991), 170 to 330 pM (Migita et al., 1991), and 400 pM (Ingley and Young, 1991); a broad spectrum of B_{max} values (260–1500 sites/cell) also has been reported (Migita et al., 1991; Lopez et al., 1991; Okada et al., 1998). Less research has been done with IL-3 and GM-CSF but they appear to interact with a single class of noninteracting sites with K_d values of 470 pM and 44 pM, respectively (Lopez et al., 1989).

Regulation of the IL-5 receptor, as well as of the synthesis, storage, and release of IL-5, clearly is important in determining eosinophil responses. However, relatively little is known of the factors that control the transcription and expression of these proteins. It has been reported that transforming growth factor (TGF) β_1 and phorbol 12-myristate 13-acetate (PMA) down-regulate IL-5 receptor α chain mRNA transcripts in vitro in a remarkably stimulus-specific manner (Zanders, 1994). Indeed, a host of other stimuli including ILs 1 to 11, G-CSF, GM-CSF, LIF, stem cell factor (SCF), erythropoetin, IFN-γ, RANTES, MIP-1α, EGF, platelet-derived growth factor (PDGF), dexamethasone, forskolin, retinoic acid, and cyclosporin A were inactive. Conversely, up-regulation of IL-5 mRNA was observed in bronchial biopsies taken from asthmatic individuals (Yasruel et al., 1997). In that study, the majority of the IL-5 receptor mRNA was associated with eosinophils, suggesting that they represent the major target for IL-5-induced responses. The gene encoding the IL-5 receptor α subunit is located in region 3p26 of chromosome 3 (Tavernier et al., 1991) and encodes a membrane-anchored form that is produced by alternative mRNA splicing (Tavernier et al., 1992). In addition, two novel soluble isoforms, which are secreted into body fluids, also are produced that arise from either normal mRNA splicing or from the absence of a splicing event (Tavernier et al., 1992). Although the soluble isoforms bind IL-5 in in vitro assays, their role in vivo is presently unclear; however, it is

TABLE 13
Some functional effects evoked by GM-CSF in eosinophils

Functional Effect	Reference(s)
Promotes proliferation and differentiation	Clutterbuck et al. (1989); Clutterbuck and Sanderson (1990); Ema et al. (1990)
Increases cytotoxicity	Lopez et al. (1986); Silberstein et al. (1986); Owen et al. (1987)
Increases cell survival	Lopez et al. (1986); Owen et al. (1987); Vancheri et al. (1989); Tai and Spry (1990); Wallen et al. (1991); Hallsworth et al. (1992)
Inhibits apoptosis	Tai et al. (1991); Alam et al. (1994); Atsuta et al. (1995)
Induces hypodense phenotype	Caulfield et al. (1990); Owen et al. (1990)
Weak chemoattractant	Warringa et al. (1991); Yamaguchi et al. (1988b)
Increases phagocytosis of *Candida albicans*	Fabian et al. (1992a,b)
Increases killing of *Staphylococcus aureas*	Fabian et al. (1992a,b)
Increases CR3 expression	Thorne et al. (1990); Walsh et al. (1991b); Hartnell et al. (1992a); Tomioka et al. (1993); Sedgwick et al. (1995)
Increases CR3 binding affinity	Blom et al. (1994)
Increases binding to glass	Walsh et al. (1990b)
Increases VLA4-mediated binding to activated endothelium	Sung et al. (1997)
Increases binding to ICAM-1/VCAM-1	Fattah et al. (1996)
Synergizes with TNFα/β to induce ICAM-1	Hansel et al. (1992); Czech et al. (1993); Horie et al. (1997a)
Stimulates IL-5 release	Alam et al. (1994)
Increases FcγRII expression	Koenderman et al. (1993); Mawhorter et al. (1996)
Increases CD69 expression	Hartnell et al. (1993)
Increases CD30L expression	Pinto et al. (1996)
Increases CD4/CD25 expression	Riedel et al. (1990)
Increases CD23/CD81 expression	Mawhorter et al. (1996)
Increases CD80/CD86 expression	Tamura et al. (1996)
Increases TGFα mRNA and protein expression	Brach et al. (1994)
Increases PAF receptor expression	Kishimoto et al. (1996a,b)
Induces IL-2 mRNA expression in combination with A23187	Bosse et al. (1996)
Synergizes with TNFα to induce CD54 expression	Horie et al. (1997b)
Enhances fMLP and PAF induced CD11b expression and adherence	Tomioka et al. (1993); Nagata et al. (1995b)
Induces EDN release and activates NADPH oxidase from adherent cells	Horie et al. (1996)
Enhances chemotaxis to PAF, LTB_4, fMLP, IL-8, C5a, RANTES, IL-4, and opsonized particles	Warringa et al. (1991); Dubois et al. (1994a)
Enhances LTC_4 release to A23187, fMLP, C5a, and PAF	Silberstein et al. (1986); Owen et al. (1991); Howell et al. (1989); Takafuji et al. (1991); Fabian et al. (1992a); Nagata et al. (1995b); Laviolette et al. (1995)
Enhances fMLP-induced NADPH oxidase activation and adherence	Nagata et al. (1995b)
Enhances fMLP-induced PAF release	Triggiani et al. (1992)
Enhances ECP and EPX release to C3b-coated Sepharose beads	Tai and Spry (1990); Carlson et al. (1993)
Promotes expression of p35 and p40 IL-12 mRNAs and protein	Grewe et al. (1998)
Enhances A23187-induced arylsulphatase and β-glucuronidase release	Fujisawa et al. (1990)
Enhances EDN release to IgA- and IgG-Sepharose-coated beads	Fujisawa et al. (1990)

likely that they neutralize the effect of IL-5 on target tissues (Tavernier et al., 1992; Devos et al., 1993). Recent studies have identified two functional promoter regions, P1 and P2, in the gene encoding the IL-5 receptor α subunit that are located in the 5′ upstream regions of exon 1 (L. Sun et al., 1995) and exon 2 (J. Zhang et al., 1997), respectively. Using the eosinophilic cell line AML14, P1 promoter activity has been localized within a 561-base pair (bp) sequence proximal to the transcriptional start site (Z. Sun et al., 1995). 5′-Deletion mutants within that region have identied a 34-bp domain (−432 to −398) that confers approximately 80% promoter activity and is highly active in a myeloid cell- and eosinophil-specific manner (Z. Sun et al., 1995). However, consensus sequences for known transcription factors are absent indicative of unique myeloid cell- and, possibly, eosinophil-specific, regulatory elements. Subsequent studies identified an enhancer element (*EOS1*) within the P1 promoter (Sun et al., 1996). A comparison with other models of transcription factor binding shows that *EOS1* is similar to the bacterial helix-turn-helix phage λ and 434 repressor-operator complexes, and the Cys4 zinc finger glucocorticoid response element (GRE) motifs. The possibility that the enhancer element may function as a GRE is supported by the identification of an AP-1-binding site adjacent to the *EOS1* domain. This is significant as AP-1:GRE is a composite response element in the regulation of a number of genes (Sun et al., 1996). The P2 promotor is located within a 66-bp region (−31 to +35) of exon 2 and features a 5′-CCAAT-3′-binding domain for the transcription factor CCAAT-enhancer binding protein (C/EBP), and two consensus motifs (−5 to +1 and +13 to +18) for the oncogene *c-ets* (J. Zhang et al., 1997). However, of particular interest is the presence of a novel 6-bp element (5′-CTAATT-3′), spanning −19 to −14, that is essential for P2 promotor activity and which is activated by a transcription factor specific to the eosinophil lineage (J. Zhang et al., 1997).

B. Signal Transduction

The binding of IL-3, IL-5, and GM-CSF to their cognate receptors leads to the activation of multiple signal-

ing pathways (Fig. 5; Koenderman et al., 1996; van der Bruggen and Koenderman, 1996; Yousefi et al., 1997). Although the α and β_c subunits of hematopoietic receptors do not exhibit intrinsic kinase activity, activating cytokines cause rapid changes in the tyrosine phosphorylation of a number of cellular proteins (van der Bruggen et al., 1993a) through the recruitment of cytoplasmic tyrosine kinases and phosphatases. Ligation of the IL-5 receptor on human eosinophils induces a rapid recruitment of the tyrosine kinases *lyn*, *syk,* and Jak-2 to the β_c subunit of the receptor (Alam et al., 1995; Pazdrak et al., 1995a,b; van der Bruggen et al., 1995; Bates et al., 1996) along with the tyrosine phosphatase SHPTP-2 (Pazdrak et al., 1997). Similarly, GM-CSF activates *lyn* and Jak-2 (Simon et al., 1997b). In addition, IL-5 promotes the phosphorylation of p52^{shc}, an adapter protein that physically links cell surface receptors to downstream signaling elements, and enhances its association to another adapter protein, Grb (Bates et al., 1998). Other early signaling events that occur in eosinophils exposed to IL-5 include the activation of PtdIns 3-kinase and the subsequent phosphorylation of PKB (Coffer et al., 1998). Despite these data, the down-stream biochemical events or the functional responses they ultimately promote are not clearly defined. However, it has been established that IL-5 stimulates the Ras-Raf1-MEK-ERK protein kinase cascade in human eosinophils (Alam et al., 1995; Pazdrak et al., 1995a; Bates et al., 1996; Coffer et al., 1998), although, at present, there are contradictory reports concerning the ERK isoform that is activated. Independent studies by Bates et al. (1996) and Hiraguri et al. (1997) found that anti-ERK antibodies immunoprecipitated three proteins of molecular weights 42, 44, 45 kDa and 40, 42, 44 kDa, respectively and, consistent with Pazdrak et al. (1995a), found that IL-5 activated the higher molecular weight species, that is probably ERK-1. However, those data contrast to the recent report of Coffer et al. (1998) who found that IL-5 only activated ERK-2. The upstream events linking the Ras-Raf1-MEK-ERK pathway to the IL-5 receptor have not been fully characterized but antisense studies have implicated a role for SHPTP-2 in ERK-2 activation (Pazdrak et al., 1997). Similarly, PtdIns 3-kinase and, possibly, PKB also are involved since the activation of ERK-1 by IL-5 and GM-CSF is inhibited by wortmannin (Hiraguri et al., 1997).

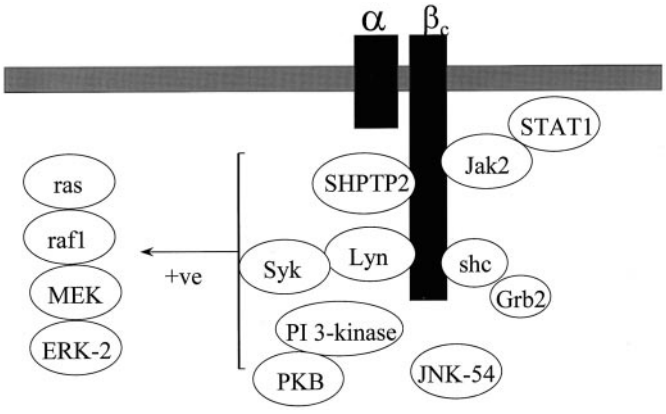

FIG. 5. IL-5-induced signaling in human eosinophils. The binding of IL-5 with its cognate receptor and dimerization of the α and β_c subunits is believed to trigger the phosphorylation of tyrosine residues upon the latter by an, as yet, undefined mechanism. The phosphotyrosine residues then permit the binding and activation of a number of kinases including *lyn*, *syk*, and Jak-2, the phosphatase SHPTP-2, and, possibly, the scaffold or adapter proteins shc and Grb. A number of downstream kinase cascades and transcription factors then are activated including ras/raf1/MEK/ERK, PtdIns 3-kinase/PKB, JNK-54, and STAT-1 with resultant gene transcription. See *VI.B* for further details.

Other proteins necessary for signaling through the IL-5 receptor include the transcription factor signal transducers and activators of transcription (STAT) 1, which probably is activated by Jak-2 (Alam and Grant, 1995; Pazdrak et al., 1995b; van der Bruggen et al., 1995). de Groot et al. (1997) also have provided evidence that TPA-responsive element (TRE)- and diad symmetry element (DSE)-dependent transcription is regulated by Jak-2 and JNK-54.

Specific domains within the common β subunit of the IL-5 receptor initiate signaling to the cells' interior. Using truncated mutants of the cytoplasmic domain of β_c subunit Sato et al. (1993) identified two functional regions: a membrane proximal domain (amino acid residues 456–517) essential for proliferation, activation of Jak-2 and induction of c-*myc*, and a second domain (amino acid residues 627–763) that is required for the binding of *shc*, activation of the p21^{ras}-Raf-l-MEK-ERK kinase cascade, and the induction of c-*fos* and c-*jun*. The association of SHPTP-2 with the IL-5 receptor β_c has been demonstrated in a cell-free reconstituted system using a synthetic peptide (residues 605–624) of the latter incorporating Y^{612} (Pazdrak et al., 1997). Binding to this phosphotyrosine-containing peptide, but not a peptide in which the phosphorylated Y^{612} had been mutated to F, increases SHPTP-2 activity implying that direct binding can induce enzyme activation (Pazdrak et al., 1997). Three additional tyrosine residues (Y^{750}, Y^{806}, Y^{869}) located carboxyl-terminal to amino acid 589 on the IL-5 receptor β_c also have been found that are surrounded by a consensus sequence that favors the binding of SHPTP-2. Thus, the exact site at which SHPTP-2 binds remains unresolved, although Pazdrak et al. (1997) have speculated Y^{612} and/or Y^{750} are likely candidates. A pentapeptide sequence at amino acids 577 to 581 also has been identified that is central to the activation of JNK-54 and DSE-dependent transcription (de Groot et al., 1997).

In addition to the β_c subunit, the cytoplasmic domain of the IL-5 receptor α subunit is apparently essential for IL-5-induced proliferation and the activation of c-*jun*, c-*fos,* and Jak-2 (Takaki et al., 1994; Cornelis et al., 1995; Muto et al., 1996).

A current model of the IL-5/IL-3/GM-CSF signaling pathway predicts that activating ligands induce a conformational change in their cognate receptors, which

thereby activate receptor-bound tyrosine kinases (Fig. 5). These then tyrosine phosphorylate the common β subunit of the receptor to provide the binding sites for the recruitment and subsequent activation of *lyn*, *syk*, and SHPTP-2. The tyrosine kinases responsible for this event are likely to be bound to a proline-rich domain, also called a box-1 motif, at residues 458 to 465 as deletion of these amino acids prevents the tyrosine phosphorylation of the IL-5 receptor β_c subunit (Itoh et al., 1996). Currently, the identity of this tyrosine kinase(s) is unknown but a case can be made for Jak-2 based on the finding that mutant cells lacking this kinase are unable to phosphorylate the IL-5 receptor β_c subunit after stimulation with GM-CSF (Watanabe et al., 1997).

C. Functional Effects

Interleukin-3, IL-5, and GM-CSF exert a range of effects on eosinophils (see Tables 11, 12, and 13). In particular, they are central in determining the number of eosinophils in the circulation and in tissues through their ability to promote production, proliferation, and differentiation (see *III.*) and to enhance their survival by suppressing apoptosis (see *XII.H*). Hematopoeitic cytokines are also implicated in the priming of mature eosinophils to a range of stimuli that evoke chemotaxis (see *XII.A.3*), degranulation (see *XII.B*), adhesion (see *XII.A.2*), and activation of the NADPH oxidase (see *XII.G*).

It is well established that administration of IL-5 to laboratory animals induces blood eosinophilia (e.g., Iwama et al., 1992) and IL-5 transgenic mice show lifelong eosinophilia in organs without overt pathology, indicating that eosinophils require other factors for activation (Dent et al., 1990). The importance of IL-5 in allergen-induced tissue eosinophilia in laboratory animals also has been examined extensively and similar investigations now are emerging in humans. Generally, exposure of sensitized mice, rats, and guinea pigs to allergen results in the appearance of IL-5 and eosinophils in the BAL fluid. The pulmonary eosinophilia is dependent upon circulating, not locally produced, IL-5 (Wang et al., 1998) and is associated with an increase in airways reactivity to a variety of stimuli including acetylcholine (ACh), arecholine, histamine, and 5-hydroxytryptamine (Chand et al., 1992a; Gulbenkian et al., 1992; Nagai et al., 1993, 1996; Brunjzeel et al., 1993). Similar effects are seen in the pleural cavity of antigen-challenged sensitized mice (Bozza et al., 1994a). Almost without exception, the effect of neutralizing IL-5 with antibodies inhibits eosinophil infiltration but has a variable effect on airways responsiveness (Gulbenkian et al., 1992; Chand et al., 1992a; van Oosterhout et al., 1993; Nagai et al., 1993, 1996). Using the technique of adoptive transfer, it has been found that IL-5-secreting $CD4^+$ Th_2-type cells in mice play a pivotal role in generating blood and airways eosinophilia and in the subsequent development of bronchial hyperreactivity and lung damage that occurs in response to aeroallergens (Hogan et al., 1998).

The effect of anti-IL-5 antibodies has not been reported in humans. However, a similar activity to that described in animals might be prediced given the reports of Shi et al. (1997, 1998) who found that IL-5 given to asthmatic subjects by the inhaled route, or instilled directly into the airways, produced pulmonary eosinophilia, and increased the number of eosinophils and the level of ECP in the induced sputum.

IL-5 is also involved in parasitosis and in helminth-induced airway hyperresponsiveness (Hall et al., 1998). Indeed, administration of the anti-IL-5 antibody TRFK-5 to mice inoculated with microfilariae of the filarial nematode *Onchocerca lienalis* reduces the ability of the animals to resist re-infection (Folkard et al., 1996). A similar approach has been adopted to show that IL-5 is important in driving eosinophilia and reducing parasite burden in mice exposed to *Aspergillus fumigatus* (Murali et al., 1993; Kurup et al., 1997), *Toxocara canis* (Buijs et al., 1995), and *Angiostrongylus cantonesis* (Sasaki et al., 1993).

VII. Interferon Receptor Superfamily

The IFN receptor superfamily, which includes receptors for IFNα/β, IFNγ, and IL-10, characteristically are single transmembrane-spanning glycoproteins with either one (IFNγ and IL-10) or two (IFNα/β) homologous extracellular regions that feature two fibronectin domains. Although, IFNα/β (type I interferons) and IL-10 (a type II interferon) exert biological actions on human eosinophils (Table 14), only a receptor for IFNγ (type II interferon) has been convincingly identified (Aldebert et al., 1996; Ishihara et al., 1997). ^{125}I-labeled IFNγ labeled a single population of noninteracting sites on intact eosinophils with a K_d and B_{max} of 3.9 pM and 183 to 233 sites per cell, respectively (Aldebert et al., 1996). Although IFNγ binds with high affinity, the ability of the agonist-occupied receptor to signal requires a species-specific accessory protein that associates with an epitope on the intracellular domain of the receptor protein.

VIII. Tumor Necrosis Factor Superfamily

The tumor necrosis factor (TNF) or nerve growth factor (NGF) superfamily is composed of cytokine receptors and leukocyte surface glycoproteins. Members of this family are characterized by three to four cysteine-rich repeats of 40 amino acids in the extracellular portion of the molecule (Mallett and Barclay, 1991).

A. Tumor Necrosis Factor α

The type I (CD120a) and type II (CD120b) TNF receptors have respective molecular masses of 55 and 75 kDa and have been identified on human eosinophils by fluorescence-activated cell sorting analysis and immune electron microscopy (Zeck Kapp et al., 1994). Generally,

TABLE 14
Functional effects evoked by interferons and IL-10 in eosinophils

Functional Effects	References
IFNα	
Inhibits IL-3-induced eosinophil differentiation	Sillaber et al. (1992)
Inhibits eosinophil colony growth	Shalit et al. (1995)
Reduces FcεRI and FcεRII expression in human cord blood-derived eosinophils	Capron et al. (1997)
Inhibits IgA- and IgE-induced ECP, EDN, and IL-5 release and antiparasitic cytotoxicity	Lamkhioued et al. (1995a); Aldebert et al. (1996)
Promotes expression of p35 and p40 IL-12 mRNAs and biologically active protein	Grewe et al. (1998)
IFNβ	
Augments FcγR expression	De Simone et al. (1986a)
IFNγ	
Down-regulates IL-3-induced eosinophil differentiation	Sillaber et al. (1992)
Decreases FcεRI expression in cord blood-derived eosinophils	Capron et al. (1997)
Enhances FcεRII expression in peripheral and core blood-derived eosinophils	Akutagawa et al. (1994); Capron et al. (1997)
Induces FcγRIII expression in peripheral eosinophil	Akutagawa et al. (1994)
Inhibits TNFα-induced CD4 expression	Hossain et al. (1996)
Increases number of cells expressing RANTES mRNA	Ying et al. (1996)
Increases Mcl-1 expression	Druilhe et al. (1998)
Induces IL-3 release	Fujisawa et al. (1994)
Induces GM-CSF mRNA expression	Moqbel et al. (1991)
Induces IL-6 mRNA expression	Hamid et al. (1992)
Induces ICAM-1 expression (response synergized with TNFα)	Czech et al. (1993)
Induces CD69 expression	Hartnell et al. (1993)
IL-10	
Inhibits LPS induced GM-CSF release and survival	Takanaski et al. (1994)
Down-regulates CD40 mRNA expression	Ohkawara et al. (1996)

TABLE 15
Functional effects evoked by TNF-α in human eosinophils

Functional Effects	Reference(s)
Induces adherence to activated HUVECs	Lamas et al. (1988)
Enhances eosinophil toxicity to *Schistosoma mansoni* larvae	Silberstein and David (1986); Thorne et al. (1986)
Enhances cytotoxicity to antibody-treated Daudi-lymphoma cells	Valerius et al. (1990)
Enhances IgE-mediated cytotoxicity to *Schistosoma japonicum*	Janecharut et al. (1992)
Enhances A23187-induced LTC_4 release	Roubin et al. (1987)
Enhances fMLP-induced LTC_4 release from normodense cells	Takafuji et al. (1992)
Increases oxidant production in eosinophils adherent to HUVEC and FCS-coated plates	Slungaard et al. (1990)
Induces superoxide release of eosinophils adherent to plastic, fibrinogen, and fibrin	Dri et al. (1991); Zeck Kapp et al. (1994)
Induces CD4 expression	Hossain et al. (1996)
Induces IL-8 mRNA/protein production and release	Nakajima et al. (1996)
Induces the expression of ICAM-1 in combination with IL-3, IL-5, GM-CSF, or IFNγ	Hansel et al. (1992); Czech et al. (1993)
Induces chemokinesis and increases PAF-induced chemotaxis	Nagata et al. (1993)
Promotes expression of p35 and p40 IL-12 mRNAs and biologically active protein	Grewe et al. (1998)

the actions of TNFα on eosinophils in culture or isolation usually are proinflammatory (Table 15). In vivo, antibodies against TNFα significantly attenuated the development of fibrosis elicited by bleomycin in mice and the associated pulmonary eosinophilia, suggesting that TNFα plays an important pathogenic role in that model (K. Zhang et al., 1997).

B. CD30 Ligand

CD30 is a transmembrane receptor that was originally identified as a surface antigen on Reed-Sternberg cells in Hodgkin's disease and found subsequently to be preferentially expressed by human activated $CD4^+$ T lymphocytes (Del Prete et al., 1995; Manetti et al., 1994). Eosinophils are $CD30^-$ cells but express an activating ligand CD30L (CD153) (Pinto et al. 1996) that has homology only with members of the TNF superfamily (Falini et al., 1995). The demonstration that native CD30L can transduce proliferative signals in $CD30^+$ targets such as Hodgkin and Reed-Sternberg cells has suggested a possible role for eosinophils in the pathology of Hodgkin's disease (Pinto et al., 1996, 1997). This contention is supported by the higher than normal levels of CD30L expression on circulating and tissue eosinophils in patients with Hodgkin's disease and hypereosinophilic syndrome compared to normal subjects. In this respect, it is interesting that the expression of CD30L on eosinophils is increased by IL-3, IL-5, and GM-CSF (Pinto et al., 1996).

C. CD40 and CD40 Ligand

Originally identified on B lymphocytes and some carcinoma cell lines, CD40 is expressed on a variety of cells including eosinophils (Ohkawara et al., 1996). Structurally, CD40 is a 45- to 50-kDa transmembrane-spanning glycoprotein and, together with its activating ligand, CD40L (CD154 also called gp39), is thought to be important for the full expression of allergic inflammatory responses in the airways of animals and possibly humans (Lei et al., 1998). mRNA and surface protein for CD40 are expressed constitutively on circulating eosinophils of allergic patients and are up-regulated in response to IgA

immune complexes and down-regulated by IL-10 (Ohkawara et al., 1996). Similarly, constitutive expression of CD40L on cells obtained from a hypereosinophilic patient has been reported along with the finding that normal eosinophils and the eosinophilic cell line Eol-3 will produce CD40L in response to fMLP, PMA, and ionomycin (Gauchat et al., 1995).

Functionally, cross-linking of CD40 increases eosinophil survival in a concentration-dependent manner by stimulating the release of GM-CSF (Ohkawara et al., 1996). In the presence of IL-4, eosinophils are able to induce CD40L-dependent B lymphocyte proliferation in vitro (Gauchat et al., 1995).

D. CD69

The CD69 antigen is a phosphorylated 28- to 32-kDa disulfide-linked homodimer that was first identified on activated T lymphocytes and natural killer cells in the late 1980s (for review, see Testi et al., 1994). Complementary DNA clones encoding human and mouse CD69 have been isolated and identified the antigen as a C-type lectin (Ziegler et al., 1994). Gene-mapping studies have placed CD69 on mouse chromosome 6 and the p13 region of human chromosome 12 (Ziegler et al., 1994). The role of CD69 as a possible marker of activated eosinophils was proposed shortly after it was originally described following the detection of significant levels of $CD69^+$ cells in the BAL fluid, but not peripheral blood, of patients with eosinophilic pneumonia (Nishikawa et al., 1992). It is now known that CD69 is expressed on eosinophils taken from the BAL fluid of patients with mild asthma (Hartnell et al., 1993, Matsumoto et al., 1998) and on peripheral blood eosinophils during human parasitosis (Mawhorter et al., 1996) consistent with an activated phenotype. Indeed, $CD69^+$ eosinophils are rapidly induced in vitro in response to IL-3, IL-5, GM-CSF, IFNγ, and IL-13 (Nishikawa et al., 1992; Hartnell et al., 1993; Luttmann et al., 1996; Mawhorter et al., 1996; Matsumoto et al., 1998). The induction of CD69 by GM-CSF is inhibited by cycloheximide, suggesting that new protein synthesis is required (Hartnell et al., 1993). However, it has been reported that protein and mRNA for CD69 are found within unstimulated eosinophils (Luttmann et al., 1996), although those data were not corroborated in a subsequent investigation (Matsumoto et al., 1998). The function of CD69 is largely unexplored but it might be involved in regulating longevity based on the finding that anti-CD69 antibodies promote apoptosis of GM-CSF-stimulated eosinophils (Walsh et al., 1996b).

E. CD95

Human CD95 (Fas/APO-1) is a membrane-associated polypeptide, has an approximate molecular mass of 48 kDa, and is comprised of 335 amino acids with a glycosylated amino-terminal extracellular domain, a hydrophobic middle, and an intracellular carboxyl terminus (Oehm et al., 1992; Smith et al., 1994). The amino terminus contains three cysteine-rich regions that are characteristic of the TNF/NGF receptor family whereas a 70-amino acid sequence at the carboxyl terminus features a, so-called, "death domain" that is necessary and sufficient for the transduction of signals that effect apoptosis (Itoh and Nagata, 1993).

Freshly purified eosinophils express CD95 at a low but consistent level (Matsumoto et al., 1995; Druilhe et al., 1996). However, following culture of eosinophils in the absence of cytokines the level of CD95 increases in a time-dependent manner that is associated temporally with reduced viability and an increase in the number of apoptotic nuclei (Druilhe et al., 1996). Similarly, cross-linking of CD95 with specific monoclonal antibodies produces a time- and concentration-dependent increase in apoptosis (Matsumoto et al., 1995; Tsuyuki et al., 1995; Druilhe et al., 1996). mRNA and protein for CD95 are up-regulated in human eosinophils cultured for 24 h with IFNγ and TNFα, and synergy occurs when both cytokines are used concurrently. These effects are functionally relevant as eosinophils now display an enhanced rate of apoptosis in response to CD95L (Luttmann et al., 1998b). Significantly, IL-3, IL-5, and GM-CSF prevent CD95 expression by an unknown mechanism and this presumably contributes to their survival-prolonging activity (Luttmann et al., 1998b; see *XII.H* for additional details). Unlike human neutrophils, the activating ligand CD95L is not constitutively expressed on eosinophils (Liles et al., 1996). However, ligation of CD95 by CD95L present on activated T lymphocytes, for example, recruits a number of intracellular pathways in human eosinophils including JNK-54, *lyn,* and IL-1-converting enzyme-like proteases that are believed to couple the activation of an upstream sphingomyelinase to the degradation of lamin B_1 (Hebestreit et al., 1998; Simon et al., 1998). Indeed, the broad-spectrum tyrosine kinase inhibitors genistein and lavendustin A prevent CD95-mediated death in human and murine eosinophils in vitro and partially resolve CD95L-induced eosinophilia in an in vivo model of inflammation in the mouse (Simon et al., 1998). Lavendustin A also inhibits CD95-mediated lamin B_1 degradation which might account in part for its antiapoptotic activity (Simon et al., 1998).

F. Nerve Growth Factor

Relatively little is known of the functional actions of NGF on eosinophils although chemotaxis, lavicidal activity and degranulation (Hamada et al., 1996; Solomon et al., 1998) all are accredited activities. NGF also suppresses fMLP-stimulated LTC_4 release (Takafuji et al., 1992).

IX. Adhesion Molecules

Adhesion molecules or receptors are thought to be central to the process of eosinophil migration from the systemic circulation into tissue (see *XII.A*). A number of

adhesion molecules are expressed by eosinophils (Fig. 6) and can be divided into three families: the selectins, integrins, and immunoglobulins.

A. Selectins

Three selectin families (denoted E, P, and L) have been described. E-selectin (CD62E) and P-selectin (CD62P) are expressed on endothelial cells whereas L-selectin (CD62P) is found on the cell surface of leukocytes including eosinophils (see Bevilacqua and Nelson, 1993; Lasky, 1995). Structurally, the selectins are characterized by an amino-terminal C-type (Ca^{2+}-dependent), lectin-like, binding domain, an EGF-like region, two to nine concensus repeats of sequence similar to those appearing in complement-regulatory proteins, such as decay-accelerating factor, a membrane-spanning domain, and a short cytoplasmic tail.

The expression of E-selectin by endothelial cells is induced by certain cytokines and requires gene transcription and protein synthesis (Bevilacqua et al., 1987). P-selectin is stored within cytoplasmic Weibel-Palade bodies from where it translocates to the plasma membrane within minutes of stimulation (Johnston et al., 1989; Geng et al., 1990). In contrast, L-selectin is constitutively expressed by eosinophils but is shed upon activation with stimuli such as A23187, PAF, fMLP, and IL-5 (Smith et al., 1992; Neeley et al., 1993). Those in vitro observations are entirely consistent with the lower than normal expression of L-selectin on eosinophils harvested from the sputum of asthmatic subjects when compared with blood eosinophils (in't Veen et al., 1998). The counterligands for selectins are a family of sialylated, fucosylated, and, in many cases, sulfated, glycosaminoglycans typified by the moiety sialyl Lewis X (Springer and Lasky, 1991). The precise carbohydrate moieties recognized by the selectins are presently unknown, although the peptide backbone appears to be important in conferring selectin specificity. The majority of selectin counterligands contain mucin regions that are characteristically serine/threonine/proline-rich peptide sequences with rigid backbones and are decorated with *O*-linked carbohydrates (Shimizu and Shaw, 1993). To date, three L-selectin counterligands have been identified upon endothelial cells: 1) GlyCAM-1, 2) MadCAM-1, and 3) CD34, which contain mucins or mucin-like domains (Lasky et al., 1992; Briskin et al., 1993; Baumhueter et al., 1994). Studies examining P-selectin-mediated binding of eosinophils to nasal polyp endothelial cells and to soluble P-selectin identified PSGL-1, a sialylated, homodimeric glycoprotein, as the eosinophil counterligand (Wein et al., 1995; Symon et al., 1996). PSGL-1 has been isolated by expression cloning from an HL-60 library and shown to be a 220-kDa homodimer with a heavily *O*-glycosylated mucin-like structure (Sako et al., 1993). Further structural analyses revealed that, in contrast to the 15-decapeptide repeat found in neutrophil PSGL-1, the corresponding eosinophil variant is 10-kDa heavier due to an extra repeat (Symon et al., 1996). The counterligand for E-selectin was identified from examining the interaction of eosinophils with soluble E-selectin immobilized upon plastic plates and identified as a sialylated, protease-resistant structure (Bochner et al., 1994).

B. Integrins

The integrins constitute a superfamily of gene products that are composed of two noncovalently linked α and β transmembrane heterodimeric glycoproteins. Eo-

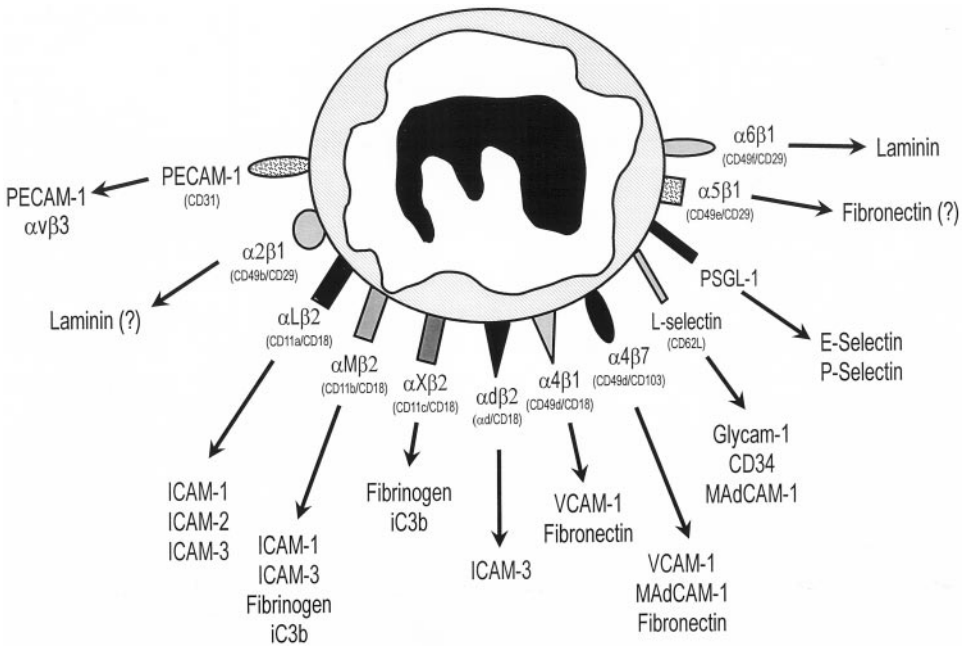

FIG. 6. Eosinophil adhesion molecules/receptors and their counterligands. See *IX* for additional details.

sinophils express the β_1 (CD29) integrins: VLA-2 (CD49b), VLA-4 (CD49d), VLA-5 (CD49e), and VLA-6 (CD49f); the β_2 (CD18) integrins (Kuijpers et al., 1993): leukocyte function-associated antigen (LFA) 1 (CD11a, $\alpha_L\beta_2$), CR3 (CD11b, $\alpha_M\beta_2$, Mac-1), and complement receptor (CR) 4 (CD11c, $\alpha_X\beta_2$, p150,95); the novel integrin, $\alpha_d\beta_2$, which interacts with intercellular adhesion molecule (ICAM) 3, and the β_7 integrin, $\alpha_4\beta_7$ (CD49d/CD103) (Kuijpers et al., 1993; Walsh et al., 1996a). The integrins bind to members of the Ig superfamily expressed upon endothelial cells as well as components of the extracellular matrix (see Fig. 6).

1. β_1 Integrins. The most extensively studied β_1 integrins are VLA-4 and VLA-6 which bind to the extracellular matrix proteins fibronectin and laminin, respectively. Relatively little is known about VLA-2 and VLA-5. It has been demonstrated that VLA-4 binds to the, so-called, IIICS region of fibronectin that features a 25-amino acid alternatively spliced connecting segment, CS-1, which is recognized by the integrin through a characteristic LDV motif (Anwar et al., 1993, 1994). VLA-4 also can bind to VCAM-1, an Ig superfamily member expressed upon cytokine-exposed endothelial cells (Bochner et al., 1991a; Dobrina et al., 1991; Weller et al., 1991b; Atsuta et al., 1998). The interaction occurs at sites within the first and fourth Ig-like domains of the protein (Osborn et al., 1992; Vonderheide et al., 1994). The VLA-4:VCAM-1 and VLA-4:fibronectin interaction is encouraged when eosinophils are preincubated with GM-CSF (Sung et al., 1997), SCF (Yuan et al., 1997), and PAF (Anwar et al., 1994) and is due to an increase in ligand affinity rather than an up-regulation of receptor expression or changes in receptor distribution (Neeley et al., 1993; Sung et al., 1997; Yuan et al., 1997). The chemoattractants, RANTES, MCP-3, and C5a transiently increase VLA-4-mediated adhesion to purified VCAM-1 and fibronectin (Weber et al., 1996). However, those data contrast with the result of other experiments. In particular, Burke-Gaffney and Hellewell (1996) have found that the activation of human lung microvascular endothelial cells by TNFα was associated with increased VLA-4-mediated adhesion of eosinophils after their exposure to eotaxin but not to RANTES or MIP-1α.

VLA-4-dependent adhesion alters the functional responsiveness of eosinophils to a number of stimuli. Thus, PAF- and A23187-induced LTC_4 release (Anwar et al., 1994; Munoz et al., 1996), the secretion of ECP in response to fMLP in cytochalasin B-treated cells (Neeley et al., 1994), and the enhanced survival of eosinophils effected by IL-3 and GM-CSF produced in an autocrine manner (Anwar et al., 1993) all are augmented. Furthermore, cross-linking of VLA-4 receptors with activating monoclonal antibodies (Laudanna et al., 1993) or after spontaneous adherence to VCAM-1-coated plates (Nagata et al., 1995a) activates the NADPH oxidase through a mechanism that might be secondary to the activation of CR3 (Nagata et al., 1995a).

Expression of VLA-6 by eosinophils was reported by Georas et al. (1993) and is elevated in mildly allergic patients. Using monoclonal antibodies directed against CD29 and CD49f, it has been shown that VLA-6 mediates the binding of eosinophils to laminin, a component of the basement membrane, and suggests that this interaction may contribute to eosinophil localization in the subendothelium (Georas et al., 1993). Consistent with the results obtained with fibronectin, the longevity of eosinophils cultured on laminin is significantly enhanced (Tourkin et al., 1993).

As described above, adhesion molecules are involved in processes other that simple cell-cell interactions such as survival, leukotriene release, and degranulation. Currently, the signaling pathways utilized by the β_1 integrins in eosinophils are under studied. However, in other cells it has been established that the cytoplasmic tail of the β subunit promotes a rearrangement of actin and cytoskeletal components to form a focal adhesion complex that predominates at sites where the cell interacts with extracellular matrix components (Clark and Brugge, 1995). In this respect, ligation of β_1 integrins results in the activation of the focal adhesion kinase $p125^{Fak}$ (Schaller and Parsons, 1994), which is believed to recruit a number of other signaling molecules to the focal adhesion complex that are intimately involved in cell spreading. Occupancy of the β_1 integrin receptor also results in the activation of *src*-related protein tyrosine kinases (Shattil et al., 1994), the mitogen-activated protein (MAP) kinase cascade (Schaller and Parsons, 1994), and a novel 59-kDa serine/threonine "integrin-linked kinase" that, as the name implies, associates with the cytoplasmic tail of β_1 integrins (Hannigan et al., 1996).

2. β_2 Integrins. Human eosinophils express the common β_2 chain CD18 and the α chains CD11a (LFA-1), CD11b (CR3), and CD11c (p150,95) that bind to ICAM-1 (CD54) (Fischer et al., 1986; Hartnell et al., 1990; Walsh et al., 1990a,b; Kyan Aung et al., 1991a; Grayson et al., 1997). A fourth integrin, $\alpha_d\beta_2$, also is expressed on human eosinophils, is up-regulated by IL-5 and Ca^{2+} ionophore A23187, and can function as an alternative ligand for VCAM-1 (Grayson et al., 1998). The expression of CD11b is greater on eosinophils found in the sputum of asthmatic subjects when compared with their peripheral blood counterparts (in't Veen et al., 1998), and this is consistent with the ability of proinflammatory mediators to up-regulate β_2 integrins in general. CR3 numbers are increased on the surface of human eosinophils by PAF, IL-3, IL-5, GM-CSF (Thorne et al., 1990; Walsh et al., 1991a; Hartnell et al., 1992a; Lundahl et al., 1993; Neeley et al., 1993; Tsai et al., 1993; Fattah et al., 1996), and, to a lesser extent, TNFα (Thorne et al., 1990), fMLP (Lundahl et al., 1993; Neeley et al., 1993), LPS (Lundahl et al., 1993), C5a (Gerard and Gerard, 1991; Lundahl et al., 1993), and RANTES (Alam et al., 1993). In contrast, the expression of LFA-1 seems to be more tightly con-

trolled in that it is up-regulated only by PAF (Hayashi et al., 1994). A number of investigators have reported that in addition to receptor number, IL-3, IL-5, GM-CSF, and RANTES also increase the affinity of β_2 integrins for their counterligands (Blom et al., 1994; Kakazu et al., 1995).

Binding through CD18 appears to be important for the activation of the NADPH oxidase (Laudanna et al., 1993). The interaction of CR3 and LFA-1 with soluble ICAM-1 promotes the production of reactive oxygen radicals (Chihara et al., 1995a), and CD18-dependent binding of eosinophils to HSA-coated plates confers sensitivity to GM-CSF and PAF which are otherwise inactive (Horie and Kita, 1994). Soluble ICAM-1 and anti-CD11b-linked to polystyrene microbeads also promote eosinophil degranulation with the release of ECP and EDN (Chihara et al., 1995b; Kato et al., 1998a). Mechanistically, the adherence of IL-5-treated eosinophils to protein-coated tissue culture plates via β_2 integrins is accompanied by inositol phosphate accumulation and the tyrosine phosphorylation of a number of proteins (Kato et al., 1998a). One of those is the product of the c-*cbl* proto-oncogene Cbl, along with two other proteins of 105 and 115 kDa (Kato et al., 1998a). Similar results have been obtained with anti-CD11b to activate directly $\alpha_M\beta_2$. Thus, the tyrosine phosphorylation of Cbl and the 115-kDa proteins along with phosphoinositide hydrolysis may play a central role in integrin-dependent functional responses such as degranulation.

3. $\alpha_4\beta_7$ Integrin. In addition to the formation of VLA-4, the α_4 subunit has been shown by immunostaining and flow cytometry to associate with the β subunit, β_7 to form $\alpha_4\beta_7$ in eosinophils (Erle et al., 1994; Walsh et al., 1996a). This integrin is expressed at the same level as $\alpha_4\beta_1$ and is believed to bind to the Ig, MadCAM-1 (Berlin et al., 1993; Briskin et al., 1993) as well as VCAM-1 and the CS-1 region of fibronectin (Chan et al., 1992; Ruegg et al., 1992; Postigo et al., 1993). Although $\alpha_4\beta_7$ is constitutively expressed upon eosinophils, it is poorly active since the presence of Mn^{2+} is required to demonstrate adherence (Seminario and Bochner, 1997). This is despite the fact that exposure of eosinophils to PAF has been shown to induce $\alpha_4\beta_7$-mediated binding to MadCAM-1 but not VCAM-1 in L-12 cells transfected with the appropriate cDNAs (Walsh et al., 1996a). Thus, in the absence of stimuli, the binding of eosinophils to VCAM-1 and fibronectin is mediated predominantly by VLA-4.

C. Intercellular Adhesion Molecule 1

Intercellular adhesion molecule 1 (CD54) is a member of the C2-type Ig family of proteins that have been implicated in cell adhesion and complement binding. Human ICAM-1 is composed of a 55-kDa core protein, an extracellular-facing fragment containing five Ig-like domains and up to eight possible sites for *N*-linked glycosylation, and a 28-amino acid cytoplasmic tail rich in lysine and arginine that is thought to be responsible for binding to the cytoskeleton (Staunton et al., 1990; Carpen et al., 1992; Kirchhausen et al., 1993).

ICAM-1 is not constitutively expressed by circulating blood eosinophils (Hansel et al., 1992; Czech et al., 1993) but has been detected on sputum eosinophils (Hansel et al., 1991a) and in eosinophils recovered from the BAL fluid of patients with eosinophilic pneumonia (Azuma et al., 1996). In contrast to data published by Hansel et al. (1992), Czech et al. (1993) reported that the inflammatory cytokines IL-3, IFNγ, and TNFα induce ICAM-1 expression on normal circulating eosinophils. Despite that discrepancy, there is a consensus that TNFα [and TNFβ (Hansel et al., 1992)] acts synergistically with IL-3, IL-5, GM-CSF, and IFNγ to up-regulate ICAM-1 expression by a mechanism that involves de novo protein synthesis (Hansel et al., 1992; Czech et al., 1993). Burke-Gaffney and Hellewell (1998) have shown that ICAM-1 mediates adhesion of eosinophils to human bronchial epithelial cells which would aid their accumulation and retention in the airways in diseases such as asthma.

Little is known of the affect of ICAM-1 binding upon eosinophil function, although it has been implicated in GM-CSF- and TNFα-induced degranulation (Horie et al., 1997a).

X. Immunoglobulins

Eosinophils can express Fc receptors for IgA, IgD, IgG, and IgM. Receptors for the Fc portion of IgE also have been detected on eosinophils but controversy surrounds the precise nature of the IgE-FcϵR interaction (Kita and Gleich, 1997).

A. Receptors for Fcα

The Fc receptor for IgA (CD89) is a transmembrane glycoprotein expressed by several granulocytes including neutrophils and eosinophils (Capron et al., 1988a). Molecular genetics has mapped the human *CD89* gene to chromosome 19 (Kremer et al., 1992), which contrasts to the genes that encode other Fc receptors that are localized to chromosome 1. Structurally, FcαR is composed of five exons spanning approximately 12 kb (de Wit et al., 1995). The first two exons (denoted S1 and S2) encode a leader sequence, the third and fourth (termed EC1 and EC2) each encode a homologous Ig-like domain and the final exon (TM/C) codes for a short intracellular region, a transmembrane segment and a short cytoplasmic tail (de Wit et al., 1995). Evidence exists for at least seven transcripts of FcαR designated FcαRa.1 to 6 and FcαRb that arise from alternative mRNA splicing (Patry et al., 1996; Pleass et al., 1996; van Dijk et al., 1996). That discovery is entirely consistent with what has been established for FcγRI (Porges et al., 1992) and FcγRII (Brooks et al., 1989) and suggests that the expression of several closely related proteins by alternative splicing provides a means of diversifying function (see *X.D*).

The cDNAs derived from the two major Fcα transcripts of both human eosinophils and neutrophils have been cloned and sequenced, and the neutrophil variants stably transfected in CHO-K1 cells (Pleass et al., 1996). The largest clone, FcαRa.1, represents the previously described full-length receptor (Maliszewski et al., 1990), whereas the splice variant, FcαRa.3, is a truncated form lacking the entire second, membrane-proximal Ig domain. The long and short forms do not bind anti-FcαR monoclonal antibodies equally or serum IgA, supporting the idea that alternative splicing of *FcαR* gene could provide a means of altering FcαR receptor function.

van Dijk et al. (1996) also have reported a novel isoform of the FcαR that is expressed in human eosinophils and neutrophils. The cloned receptor, FcαRb, differs from previously described splice variants in that it lacks the exon (TM/C) encoding the transmembrane/intracellular region of the wild-type receptor and exon EC2 is extended to encode 23 additional amino acids. Transfection of IIA1.6 murine pro-B lymphocytes with the cDNA for FcαRb results in high levels of expression at the plasma membrane, along with the secretion of a significant amount of protein. The expression of FcαRb at the cell surface is not affected by phosphatidylinositol-specific PLC, indicating that glycosyl phosphatidylinositol (GPI) linkage of FcαRb is unlikely. In IIA1.6 murine pro-B lymphocytes expressing FcαRb and FcγR, which is necessary for signal transduction by wild-type FcαR, neither Ca^{2+} mobilization nor tyrosine phosphorylation is observed upon receptor cross-linking (van Dijk et al., 1996), suggesting that FcαRb has a different functional role to FcαR.

The molecular mass of FcαR on eosinophils (70–100 kDa) is significantly higher when compared to its counterpart on human neutrophils (55–75 kDa). However, removal of *N*-linked carbohydrates from both cell types yields a protein of 32 kDa, indicating differential degrees of glycosylation between neutrophils and eosinophils (Monteiro et al., 1993). The expression of FcαR is up-regulated approximately 3-fold on human eosinophils exposed to the Ca^{2+} ionophore A23187 (Monteiro et al., 1993). Similarly, eosinophils harvested from allergic individuals express higher levels of FcαR when compared with control subjects (Monteiro et al., 1993).

Functionally, anti-IgA induces eosinophil migration in atopic and healthy volunteers (Rihoux et al., 1990). A number of studies also have demonstrated the expression of functional IgA receptors on human eosinophils (Capron et al., 1988a; Abu Ghazaleh et al., 1989; Kita et al., 1991b) with particular reference to degranulation. Secretory IgA and IgA-coated Sepharose beads are particularly effective at promoting EDN release by a mechanism that is enhanced by IL-3 and GM-CSF (Abu Ghazaleh et al., 1989; Kita et al., 1991b).

B. Receptors for Fcδ and Fcμ

It has been reported (Wardlaw et al., 1995) that human eosinophils express Fc receptors for IgD but this assertion has not received further documentation. In contrast, normal blood eosinophils lack FcμR (CD7) (Ottesen et al., 1977; Walsh and Kay, 1986) although binding of IgM can apparently occur when cells are cultured in vitro (De Simone et al., 1982a).

C. Receptors for Fcϵ

It has been known for some time that certain allergic diseases and parasitic infections are associated with peripheral blood and tissue eosinophilia along with an increase in total and antigen-specific IgE. Indeed, there is a close correlation between serum IgE and the prevalence and severity of allergic diseases such as asthma (Sears et al., 1991). Similarly, the acquisition of immunity against *Schistosoma hematobium* is positively correlated with the appearance of anti-schistosome IgE antibodies (Hagan et al., 1991). IgE is known to promote mast cell and eosinophil degranulation (Khalife et al., 1986; Galli et al., 1991; Tomassini et al., 1991), enhance antigen presentation to, and internalization by, T lymphocytes when bound to antigen-presenting cells (APCs) (Mudde et al., 1995; Maurer et al., 1996) and mediate killing of invading parasites by acting as a ligand for antibody-dependent, cell-mediated cytotoxicity (Capron et al., 1982; Truong et al., 1993; Capron and Capron, 1994; Gounni et al., 1994a). Thus, these and other data have led to the general view that IgE is implicated in the direct and indirect activation of eosinophils in allergic diseases and following parasite infestation.

Arbesman and coinvestigators were the first to demonstrate that complexed IgE binds to human eosinophils (Ishikawa et al., 1974; Fujita et al., 1975), an observation that was extended several years later by the identification of cell surface receptors for IgE (Capron et al., 1981). Subsequently, it was established that IgE bound to a receptor on eosinophils with low affinity ($K_d \sim 100$ nM) that was similar, but not identical, to CD23 (FcϵRII) expressed by B lymphocytes (Capron et al., 1986, 1991; Jouault et al., 1988; Yokota et al., 1988; Capron and Joseph, 1991). However, in 1998, definitive evidence was provided that human eosinophils express FcϵRII that is identical with CD23 expressed by B lymphocytes (Abdelilah et al., 1998). Structurally, FcϵRII is a 45-kDa type II glycoprotein that can exist in at least two isoforms, FcϵRIIa and FcϵRIIb, that differ only in their amino-terminal cytoplasmic tail and arise through differential mRNA splicing (Yokota et al., 1988). Eosinophils express both forms of FcϵRII (Abdelilah et al., 1998). Since those original observations, two other receptors for IgE have been identified on eosinophils and to some extent characterized. In mice, one of these, FcϵRI, is a tetrameric protein composed of an α chain, which binds IgE, a β chain, and two disulfide-linked γ chains (Ravetch and Kinet, 1991), and is recognized by IgE with high affinity ($K_d \sim 0.1$ nM) (Gounni et al., 1994a,b). Interestingly, the human homolog of FcϵRI

lacks the β subunit. The other receptor is a galactose-specific, thiol-dependent S-type lectin called Mac-2 that has a high degree of sequence homology to rat ϵBP and carbohydrate-binding protein 35 and binds to IgE with relatively low affinity (Truong et al., 1993).

Evidence is available that eosinophils can express each variant of Fcϵ, although the extent to which this occurs depends on whether the cells are purified from normal subjects or from individuals with eosinophilia associated with allergic inflammation or parasitosis. Further distinctions probably can be made based on the type and severity of disease. For example, the expression of FcϵRII is seemingly restricted to a hypodense population of eosinophils harvested from subjects with prominent eosinophilia and certain allergic disorders (Capron et al., 1986, 1989; Rumi et al., 1998), whereas little, if any, expression is detected on eosinophils from "normal" individuals (Hartnell et al., 1989; Rumi et al., 1998). Comparable data also have been reported for FcϵRI (Terada et al., 1995; Rajakulasingam et al., 1997, 1998; Sihra et al., 1997) and Mac-2/ϵBP (Truong et al., 1993). In the later case, Northern blot analysis using eosinophil RNA from several eosinophilic donors probed with human Mac-2 and human ϵBP cDNAs routinely identified a single 1.2-kb product (Truong et al., 1993). In 50% of those eosinophil preparations, complementary flow cytometry experiments identified cell surface Mac-2 expression. This was confirmed by Western immunoblotting which resulted in the labeling of a 29-kDa band, consistent with Mac-2 protein, and two smaller anonymous peptides of 20 and 15 kDa (Truong et al., 1993).

It is clear from the aforementioned discussion that there is a wide body of evidence from studies in humans that IgE can activate eosinophils and promote antibody-dependent, cell-mediated cytotoxicity through FcϵRI, FcϵRII, and Mac-2/ϵBP. However, some controversy still surrounds the functional role of Fcϵ on eosinophils (Kita and Gleich, 1997). In particular, allergen-antibody complexes also can activate eosinophils via FcγR (Kaneko et al., 1995a). Similarly, FcγR are involved in IgG-dependent cytotoxicity toward parasites (Butterworth et al., 1977). Moreover, eosinophils purified from the BAL fluid, liver granulomas, and bone marrow cultures of parasite-infected mice do not express cell surface receptors for Fcϵ and fail to bind IgE under conditions where eosinophils can be activated following ligation of FcγR by IgG (Jones et al., 1994; de Andres et al., 1997b). A major implication of those findings is that the mouse might not be a suitable species to study human immunological disease and, clearly, this requires careful investigation. Another enigmatic set of observations that currently defies explanation is the finding that antibodies raised against *all* three Fcϵ receptors abolish IgE binding and antibody-dependent, cell-mediated cytotoxicity (see Kita and Gleich, 1997 and references therein).

D. Receptors for Fcγ

Three functional receptors for IgG have been identified and characterized on human leukocytes. On resting human eosinophils, only one of these, FcγRII (CDw32), is constitutively expressed to any extent (Hartnell et al., 1990) although murine eosinophils also express FcγRIII (CD16) in reasonable numbers (de Andres et al., 1997b). IFNγ up-regulates FcγRII expression (Valerius et al., 1990; Hartnell et al., 1992b) and is associated with enhanced IgG-induced antibody-dependent cellular cytotoxicity (Valerius et al., 1990). Those data are consistent with an earlier publication that described an increase in Fcγ receptor density and cytotoxicity in response to IFNβ (De Simone et al., 1986). Structurally, FcγRII is a 40-kDa protein, for which IgG has low affinity, and is widely distributed among leukocytes.

Hartnell et al. (1992b) have reported that the expression of FcγRII is up-regulated by IL-3. However, although a subsequent study (Koenderman et al., 1993) found that the addition of IL-3, IL-5, and GM-CSF to freshly prepared eosinophils produced a transient 3-fold increase in their ability to form rosettes with IgG-sensitized erythrocytes, no change in FcγRII receptor expression was noted. Indeed, it was concluded that changes in FcγRII signaling might reflect alterations in CR3 receptor function since the increase in rosetting activity was accompanied by a commensurate augmentation in the binding of iC3b to CR3. Further support for that contention was that an antibody against CD11b prevented the effects of IL-3, IL-5, and GM-CSF (Koenderman et al., 1993). The reason for the discrepancy between the two studies is unclear. In murine eosinophils, ligation of FcγRII results in phosphatidylinositol hydrolysis which has been linked to the activation of 5-lipoxygenase (de Andres et al., 1991b).

The other Fcγ receptor for which IgG has low affinity is the 50- to 70-kDa FcγRIII (CD16). Although not expressed constitutively by human eosinophils, high levels are found intracellularly that can be mobilized to the cell surface by mediators such as IFNγ, PAF, fMLP, and C5a in a cycloheximide- and dexamethasone-sensitive manner, indicating a requirement for new protein synthesis (Hartnell et al., 1992b; Zhu et al., 1998). The up-regulation of FcγRIII is transient and protein is rapidly released into the medium and then is reabsorbed. The role of FcγRIII on eosinophils in unknown but it is likely that the secretion of the soluble receptor might neutralize bioavailable IgG. Treatment of human eosinophils with phosphatidylinositol-specific PLC markedly reduces cell surface CD16 expression, indicating that it is a GPI-linked receptor (Zhu et al., 1998). The finding that anti-CD16 effects membrane depolarization and LTC_4 release in cytokine-treated cells (Hartnell et al., 1992b) indicates that this effect is functionally relevant. Those data are concordant with the enhanced expression of IgG receptors in general by eosinophils purified from

subjects with eosinophilia (that have presumably become exposed to a host of mediators in vivo) when compared with normal individuals (Kishimoto, 1988).

The cDNA of each FcγR has been cloned (Stuart et al., 1987; Simmons and Seed, 1988; Allen and Seed, 1989) which led to the discovery of two FcγRIII isoforms [FcγRIII-1 (CD16-1), FcγRIII-2 (CD16-2)] that are encoded by distinct genes (Ravetch and Perussia, 1989; Scallon et al., 1989). FcγRIII-1 is expressed predominantly by resting neutrophils and is anchored to the cell surface by a GPI linkage; however, after activation of the cell, this receptor is shed and is detected in the plasma (Huizinga et al., 1988, 1989, 1990). In contrast, FcγRIII-2 is a transmembrane-spanning receptor expressed by natural killer cells (Hibbs et al., 1989; Kurosaki and Ravetch, 1989; Lanier et al., 1991). The FcγRIII variant(s) expressed on eosinophils and the function(s) it specifically subserves is currently unclear, although treatment of IFNγ-exposed eosinophils with phosphatidylinostitol-specific PLC reduces FcγRIII expression, suggesting that eosinophils express a functionally active GPI-linked form (FcγRIII-1) of the receptor (Hartnell et al., 1992b).

FcγRI (CD64) is a 72-kDa protein for which IgG has high affinity and is expressed almost exclusively by monocytes. However, receptors for FcγRI can be induced in human eosinophils treated with IFNγ (Hartnell et al., 1992b).

IgG, immobilized to Sephadex beads, evokes a host of functions in eosinophils. Of significance was the demonstration in 1997 that ligation of Fcγ receptors on murine eosinophils provided a napoptotic signal. de Andres et al. (1997a) reported that culture of murine eosinophil precursors with a rat monoclonal antibody (2.4G2) that reacts with CD32 and CD16 promoted several hallmarks of apoptosis: chromatin condensation, annexin V binding, and induction of CD95. Since murine eosinophils express CD16 and CD32, additional experiments were performed with precursors obtained from mice in which the *CD16* gene was disrupted. The results of those experiments established that apoptosis was absolutely dependent on CD32 and the activation of CD95 (de Andres et al., 1997a). Collectively, those data highlight a novel mechanism of inducing apoptosis which, if reproduced in human eosinophils, could be relevant to cell-mediated tissue injury and antibody-dependent cellular cytotoxicity (see *XII.H*).

Other functional effects for which IgG has been accredited include degranulation (Kita et al., 1991b,d; Tomassini et al., 1991; Kaneko et al., 1995a,b), activation of the NADPH oxidase (de Andres et al., 1997b), and the generation of LTC_4 (Shaw et al., 1985; Cromwell et al., 1988; Moqbel et al., 1990a) and PAF (Cromwell et al., 1990).

XI. Miscellaneous

A. Interleukin-1

The type I (CDw121a) and type II (CDw121b) IL-1 receptor have been identified in mice and humans. Structurally, these receptors are single transmembrane glycoproteins with approximate molecular masses of 80 kDa and 60 kDa, respectively. Both receptors bind the predominately membrane associated IL-1α, IL-1β that is secreted, and IL-1RA. Studies with human eosinophils suggest that IL-1β induces the secretion of arylsulfatase and β-glucuronidase whereas a combination of IL-1α and IL-1β inhibits the activation of the NADPH oxidase in response to the phorbol diester PMA (Pincus et al., 1986; Whitcomb et al., 1989). Prolonged exposure (30–180 min) of guinea pig eosinophils to IL-1β suppresses A23187-induced [^{3}H]AA and phosphatidylcholine release by down-regulating the activity of PLA_2 (Debbaghi et al., 1992).

Sanz et al. (1995) demonstrated that intradermal administration of IL-1β to rats stimulates the accumulation of radiolabeled eosinophils to the sites of injection (Sanz et al., 1995). Moreover, persuasive evidence for a role of IL-1β in allergen-induced eosinophil migration derives from the ability of IL-1RA to prevent pulmonary eosinophilia when given by aerosol to sensitized guinea pigs immediately before challenge (Watson et al., 1993). Similarly, IL-1RA blunts the LPR and the number of hypodense eosinophils that appear in the BAL fluid of allergen-challenged sensitized guinea pigs (Okada et al., 1995). These findings have prompted clinical trials with IL-1RA in asthma, the results of which are eagerly awaited. It is noteworthy that the in vivo chemotactic activity of IL-1β is likely to be secondary to the activation of the endothelium (Lamas et al., 1988; Bochner et al., 1991a; Kyan Aung et al., 1991b; Ebisawa et al., 1992) and/or to the release of other chemoattractants such as PAF and IL-8 (Sanz et al., 1995).

B. Interleukin-2

The IL-2 receptor is composed of three polypeptide chains; an α chain (p55, CD25), a β chain (p 75, CD122), and a γ chain (γ_c) that is common to several cytokine receptors. IL-2 binds to the α and β chains with low affinity but does not interact with γ_c. However, a high-affinity IL-2:receptor complex is achieved when the ligand ligates the $\alpha\beta\gamma_c$ heterotrimer; interactions of intermediate affinity also can occur with $\alpha\gamma_c$ and $\beta\gamma_c$ heterodimeric forms of the receptor. In T lymphocytes, the β chain of the IL-2 receptor is essential for activation and features critical sequences within its intracellular domain necessary for effective signaling (Hatakeyama et al., 1989). The same appears to be true for γ_c (Zurawski and Zurawski, 1992) whereas the α chain alone cannot transduce the IL-2 signal.

In 1991, Rand et al. (1991a) reported that IL-2 was chemotactic for eosinophils obtained from both normal and hypereosinophilic individuals, which implied that cognate receptors for this cytokine were expressed. The demonstration that eosinophils were exquisitely sensitive to IL-2 (EC_{50} = 1 pM) and that chemotaxis was blocked by antibodies against p55 and p75 led to the proposal that IL-2 mediates its effect via the $\alpha\beta\gamma_c$ het-

erotrimer (Rand et al., 1991a). The expression of p55 is increased on eosinophils taken from hypereosinophilic individuals and after exposure (24–48 h) of normal eosinophils to supernatant obtained from U937 cells. GM-CSF, IL-3, and IL-16 (lymphocyte chemotactic factor) act similarly, albeit to a lesser extent (Riedel et al., 1990; Rand et al., 1991a), implying that the IL-2 receptor may represent an activation marker. The IL-2 receptor is induced on eosinophils taken from rats with experimental adjuvant arthritis (Meacock et al., 1991).

In vivo, infusion of IL-2 into the systemic circulation, as part of cancer chemotherapy, results in eosinophilia and an attendant increase in colony-stimulating activity (Macdonald et al., 1990; Sedgwick et al., 1990a). This effect is indirect and is due to the release of IL-3, IL-5, or GM-CSF. In Brown Norway rats, IL-2 promotes pulmonary eosinophilia with localization around the airways (Renzi et al., 1992).

C. Interleukin-4

The IL-4 receptor is composed of at least two chains, a 140-kDa α chain (CD124) to which IL-4 binds with high affinity and is responsible for signal transduction and γ_c, found also in the IL-2 receptor, which acts as an amplifier (Russell et al., 1993; Kondo et al., 1993). In vivo studies have implicated IL-4 in selective eosinophil recruitment, although that response may be secondary to the activation of the endothelium (see *XII.A.2*). In human and murine eosinophils, IL-4 exerts a number of actions upon eosinophils (Table 16) by mechanisms that may involve the activation of PtdIns 3-kinase and PKB (Coffer et al., 1998).

D. Interleukin-10

No evidence is available that eosinophils express receptors for IL-10. However, administration of IL-10 to sensitized Brown Norway rats attenuates the LPR and coincident eosinophilia following allergen provocation (Woolley et al., 1994b). Similarly, intranasal IL-10 significantly suppresses eosinophil recruitment into the lungs and peritoneum of immunized mice in response to ovalbumin (Zuany-Amorim et al., 1995, 1996). These data have lead to the suggestion that IL-10 may have utility in the treatment of eosinophil-dependent inflammatory diseases such as asthma (Pretolani and Goldman, 1997) although clinical studies have yet to be performed.

E. Interleukin-12

Interleukin-12 is a cytokine that promotes Th1-driven cell immunity while suppressing Th2-mediated responses. Although the direct effect of IL-12 on eosinophil function is not established, this cytokine has a marked impact on allergen- and parasite-induced eosinophil recruitment in vivo. Generally, antigen-induced airway eosinophilia in sensitized mice is suppressed by IL-12 (Gavett et al., 1995; Kips et al., 1995; Wynn et al., 1995; Iwamoto et al., 1996; Sur et al., 1996; Pauwels et al., 1997) through its ability to enhance the secretion of IFNγ, which subsequently suppresses the secretion of IL-5. Thus, Th2-cells are believed to represent a primary, albeit indirect, target for IL-12. Furthermore, in IFNγ-treated knockout mice, IL-12 is proinflammatory (Wynn et al., 1995). However, Pearlman et al. (1997) have reported that the administration of IL-12 to mice infected with *Onchocerca volvulus*, which promotes onchocercal keratitis, enhanced the pathology and the associated eosinophil recruitment into the corneal stroma by augmenting the elaboration of eosinophil chemokines even though the level of IFNγ was also elevated (Pearlman et al., 1997). Thus, it would appear that the ultimate effect of IL-12 in inflammation is more complex and not simply due to changes in the relative expression of IFNγ and IL-5.

F. Interleukin-13

A recent report has documented the ability of IL-13 to increase eosinophil survival by promoting the synthesis and/or release of IL-3 and GM-CSF in a sufficient concentration to act in an autocrine manner (Luttmann et

TABLE 16
Functional effects evoked by interleukin-4 in eosinophils

Species	Functional Effect	Reference
Human	Induces chemotaxis in peripheral blood of individuals with atopic dermatitis but not normal subjects	Dubois et al. (1994) Dubois and Bruijnzeel (1994)
Human	Inhibits IL-3 and IL-5 induced differentiation of cord blood mononuclear cells	Ochiai et al. (1995)
Human	Down-regulation of FcεRI and up-regulation of FcεRII expression during differentiation of eosinophils from cord blood precursors	Capron et al. (1997)
Human	Stimulates FcεRI mRNA expression in individuals with allergic rhinitis	Terada et al. (1995)
Human	Stimulates HLA-DR expression	Weller et al. (1993)
Human	Up-regulates TGFβ_1 and down-regulates TGFα mRNA and protein	Elovic et al. (1998)
Human	Promotes expression of p35 and p40 IL-12 mRNAs and biologically active protein	Grewe et al. (1998)
Human	Promotes apoptosis and overcomes survival-enhancing effect of IL-3, IL-5, and GM-CSF	Wedi et al. (1998)
Mouse	Inhibits constitutive mRNA but not protein expression of FcγRII and FcγRIII	de Andres et al. (1994)
Mouse	Increases FcγRII binding but not expression in BAL cells obtained mice with *Toxoccara cais*-infected lungs	Jones et al. (1994)
Mouse	Inhibits β-glucuronidase and arylsulfase release stimulated by IgG- but not IgE-coated beads as well as antibody-dependent killing of *S. mansoni* through down-regulation of IgG FcR but not IgE FcR	Baskar et al. (1990)
Mouse	Stimulates class II MHC expression	Mawhorter et al. (1993)

al., 1996). IL-13 also is chemotactic for human eosinophils (Horie et al., 1997b) and induces CD69, a putative marker of activated eosinophils, at the mRNA and protein level (Luttmann et al., 1996). These effects of IL-13 are mediated via the IL-4 receptor.

G. Transforming Growth Factor β

Three receptors for TGFβ have been identified in humans. Two of these (the type I and type II receptors) are bound by TGFβ with high affinity while the third, or type III receptor, is recognized by $TGF\beta_1$ with relatively low affinity (Wang et al., 1991; Massague, 1992). The type I and II receptors have intrinsic serine/threonine kinase activity, are related to the activin receptor, and are believed to aggregate when transducing the signal conferred by TGFβ. In contrast, the type III receptor, which includes β-glycan and endoglin in its structure, does not signal but may concentrate TGFβ molecules on the cell surface and present them to the other TGFβ receptor subtypes.

The complement of TGFβ receptors expressed by eosinophils is unknown although the functional effects of $TGF\beta_1$ have been studied to some extent. In general, $TGF\beta_1$ is inhibitory: it suppresses IL-5-induced degranulation (Alam et al., 1994; Atsuta et al., 1995), abrogates IL-3-, IL-5-, GM-CSF-, and IFN-γ-mediated survival through the induction of apoptosis (Alam et al., 1994; Atsuta et al., 1995; Luttmann et al., 1998a), attenuates the secretion of GM-CSF and IL-5 by IL-3 and GM-CSF, respectively (Alam et al., 1994), and inhibits IL-3-dependent differentiation of human eosinophils in a bone marrow suspension system (Sillaber et al., 1992). However, low concentrations of TGFβ induce chemotaxis (Luttmann et al., 1998a), indicating that it also has the ability to activate eosinophils which might reflect expression of multiple receptors for TGFβ. At the biochemical level, $TGF\beta_1$ prevents the activation of *lyn*, Jak-2, and ERK-2 by IL-5 as well as the phosphorylation of STAT-1 (Pazdrak et al., 1995c) although the extent to which these effects relate to the aforementioned functional responses remain largely unexplored.

H. Platelet-Derived Growth Factor

PDGF is composed of two chains (A and B) that can dimerize so that three possible conformations can be secreted (AA AB and BB). Receptors for PDGF are members of the subclass III of receptor tyrosine kinases which also includes the receptors for c-*kit* and CSF-1 (c-*fms*; Yarden et al., 1986; Heldin, 1992). PDGFs exert their effects through at least two subtypes of the PDGF receptor denoted α and β. Binding of dimeric PDGF promotes receptor dimerization with three possible configurations (αα, αβ, and ββ). Although the PDGF receptor subtype(s) on eosinophils is not known, PDGF-BB, which binds all three receptor isoforms, elicits a number of responses including the release of EPO and EDN (Bach et al., 1992) and, at higher concentrations, the generation of superoxide, which is primed by prior exposure of eosinophils to phorbol esters (Bach et al., 1991, 1992).

I. Stem Cell Factor

Stem cell factor is expressed either as a soluble or membrane-bound form and is the endogenous ligand for *c-kit* a receptor with intrinsic protein kinase activity (Ogawa et al., 1991). Recent cytofluorographical analyses have identified *c-kit* on peripheral blood eosinophils from both nonatopic and atopic individuals (Yuan et al., 1997). Although little is known of the role of SCF, it has been shown to augment GM-CSF- and IL-3-induced eosinophil colony formation (Ichihara et al., 1994), encourage the proliferation of eosinophils precursors in the presence of IL-3, IL-5, and GM-CSF (Kobayashi, 1993), and increase VLA-4 avidity and the subsequent binding to fibronectin and VCAM-1 (Yuan et al., 1997). In a murine model of allergic airway inflammation, Lukacs et al. (1996b) discovered that the concentration of SCF in the lungs and serum were markedly increased following allergen challenge with attendant eosinophilia. Of significance was the additional observation that the inflammation was prevented in mice given a neutralizing antibody against SCF, suggesting that it acts as a direct or indirect eosinophil chemoattractant (Lukacs et al., 1996b).

J. CD4

The expression of CD4 upon eosinophils obtained from both normal and eosinophilic individuals was first reported by Lucey et al. (1989a). Immunoprecipitation of a 55-kDa polypeptide using two anti-CD4 antibodies subsequently led to the identification of gp120, a ligand for human CD4 (Lucey et al., 1989a). Since that original report other experiments have established that the CD4 ligands, gp120, OKT4 (a CD4 binding antibody), and LCF [a potent chemotactic factor with activity in the low picomolar range (Rand et al., 1991b), subsequently identified as IL-16—see *XII.D.9*] stimulate eosinophil migration. The expression of CD4 on human eosinophils is induced by TNFα, IL-3, and GM-CSF (Riedel et al., 1990; Hossain et al., 1996).

K. CD9

The CD9 antigen is a 24-kDa cell surface glycoprotein that belongs to the transmembrane 4 superfamily that is characterized by four hydrophobic transmembrane-spanning domains (Wright and Tomlinson, 1994). The role of CD9 in eosinophils is unknown although immobilization of an anti-CD9 monoclonal antibody, ALB6, to tissue culture plates induces degranulation (Kim et al., 1997). That response was inhibited by an antibody against CD18, suggesting a role for β_2 integrins. In addition, a number of anti-CD9 clones (ALB6/FMC56/ML13) have been shown to enhance cell survival by stimulating the production and release of GM-CSF (Kim

et al., 1997). Fernvik et al. (1995) have identified an intracellular pool of CD9 in human eosinophils that can be mobilized in response to PMA. Those investigators also demonstrated increased CD9 expression on circulating eosinophils of patients with allergic rhinitis before and during the pollen season (Fernvik et al., 1996).

L. CD44

The designation CD44 encompasses several closely related type I transmembrane proteins that share amino- and carboxyl-terminal sequences but differ in their central extracellular domain. Perhaps the single most important property of CD44 is its ability to bind hyaluronic acid that exerts a number of effects including homotypic cell aggregation, the binding of T lymphocytes to bone marrow stromal cells (Borland et al., 1998), and in cell trafficking where it mediates rolling and firm adhesion to matrix constituents (Miyake et al., 1990; Degrendele et al., 1996). Although CD44 ligands other than hyaluronic acid have been identified, there is little to indicate a significant role for any of them. In 1998, Matsumoto et al. (1998a) detected cell surface CD44 on human eosinophils by flow cytometry with the monoclonal antibody J173 and established that expression was increased (~ 2-fold), in a time- and concentration-dependent manner, after culture of cells with IL-3, IL-5, and GM-CSF. Significantly, the authors of that study also noted that expression of CD44 was higher (3- to 8-fold) in hypodense eosinophils purified from eosinophilic donors and proposed that CD44 might, therefore, represent a useful cell surface marker of activation. A perplexing finding is that normodense, hypodense, and cytokine-treated eosinophils do not bind hyaluronic acid (Matsumoto et al., 1998a). However, it is likely that this anomaly relates to the level of glycosylation which tends to impede CD44-hyaluronate interactions (Katoh et al., 1995; Bartolazzi et al., 1996). The function of CD44 on human eosinophils is unknown.

M. CD52

CD52 is a GPI-linked membrane protein that is expressed constitutively at the protein and mRNA level in eosinophils but not neutrophils (Elsner et al., 1996c). There is a paucity of information on the functions CD52 mediates. Receptor cross-linking results in the inhibition of C5a-, PAF-, and GM-CSF-induced oxygen radical production which has led to the suggestion that this could be used to selectively down-regulate eosinophil activity during inflammation (Elsner et al., 1996c). The phorbol ester PMA can down-regulate CD52 expression, but this effect is not mimicked by IL-3, IL-5, GM-CSF, IFNγ, C5a, RANTES, or PAF (Elsner et al., 1996c).

N. Complement Receptors Not Coupled Through G Proteins

In addition to receptors for C3a and C5a, eosinophils also express receptors for a number of other complement fragments that signal by G protein-independent mechanisms. Henson (1969) first demonstrated that guinea pig eosinophils could form rosettes with C3-coated erythrocytes, a finding that was extended to human eosinophils 7 years later (Tai and Spry, 1976). A number of investigations subsequently established that eosinophils from several species, including human, express CR1 (CD35), CR3 (CD11b/CD18), CR4 (CD11c/CD103; p150,95), and receptors for C1q (Fearon, 1985; Fischer et al., 1986; Hamada and Greene, 1987; Hartnell et al., 1990, 1992a) but not CR2 (Spry and Tai, 1976).

1. CR1. CR1 was originally purified as a 205-kDa single-chain polypeptide from human erythrocyte membranes and subsequently found to be expressed as several allotypes—A (190 kDa), B (220 kDa), C (160 kDa), and D (250 kDa)—that are encoded by a single gene (Fearon, 1979; Gerdes et al., 1982; Dykman et al., 1983a, b; Hogg et al., 1984; Ahearn and Fearon, 1989). In all cases, CR1 is recognized by the complement fragments C3b, C4b, and iC3b in increasing order of affinity (Ross et al., 1983; Gordon et al., 1987) as well as C1q (see below) (Klickstein et al., 1997). Low levels of CR1 generally are expressed by human eosinophils (Hartnell et al., 1992a) although the density is increased in response to certain stimuli including LTB_4, 5-HETE, and 5-HPETE (Nagy et al., 1982; Fischer et al., 1986). Nothing is known of the cell signaling pathways activated following ligation of CR1 on eosinophils. However, exposure of human neutrophils to monomeric C3b increases CR1 expression (Porteu et al., 1987), and cross-linking of anti-CR1 antibodies bound to cell surface CR1 induces Ca^{2+} mobilization and the activation of PLD (Fallman et al., 1993). Significantly, these effects are enhanced when anti-CR1 antibodies are bound to a particle (Fallman et al., 1993), indicating that CR1 is involved in neutrophil activation.

Functionally, CR1 mediates several effects including all of those described for the cC1q receptor (see below). In addition, fMLP (Kay et al., 1979) and high concentrations of histamine (Anwar and Kay 1977, 1980) significantly increase the percentage of human eosinophils that form rosettes with C3b-coated ovine erythrocytes.

2. CR3. CR3 was originally identified as an iC3b receptor by rosette formation of iC3b-coated erythrocytes with neutrophils and other leukocytes (Ross and Lambris, 1982). Shortly after that observation, CR3 was found to be identical with Mac-1 and an integrin (Beller et al., 1982). Of the varied complement fragments that can be generated, iC3b is the preferred ligand of CR3 although C3b and C3d will bind, albeit with relatively lower affinity (Ross et al., 1983; Gordon et al., 1987). The basic structure of the integrins is described in *IX.B*. The α_M (CD11b) and β_2 (CD18) subunits of CR3 are approximately 170 kDa and 95 kDa, respectively (Sanchez Madrid et al., 1983). The β_2 subunit is an integral transmembrane glycoprotein composed of three distinct regions: a short, 46-amino acid carboxyl-terminal cyto-

plasmic domain, a membrane-spanning region, and an extracellular component that features conserved residues required for ligand binding and association with the α subunit (Kishimoto et al., 1987; Wardlaw et al., 1990). The topology of α_M is similar to the β_2 subunit, and the extracellular domain contains several putative binding sites for a variety of ligands. In particular, a so-called "I" (inserted) region is present that has a unique sequence that binds divalent cations and the complement fragment iC3b (Diamond et al., 1993; Michishita et al., 1993).

In addition to the ability of a diverse range of agents to activate CR3 on eosinophils and to increase its expression, ligands such as iC3b, ICAM-1, or fibrinogen can activate eosinophils, resulting in homotypic aggregation (Koenderman et al., 1991), degranulation, and superoxide anion generation (Metcalfe et al., 1977; Zeiger and Colten, 1977). In many instances, adhesion is prerequisite for those functional effects to be manifest (Horie and Kita, 1994). The biochemical pathways involved in activating eosinophils have not been delineated. However, in human neutrophils cross-linking of either CD18 or CD11b by antibodies evokes a PTX-insensitive Ca^{2+} transient (Sengelov, 1995). PLD is similarly activated in response to iC3b-opsonized particles, and the *src*-related tyrosine kinase $p58^{fgr}$ and the cytoskeletal protein paxillin are phosphorylated when neutrophils adhere to fibronectin in the presence of iC3b (Sengelov, 1995).

3. CR4. CR4 shares the same β subunit as CR3 but has a novel 150-kDa α chain, α_X (also known as p150,95 and CD11c), which is 63% homologous to α_L (Corbi et al., 1987). Significantly, the extracellular domain of α_X also features a unique I domain similar to that found in α_L that specifically binds ICAM-1, iC3b, and, to a lesser extent, C3b (Ross et al., 1983; Corbi et al., 1987; Gordon et al., 1987). The endogenous cellular ligands for CR4 on eosinophils are currently unknown and the function this receptor subserves is obscure. In neutrophils, it has been suggested that CR4 may have a role as an "assisting" adhesion protein (Anderson et al., 1986), although whether this occurs with eosinophils is unexplored. In macrophages, CR4 is immobile in the plane of the plasma membrane, and it has been proposed that it is linked to the cytoskeleton and is responsible for the phagocytosis of iC3b-coated particles (Ross et al., 1992; Ross, 1994).

4. The C1q Receptor. The C1 proteolytic complex is the first molecule in the so-called classical complement pathway and is activated after an interaction with a microbe-bound antibody. C1 is composed of six C1q subunits which collectively take the form of a bouquet of flowers with a central collagen-like trunk branching into six globular peptide chains that feature an antibody-binding site. C1q is associated with two other subunits, C1r and C1n, stabilized by Ca^{2+} in a trimolecular complex. Receptors for C1q on human eosinophils were initially detected by Hamada and Greene (1987), using ^{125}I-labeled C1q, at a density (1.9×10^7/cell) approximately twice that of autologous neutrophils. Differential modulation of C1q-mediated functional activities by monoclonal antibodies across cell types and the subsequent finding that ligation of the C1q receptor can occur through both the collagen-like and globular components of the molecule led to the proposal of C1q receptor heterogeneity (Tenner, 1993). A 60-kDa receptor (cC1qR), shown in 1993 to be calreticulin (Malhotra et al., 1993), recognizes the collagen-like amino terminus of C1q and is constitutively expressed on eosinophils (Kuna et al., 1996). In addition, a novel 33-kDa receptor (gC1qR) has been identified on eosinophils through which C1q interacts at the globular carboxyl terminus of the protein (Peerschke et al., 1994; Kuna et al., 1996). However, experimental evidence supports the idea that gC1qR is not membrane-bound, but rather a secreted soluble protein (van den Berg et al., 1997). It is possible that low levels may be expressed on the surface of certain cells complexed with other fluid-phase molecules (van den Berg et al., 1997). A third, 126-kDa, receptor for C1q, C1qRp, also has been identified that modulates monocyte phagocytosis (Guan et al., 1991, 1994; Nepomuceno et al., 1997). Whether this form is expressed by eosinophils is unclear. In 1997 it was reported that ^{125}I-labeled C1q bound specifically to human CR1 transfected into K562 cells (Klickstein et al., 1997), suggesting that if this translates to normal cells, a common receptor is recognized by all three complement opsonins: C1q, C3b, and C4b. The observation that collagen completely inhibited ^{125}I-labeled C1q binding to K562 cells strongly suggests that CR1 represents the previously defined cC1qR (see above). The nature and identity of the gC1qR is ill-defined.

The functional effects that are mediated by cC1qR are little studied. Work by Hamada and Greene (1987) demonstrated that, in the presence of a small amount of IgG antibody, C1q enhances the cytotoxic capacity of eosinophils against schistosomula. More recently it was shown that ligation of the human C1qR induces eosinophil migration (Kuna et al., 1996) and promotes superoxide anion generation (Tenner, 1993) with cC1q being the most potent opsonin. Collectively, these findings support the original suggestion that the C1qR might play a role in the effector functions of eosinophils (Hamada and Greene, 1987).

O. Melittin

Several naturally occurring peptides have been implicated in the activation of proinflammatory cells. One of the most thoroughly investigated of these is melittin, an abundant component of the venom of honeybees. Melittin is an amphiphilic 26-amino acid peptide that is reported to stimulate, at noncytotoxic concentrations and by a nonreceptor-mediated mechanism, the exocytosis of EPO from the specific granules and the generation of TX (Kroegel et al., 1990b). The latter observation is consis-

tent with the effect of melittin on human neutrophils and rat mast cells (Kroegel et al., 1981) and seemingly results following a direct intracellular action at the level of PLA_2 or a closely associated protein (Kroegel et al., 1981).

P. Secretory Component

Secretory component is an intrinsic protein localized to the basolateral surface of secretory epithelial cells, and is believed to mediate the *trans*-epithelial transport of polymeric immunoglobulins, in particular IgA (Brandtzaeg, 1981). During that process, the polymeric Ig receptor is cleaved and SC binds to IgA, forming secretory IgA with the excess SC being released into secretions (Mostov et al., 1980).

Secretory IgA is a very effective degranulation-promoting stimulus in human eosinophils compared to serum IgA (Abu Ghazaleh et al., 1989). Lamkhioued et al. (1995a) hypothesized that this discrepancy might simply be due to the association of SC with IgA that follows *trans*-epithelial transport and the subsequent binding of SC to a specific receptor expressed by eosinophils distinct from FcαR. Indeed, saturable, high-affinity (K_d = 30 nM), low-capacity (B_{max} = 1100/cell) binding sites (receptors ?) for ^{125}I-labeled human SC have been identified on human eosinophils (see Lamkhioued et al., 1995a; Motegi and Kita, 1998). Moreover, it was shown by flow cytometry that purified SC bound to a subpopulation (4–59%) of blood eosinophils purified from 19 patients with eosinophilia in a manner that was prevented by unlabeled SC and secretory IgA, but not by serum IgA or IgG (Lamkhioued et al., 1995a). Significantly, exposure of the same eosinophils to free SC and secretory IgA, in the presence of a cross-linking agent, resulted in degranulation whereas serum IgA was ineffective. Similar results have been reported for normal eosinophils where SC enhances cytokine- and IgG-induced superoxide generation (Motegi and Kita, 1998). Preliminary data indicate that the receptor for SC is a 15-kDa GPI-anchored protein that might be a lectin (Lamkhioued et al., 1995a). Collectively, these data provide an explanation for the preferential activation of human eosinophils by secretory IgA over serum IgA, and imply that FcαR and/or the 15-kDa receptor for SC could mediate IgA-driven immune responses.

Q. Human Leukocyte Antigen

In addition to their ability to synthesize and release a plethora of proinflammatory mediators, eosinophils have the capacity to act as APCs, which identifies a new role of eosinophils in regulating the immune response. As eosinophils mature, they lose Ia antigen (Koeffler et al., 1980) and, accordingly, express little if any major major histocompatibility complex (MHC) class II antigens (Lucey et al., 1989b; Weller et al., 1993; Magyar et al., 1995; Tamura et al., 1996) which is prerequisite for antigen presentation to $CD4^+$ T lymphocytes. However, in the presence of certain cytokines (GM-CSF, IL-4, IL-5, IFNγ) and in disease states such as chronic eosinophilic pneumonia (Beninati et al., 1993) and asthma (Hansel et al., 1991a,b, 1992; Sedgwick et al., 1992b), human leukocyte antigen (HLA) DR^+ eosinophils have been detected (Lucey et al., 1989b; Walsh et al., 1990a; Hansel et al., 1991a, 1992) that are capable of acting as APCs (Lucey et al., 1989b; del Pozo et al., 1992; Hansel et al., 1989, 1992; Weller et al., 1993; Mawhorter et al., 1994) by a mechanism that, in mice (Tamura et al., 1996) and possibly humans (Bosse et al., 1998), is dependent on CD80 (B7-1) and CD86 (B7-2). Furthermore, Hansel et al. (1992) demonstrated that eosinophil-mediated, antigen-specific proliferation of an autologous HLA-DR-restricted clone was prevented by a monoclonal antibody against HLA-DR. Identical results also have been obtained with murine eosinophils which have been shown to present antigens derived from the parasite *Mesocestoides corti* to specific T cell clones with an associated increase in IL-2 generation and consequent proliferation (del Pozo et al., 1992). In an independent study, the ability of GM-CSF-treated, human peripheral blood eosinophils (in which the expression of ICAM-1 is increased) to bind human RV16 and to present antigen to RV16-specific T cells was documented (Handzel et al., 1998). Those observations were associated with attendant T cell proliferation and the elaboration of IFNγ (Handzel et al., 1998) which provide an explanation for the exacerbations of asthma symptoms that sometimes occur after viral infections. As described in *XI.J*, human eosinophils also express CD4 which is up-regulated by IL-3 and GM-CSF and enhances eosinophil migration when bound by IL-16 for which it is a receptor (Rand et al., 1991b). This functional property, along with the possibility that $CD4^+$ eosinophils could interact with $HLA\text{-}DR^+$ cells, including other eosinophils, suggests potentially complex cell-cell interactions during antigen presentation.

XII. Functional Consequences of Eosinophil Activation

A. Locomotion

The selective migration of circulating eosinophils across the endothelium and into tissue occurs sequentially in a number of characteristic, well defined steps (see Teixeira et al., 1995b; Wardlaw et al., 1995; Knol and Roos, 1996). These include the 1) reversible binding of the eosinophil to activated endothelial cells and subsequent "rolling" of the eosinophil along the lumenal surface of the vessel; 2) firm "adhesion" of the eosinophil to the endothelium; and 3) "transmigration" of the adherent eosinophil through the endothelium into tissues. The mechanisms underlying these processes have aided our understanding of the selective recruitment of eosinophil to sites of chronic inflammation in response to allergens and parasite infestation. Thus, eosinophils,

unlike neutrophils, express VLA-4. Moreover, CC chemokines, C3a, and LTD_4 are selective eosinophil chemoattractants.

1. Rolling. Eosinophil-endothelial cell interactions are enhanced after the release of inflammatory mediators from tissue and/or resident cells. Like neutrophils, the so-called "rolling" step is thought to be predominately mediated by selectins expressed on eosinophils (L-selectin) and endothelial cells (E- and P-selectin) that bind to their respective counterligands (see Fig. 6). Indeed, evidence that all three selectins contribute to primary tethering or rolling has been provided.

A role for L-selectin in rolling was first suggested from studies performed under nonstatic conditions which showed that the adherence of eosinophils to IL-1β-activated HUVECs was inhibited by blocking antibodies against L-selectin (Knol et al., 1994). Furthermore, under conditions where L-selectin is shed, the ability of eosinophils to bind is reduced (Knol et al., 1994). An examination of the effect of a range of neutralizing antibodies confirmed a role for eosinophil L-selectin, but not CD18, in the mechanism of rolling along activated (IL-1) venule endothelial cells of the rabbit mesentery. That report also suggested a role for VLA-4 through binding to an unidentified ligand expressed by the endothelium (Sriramarao et al., 1994). However, a recent study examining eosinophil rolling under flow conditions showed that L-selectin was not required for primary tethering but, instead, contributed to homotypic (cell-cell) aggregation and secondary binding in collaboration with PSGL-1 (Kitayama et al., 1997). Evidence in vitro for a role for L-selectin in C5a-induced homotypic aggregation has been demonstrated with guinea pig eosinophils (Teixeira et al., 1996b).

Although cytokine-activated endothelial cells can bind eosinophils via E-selectin (Bochner et al., 1991a; Kyan Aung et al., 1991a; Weller et al., 1991b), recent studies, using specific blocking antibodies, have excluded E-selectin in eosinophil rolling along rabbit-activated mesenteric venules (Sriramarao et al., 1996). Furthermore, eosinophils, unlike neutrophils, do not "roll" on monolayers of E-selectin-transfected cells (Sriramarao et al., 1996) or E-selectin-coated surfaces under flow conditions (Kitayama et al., 1997), possibly because of relatively low expression of an E-selectin counterligand(s) (Bochner et al., 1994). In contrast, a role for P-selectin is suggested following the demonstration of eosinophil rollingon P-selectin-coated surfaces (Symon et al., 1996; Kitayama et al., 1997) that was mediated by the binding of P-selectin to the amino-terminus of PSGL-1 (Patel and McEver, 1997). That conclusion is supported from studies of eosinophil binding to eosinophil-rich nasal polyp endothelial cells (Symon et al., 1994), (that express E-selectin, P-selectin, and ICAM-1), which was almost completely inhibited by monoclonal antibodies raised against P-selectin. In addition, binding was partially blocked by a monoclonal antibody against eosinophil CR3 but was unaffected by neutralizing E-selectin, L-selectin, ICAM-1, VCAM-1, VLA-4, and LFA-1 (Symon et al., 1994). A central role for endothelial cell P-selectin in eosinophil rolling is supported by a number of recent reports. Thus, in vivo studies using a mouse model of LPS-induced pleurisy established that eosinophil infiltration into the pleural cavity at 24 h was inhibited by monoclonal antibodies to L-selectin (97%) and P-selectin (54%) but not E-selectin (Henriques et al., 1996). In contrast, although the more rapid influx of neutrophils at 4 and 24 h was inhibited by anti-L-selectin, that response was unaffected by antibodies directed against either P- and E-selectins unless they were used in combination (Henriques et al., 1996). Eosinophil rolling and adhesion studied by intravital microscopy in P-selectin-deficient mice and after the induction of eosinophilic peritonitis was reduced by 75% when compared to that of wild-type animals (Broide et al., 1998a). Similarly, the number of eosinophils recruited into the lung and BAL fluid of allergen-challenged, P-selectin-deficient mice was greatly attenuated when compared to that of genetically naïve animals (Broide et al., 1998b).

2. Adhesion. The rolling of eosinophils along activated endothelial cells is thought to facilitate their subsequent adherence. Using blocking antibodies, it has been reproducibly shown that firm binding of eosinophils to TNFα-, IL-1β-, and LPS-activated HUVECs is mediated by CR3/ICAM-1 and VLA-4/VCAM-1 interactions (Kimani et al., 1988; Lamas et al., 1988; Walsh et al., 1990b, 1991a; Bochner et al., 1991a; Dobrina et al., 1991; Kyan Aung et al., 1991a,b; Weller et al., 1991b). The observation that VLA-4 is found only on the cell surface of a restricted number of leukocytes, including eosinophils, monocytes, basophils, and T lymphocytes, has led to the suggestion that the expression of VCAM-1 on endothelia can facilitate the selective recruitment of these cells. Of potential importance is the knowledge that IL-4 (Schleimer et al., 1992) and IL-13 (Sironi et al., 1994; Bochner et al., 1995) selectively up-regulate the expression of VCAM-1 on endothelial cells. Since the level of these two cytokines is elevated in asthma they may, indirectly, govern eosinophil trafficking into the lung. Endothelial cell-derived chemokines that act through CCR3 also are able to promote firm adhesion of eosinophils to TNFα- and IFNγ-treated HUVECs by α_4 and β_2 integrins. This is seen even under conditions of shear flow where transiently tethered eosinophils become arrested (Kitayama et al., 1998).

Similar mechanisms of adhesion occur in vivo although the relative contribution of CCR3 and VLA-4 to eosinophil migration appears to be variable and is probably related to species, stimuli, and differences in specificity of blocking antibodies. Using ovalbumin-sensitized mice, Nakajima et al. (1994) documented that allergen challenge resulted in an increased expression of VCAM-1 by endothelial cells and coincident pulmonary eosinophilia. Moreover, by using neutralizing antibod-

ies, that effect was shown to be due to an interaction between VLA-4 on the eosinophil and endothelial cell VCAM-1 (Nakajima et al., 1994). More contemporary experiments also have implicated ICAM-1 in eosinophil recruitment. An example is provided by Chin et al. (1998) who found that anti-ICAM-1 attenuated by >70% the accumulation of eosinophils into the bronchial lumen of allergen-challenged sensitized mice. That finding was subsequently confirmed in ICAM-1 knockout animals (Broide et al., 1998b). However, a study using P-selectin/ICAM-1 double-mutant mice found that the recruitment of eosinophils into the peritoneum was inhibited by only 62%, whereas the simultaneous administration of anti-VCAM abolished the eosinophilia (Broide et al., 1998a). Thus, P-selectin, VCAM-1, and ICAM-1 all appear to be important for the adhesion and/or subsequent induction of peritoneal eosinophilia in vivo in the mouse.

In guinea pigs, cutaneous eosinophilia in response to a range of stimuli including LTB_4, PAF, and C5a *des arg* is inhibited by anti-CD18 neutralizing antibodies (Teixeira et al., 1994a; Macari et al., 1996). Similarly, airway hyperresponsiveness, the LPR, and the associated infiltration of eosinophils into the tracheal wall and nasal mucosa that follows allergen challenge of ovalbumin-sensitized guinea pigs or naïve animals given IL-5 is blunted by anti-VLA-4 antibodies (Terada et al., 1996; Sagara et al., 1997; Kraneveld et al., 1997). Thus, comparable mechanisms of eosinophil adherence apply in the skin of guinea pigs.

Human investigations have provided evidence that adhesion molecules might play a central role in pulmonary eosinophil recruitment. Several independent studies have established that ICAM-1 and HLA-DR are induced on eosinophils present in the BAL fluid and sputum of asthmatic subjects along with an up-regulation of CR3 and reduced expression of L-selectin (Hansel et al., 1991a; Kroegel et al., 1994a; Mengelers et al., 1994). In addition, bronchial biopsies of asthmatic subjects showed increased expression of CR3, LFA-1, and VLA-4 within the eosinophil-rich mucosa and submucosa, and increased expression of ICAM-1, VCAM-1, and E-selectin upon the luminal membrane of the endothelial cells (Ohkawara et al., 1995). Those data are consistent with an activated eosinophil phenotype and suggest that eosinophils could interact with T lymphocytes leading to immunomodulation and cell activation. Similar data have been generated experimentally in *Nippostrongylus brasiliensis*-infected mice where eosinophils in the BAL fluid show increased expression of ICAM-1, LFA-1, and VLA-4 (Watkins et al., 1996).

3. Transmigration and Chemotaxis. In vitro, a number of stimuli have been identified that are potent and effective eosinophil chemotaxins and the most notable of these are PAF, LTD_4, C5a, IL-2, and RANTES (see Table 17). However, of particular interest has been the demonstration that the CC chemokines eotaxin and eotaxin-2 are selective eosinophil chemoattractants although their potency is less than the aforementioned stimuli (Forssmann et al., 1997). In the presence of IL-5 (or IL-3/GM-CSF), eotaxin and PAF promote the migration of eosinophils across Matrigel membranes (to simulate the basement membrane) by a mechanism inhibited by antibodies against CD29 and CD18, implicating β_1 and β_2 integrin adhesion molecules, respectively (Okada et al., 1997). Migration is not seen in the absence of hematopoietic cytokines which has led to the hypothesis that directional migration of eosinophils into tissue requires both a specific chemoattractant, such as eotaxin, and an activating cytokine that also enhances eosinopoeisis, such as IL-5 (see below). Other studies have found that the migration of primed eosinophils across an intestinal epithelial cell line, T84, in response to PAF is dependent on VLA-4, CD11b, and ICAM-1 (Resnick et al., 1995) while PAF-induced migration across IL-1-activated endothelium is inhibited by anti-VLA-4 (Ebisawa et al., 1994).

Detailed investigations of the mechanism(s) of eosinophil accumulation following allergen challenge of ovalbumin-sensitized guinea pigs have identified eotaxin as a major chemotactic factor that is present in the BAL fluid and lung (Jose et al., 1994a,b). Eotaxin also has been implicated in IL-4-, but not TNFα- or LTB_4-, induced eosinophil accumulation in rat skin by use of neutralizing antieotaxin antibodies (Sanz et al., 1998). A comparison of eotaxin release into the lung and BAL fluid with the extent of eosinophil accumulation in tissue revealed parallel increases during the first 6 h after allergen challenge. However, although eotaxin levels then declined, eosinophil numbers remained constant and BAL levels were increased. It has been speculated that eotaxin may be rapidly degraded in tissue but not in the BAL fluid, thereby creating a concentration gradient for the attraction of eosinophils into the airway lumen (Humbles et al., 1997). An additional activity attributed to eotaxin is a rapid and selective release of eosinophils and their progenitors from the bone marrow by a mechanism that is markedly enhanced by IL-5 (Palframan et al., 1998a). Thus, eotaxin may be involved both in the egress of eosinophils from the bone marrow as well as their subsequent recruitment into tissues.

Other indirect evidence that could support a role for eotaxin in eosinophil migration is provided from histological studies. Ishi et al. (1998) have reported that exposure of rats to ozone induces large increases in the expression of eotaxin mRNA transcripts and the recruitment of eosinophils into the BAL fluid. Comparable data also are available from human studies. Indeed, the number of cells expressing eotaxin mRNA transcripts is increased in the epithelial and subepithelial layers of individuals with chronic sinusitis (Minshall et al., 1997). A comparison of the levels of epithelial/endothelial cell-associated eotaxin and eosinophil CCR3 mRNA tran-

TABLE 17
Eosinophil chemoattractants

Stimuli	Potency	Reference(s)
Lipids		
PAF	High	Wardlaw et al. (1986); Sigal et al. (1987); Tamura et al. (1987); Czarnetzki and Csato (1989); Kurihara et al. (1989); Martins et al. (1989); Little and Casale (1991); Sun et al. (1991); Foster et al. (1992); Numao and Agrawal (1992); Fukuda et al. (1992); Miyagawa et al. (1992); Warringa et al. (1992b); Towney et al. (1994); Elsner et al. (1996a); Erger and Casale (1996); Schweizer et al. (1996)
LTB_4	Low (human)/ Medium (guinea pig)	Czarnetski and Mertensmeir (1985); Czarnetzki and Rosenbach (1986); Czarnetzki and Csato (1989); Coffier et al. (1991a); Maghni et al. (1991); Ng et al. (1991); Taylor et al. (1989, 1991); Sehmi et al. (1992a); Kim et al. (1994); Spada et al. (1994)
LTD_4	High	Spada et al. (1994)
ETEs, HETEs, and diHETEs	High	Morita et al. (1990a); Sehmi et al. (1991); Schwenk et al. (1992); Powell et al. (1995); Schwenk and Schroder (1995); O'Flaherty et al. (1996a); Czech et al. (1997)
LXA_4	Low	Soyombo et al. (1994)
Peptides		
fMLP	Low	Ogawa et al. (1981b); Morita et al. (1989b)
C5a	High	Kay et al. (1973); Klebanoff et al. (1977); Ogawa et al. (1981a); Fischer and Czarnetzki (1982); Morita et al. (1989b); Elsner et al. (1996a,b)
C3a	Medium	Daffern et al. (1995)
SP	High	Wiedermann et al. (1993)
Secretogranin	Medium	Dunzendorfer et al. (1998)
CGRP	?	Manley & Hayes (1989)
Cytokines		
IL-3	Low	Yamaguchi et al. (1988b); Coeffier et al. (1991b); Warringa et al. (1991); Sehmi et al. (1992b); Hakansson et al. (1994)
IL-2	High	Rand et al. (1991a)
IL-4	?	Dubois et al. (1994); Dubois and Bruijnzeel (1994)
IL-13	?	Horie et al. (1997b)
TGFβ	?	Luttmann et al. (1998a)
TNFα	Low	Nagata et al. (1993)
CC chemokines		
RANTES	High	Kameyoshi et al. (1992); Rot et al. (1992); Alam et al. (1993); Dahinden et al. (1994); Kameyoshi et al. (1994)
Eotaxin	Low	Jose et al. (1994a,b); Elsner et al. (1996b)
Eotaxin-2	Medium	Forssmann et al. (1997)
MIP-1α	Low	Rot et al. (1992); Dahinden et al. (1994)
MCP-2	Medium	Noso et al. (1994); Weber and Dahinden (1995)
MCP-3	High	Dahinden et al. (1994); Noso et al. (1994); Elsner et al. (1996b)
MCP-4	High	Garcia Zepeda et al. (1996a)
MCP-5	Low	Sarafi et al. (1997)
CXC chemokines		
IL-8	Low (primed cells)	Schweizer et al. (1994)
Lectins		
Ecalectin	High	Matsumoto et al. (1998b)

scripts demonstrated significantly enhanced expression in biopsies obtained from asthmatic patients when compared to normal individuals (Ying et al., 1997). Interestingly, there was a highly significant inverse correlation between eotaxin mRNA-positive cells and the PC_{20} to histamine (Ying et al., 1997).

In addition to eotaxin, a number of other chemoattractants have been implicated in eosinophil recruitment. Lukacs et al. (1996a) have shown that MIP-1α and RANTES are produced in murine lung following allergen challenge and that a homogenate of that lung tissue induces eosinophil chemotaxis ex vivo by a mechanism inhibited by neutralizing antibodies to those chemokines. Similarly, the chemotaxis induced by a lung homogenate prepared from *Toxocara canis*-infected rats was shown to be mediated by PAF, LTB_4, and IL-5 (Okada et al., 1996), whereas BAL fluid from asthmatic patients with birch pollen allergy evoked eosinophil chemotaxis that was inhibited by anti-RANTES and anti-IL-5 antibodies (Venge et al., 1996). Collectively, those findings are consistent with human studies where a significant influx of eosinophils, basophils, and mononuclear cells into the nasal mucosa has been reported after RANTES challenge that induces a clinically symptomatic response (Kuna et al., 1998).

In general, the most effective chemotaxins are those acting through G protein-linked receptors while the cytokines IL-3, IL-5, and GM-CSF seem predominately to be involved in eosinophil priming (see *VI.C*). This two-step model of eosinophil chemotaxis has been demonstrated in vivo. Injection of IL-5 into guinea pigs increases the circulating levels of eosinophils and, although inactive itself, primes the ability of LTB_4 and eotaxin to promote cutaneous eosinophilia (Collins et al., 1995). Similarly, intranasal and intradermal induction of eosinophilia by eotaxin is observed in IL-5 transgenic mice but not in wild-type animals (Rothenberg et al., 1996). Moreover, IL-5 alone is unable to evoke cutaneous eosinophilia in sensitized BALB/c mice but effectively primes for IL-1β-, IL-4-, TNFα-, RANTES-, and MIP-1α-induced recruitment (Satoh et al., 1997). Thus, the priming of circulating eosinophils in diseases such as atopic

dermatitis and allergic asthma may explain their increased sensitivity to a selective number of chemoattractants that act through G protein-coupled receptors (Morita et al., 1989a,b; Bruijnzeel et al., 1993a; Warringa et al., 1993b).

At present little is known of the biochemical pathways mediating eosinophil rolling, adhesion, and locomotion. Chemotaxis resulting from the activation of seven transmembrane-spanning receptors is associated with rapid increases in the $[Ca^{2+}]_i$ and actin polymerization. Whether these changes are essential to chemotaxis is equivocal; thus, Elsner et al. (1996a) have provided evidence that Ca^{2+} fluxes are required for chemotaxis and actin polymerization, whereas another study showed that depletion of Ca^{2+} failed to effect chemotaxis and actually enhanced actin polymerization (Schweizer et al., 1996). Recently, caged peptides have been used to probe the role of calcium-calmodulin and myosin light chain kinase in eosinophil motility (Walker et al., 1998). Flash photolysis of polarized eosinophils containing caged peptides against the aforementioned proteins with near UV light promptly blocked amoeboid locomotion from which it can be inferred that myosin is involved in this response.

B. Cytolysis, Secretion, and Piecemeal Degranulation

At least three discrete processes have been defined that result in the release of granule contents from eosinophils: secretion, piecemeal degranulation, and cytolysis (necrosis). Many stimuli have been identified that promote release by the former two mechanisms including opsonized particles, deuterium oxide, immunoglobulins, metazoan parasites, Sepharose beads, and various proinflammatory mediators and cytokines such as PAF, fMLP, and complement (Winqvist et al., 1984; Capron and Capron, 1987; Gorski et al., 1988; Kroegel et al., 1988, 1989c, 1990; Abu Ghazaleh et al., 1989; Carlson et al., 1991,1992; Kita et al., 1991a; Takafuji et al., 1994; Kaneko et al., 1995a; Munoz et al., 1995; Horie et al., 1996). In addition, although ignored for many years, a possible role for eosinophil cytolysis in the genesis of inflammatory lesions is being considered (Erjefalt et al., 1996, 1997a,b). In the succeeding sections, the main proteins stored within eosinophil granules and their characteristics are described, along with the current understanding of the morphological, biochemical, and electrophysiological basis of granule protein release.

1. Granule Proteins

a. MAJOR BASIC PROTEIN. MBP is a 13.8-kDa arginine- and cysteine-rich polypeptide composed of 117 amino acids that features alternating hydrophobic and hydrophilic sequences (Barker et al., 1988; Wasmoen et al., 1988). This protein was first isolated from guinea pig eosinophils and shown subsequently to have a very high tendency to form aggregates (Lewis et al., 1978). However, despite its prominence in eosinophils, it is also expressed in placental X cells and placental giant cells during pregnancy. As the name implies, MBP accounts for the majority (50%, approximately 250 pg/cell) of the granule protein found in guinea pig eosinophils and was so named for that fact (Gleich et al., 1973). However, in normal human eosinophils, the MBP content lies between 5 and 10 pg/cell (Ackerman et al., 1983; Gleich and Loegering, 1984; Peters et al., 1988) and is lower still in disease states associated with peripheral blood eosinophilia (Peters et al., 1988). The high number of arginine residues renders the protein so basic that its isoelectric point cannot be measured, although it has been calculated as 10.9 (Hamann et al., 1991). MBP has been purified and/or cloned from several species including humans (McGrogan et al., 1988; Barker et al., 1990; Hamann et al., 1991; Popken-Harris et al., 1994, 1995; Li et al., 1995), guinea pigs (Gleich et al., 1973, 1974; Aoki et al., 1991), rats (Nittoh et al., 1995; Watanabe et al., 1995), and mice (Larson et al., 1995; Denzler et al., 1997a), and characterized extensively. Although MBP is localized to the crystalloid core of the specific granules of most species (Egesten et al., 1986), an equivalent protein is present in the homogeneous granules of bovine and equine eosinophils (Archer, 1963; Duffus et al., 1980). The gene encoding human MBP is 3.3 kb, composed of nine upstream exons, five coding exons, and five introns, and is localized to chromosome 11 (Barker et al., 1990; Hamann et al., 1991; Li et al., 1995); the murine *MBP* gene maps to chromosome 2 (Denzler et al., 1997a). Detailed molecular biological studies suggest that MBP is translated as a more neutral preproprotein, to protect the cell from the toxic actions of the mature form, before it is taken up into the secondary granules and processed (Hamann et al., 1991; Popken-Harris et al., 1994). Indeed, evidence now exists that pro-MBP is converted into mature MBP within granules during the process of eosinophil differentiation (Popken-Harris et al., 1998).

Original studies identified a MBP gene promoter, P2, that generated a 1-kb transcript for prepro-MBP (Barker et al., 1988). However, in 1995, Li et al. (1995) demonstrated that the *MBP* gene is expressed from two upstream promoters: a distal promoter, P1, generating a 1.6-kb product, in addition to the previously described P2 promoter resulting in a smaller transcript. It has since been established that the long and short forms arise by differential splicing of alternate MBP mRNA transcripts from promoters P1 and P2, respectively (Li et al., 1995). Both cDNAs have identical coding and 3′-untranslated regions but differ in their 5′ sequences (Li et al., 1995). Distribution studies have identified high levels of the 1-kb variant in immature cells such as those found in the bone marrow, when compared to the long form of the protein which predominates in differentiated blood eosinophils. Those data are consistent with differential use of the P1 and P2 promoters as a mechanism for regulating MBP expression in eosinophil maturation (Li et al., 1995).

Little is known of the regulation of the *MBP* gene although the detection of mRNA transcripts for GATA-1, GATA-2, and GATA-3 in eosinophils (Zon et al., 1993) has led to the proposal that gene transcription in the eosinophil lineage is regulated by the GATA family of transcription factors. Indeed, Yamaguchi et al. (1998) have reported that the GATA-binding proteins can have a significant impact on the *trans*-activation of the MBP promoter. A consensus sequence, conserved between the human and murine MBP promoter, has been identified that binds the transcription factors GATA-1 and GATA-2. Transfection of Jurkat T-cells with a GATA-1 expression vector significantly enhanced MBP promoter activity while a GATA-2 expression vector was inactive (Yamaguchi et al., 1998). Interestingly, cotransfection experiments with both vectors resulted in less *trans*-activation than the single GATA-1 construct, suggesting that GATA-2 can negatively modulate the ability of GATA-1 to *trans*-activate the MBP promoter (Yamaguchi et al., 1998).

Human recombinant prepro-MBP has been expressed in CHO cells, purified, and characterized (Popken-Harris et al., 1995). The cDNA for prepro-MBP encodes a 25.2-kDa protein of 222 amino acids. Structurally, prepro-MBP is composed of a typical 15-amino acid hydrophobic signal peptide, a "pro" portion—that is 90 amino acids in length (9.9 kDa)—and MBP itself, which accounts for the majority (117 amino acids) of the polypeptide. It is of interest that purified MBP (33 kDa) is considerably heavier (~ 8 kDa) than the molecular mass predicted from the cDNA, indicating that a considerable amount of carbohydrate must be added to the polypeptide to account for the discrepancy in mass. Indeed, analysis of purified prepro-MBP has established that S^{24} and T^{25} are *O*-glycosylated. Other likely residues that could be modified include N^{86}, which is a candidate for *N*-linked glycosylation, and S^{62}, to which glycosaminoglycans can attach (Shikata et al., 1993). Significantly, glycosylation of prepro-MBP occurs exclusively in the prepro part of the protein which is consistent with the knowledge that mature MBP is nonglycosylated (Wasmoen et al., 1988).

MBP has the potential to act in a paracrine manner to modify the activity of other eosinophils. At low, noncytotoxic concentrations (<0.1 μg/ml), MBP is as effective as secretory IgA in evoking the exocytosis of EDN (Kita et al., 1995). MBP-induced degranulation is partially dependent on extracellular Ca^{2+} although it does not evoke a Ca^{2+} transient in eosinophils. MBP also increases the expression of IL-8 mRNA transcripts and protein in an actinomycin D-sensitive manner and acts synergistically with the Ca^{2+} ionophore A23187 in the production of LTC_4 (Kita et al., 1995).

MBP is a potent helminthotoxin and cytotoxin. It also degranulates basophils and possesses bactericidal activity. The ability of MBP to damage target cells is due to its ability to increase membrane permeability through surface charge interactions rather than by the formation of distinct pores (Young et al., 1986). It is believed that the high cationic nature of MBP allows it to bind avidly to anionic domains on target cells and parasites that results in perturbation of the lipid bilayer following insertion of apolar residues into the membrane (Wasmoen et al., 1988).

Recently, a novel homolog of MBP was identified in human eosinophil granules (Plager et al., 1998). Although it has similar biological activity to MBP, it is considerably less abundant and does not interact with MBP in a synergistic manner with respect to its cytotoxicity (Plager et al., 1998). Its role in eosinophil-driven histopathology is unknown.

b. EOSINOPHIL CATIONIC PROTEIN. Present within the matrix of the specific granules are a number of other proteins including the variably glycosylated, zinc-rich single-chain peptide ECP, which has a molecular mass ranging between 16 and 21.4 kDa and shows significant primary sequence identity across species (Peterson et al., 1988; Watanabe et al., 1995). Approximately 15 pg of ECP are present in a single human eosinophil, although marked variation between individuals is apparent (Venge, 1993). The isoelectric point of ECP is very basic (10.8 and 9.85 in humans and rats, respectively) due to a high content of arginine residues, although it shares more sequence homology to EDN (66%) and pancreatic ribonuclease (31%) than to the similarly charged MBP (Rosenberg et al., 1989a,b; Nittoh et al., 1997). ECP is a member of a subfamily of rapidly evolving, primate RNase A multigenes that emerged through gene duplication in primates 25 to 40 million years ago (Hamann et al., 1990; Rosenberg, 1995). Accordingly, ECP possesses ribonuclease activity which has the characteristics of the "nonsecretory" liver-type (Sorrentino and Glitz 1991), although it is approximately 100 times less active than another eosinophil product, EDN (Gleich et al., 1986; Gullberg et al., 1986; Slifman et al., 1986; Barker et al., 1989).

Two peaks of ECP activity, denoted ECP-1 and ECP-2, are resolved following chromatography of human eosinophils on heparin Sepharose (Gleich et al., 1986). Endoglycosidase F digestion of both activities decreases their molecular mass, indicating that they feature at least one complex oligosaccharide (Gleich et al., 1986). Two forms of ECP also have been identified immunologically; one of these is found within the granules of resting ($EG1^+$) eosinophils while the other represents a secreted ($EG2^+$) form thought to be derived from activated cells (Tai et al., 1984). The difference between these two forms is currently unknown, although it is possible that structural changes occur to the protein when it is released by exocytosis. Their possible relationship to ECP-1 and ECP-2 has not been formally explored.

The gene for human ECP (*RNS3*) is localized to the q24-q31 region of chromosome 14 which encodes a pre-

protein (Olsson et al., 1986; Rosenberg et al., 1989a,b; Mastrianni et al., 1992) that subsequently is processed to the form stored in the matrix of the specific granules. Structurally, *RNS3* is ~ 1.2 kb and contains a single intron (230 bases) in the 5′-untranslated region and an intronless coding domain that are characteristic features of members of the RNase gene superfamily (Hamann et al., 1990). Rosenberg et al. (1989a,b) have isolated a 725-bp full-length cDNA for human ECP; the open reading frame encodes a preprotein with a 27-amino acid "leader" sequence preceding a 133-residue mature ECP polypeptide which has a mass of 15.6 kDa. Comparable data also were reported by Barker et al. (1989). An intronic enhancer element has been identified within the *ECP* gene that features a consensus sequence for NF-AT-1 (Handen and Rosenberg, 1997). However, it is of interest that no "super shift" was observed in gel-shift assays performed in the presence of an anti-NF-AT serum, suggesting that a nuclear factor other than NF-AT may be acting at this site (Handen and Rosenberg, 1997).

In addition to its weak RNase activity, ECP exhibits a number of other properties: it is bactericidal, promotes degranulation of mast cells, and is helminthotoxic (Gleich et al., 1986; Lehrer et al., 1989). The mechanism of action of ECP has not been studied in detail but it is believed to exert many of its effects by creating functional pores or channels that traverse the plasmalemma of target cells, which are neither voltage- nor ion-sensitive (Young et al., 1986). It is noteworthy that the cytotoxicity of ECP is not apparently dependent on its RNase activity (Rosenberg et al., 1995b). Perhaps the most notable property of ECP is its ability to elicit the Gordon phenomenon when injected into rabbits by the i.c.v. route. This is characterized by the destruction of Purkinje cells and a spongiform change in the structure of the white matter of the cerebellum, pons, and spinal cord (Durack et al., 1979; Fredens et al., 1982).

c. EOSINOPHIL-DERIVED NEUROTOXIN. Another member of the RNase A multigene family localized to the matrix of the specific granules is EDN. It is now appreciated that EDN is indistinguishable from another protein, EPX, that was purified and characterized from human eosinophils by Peterson and Venge (1983). EDN and EPX are almost certainly the same protein; they exhibit identical physiochemical, immunological, and neurotoxicological properties and have equivalent RNase activity (Slifman et al., 1989). The human form of EDN has been expressed, purified, and extensively characterized from eosinophils taken from normal subjects and individuals with hypereosinophilic syndrome (Durack et al., 1981; Peterson and Venge, 1983; Newton et al., 1994; L. Sun et al., 1995), and the crystal structure has been resolved (Mosimann et al., 1996). Structurally, EDN is an 18.5-kDa, single-chain polypeptide (Gleich et al., 1986; Rosenberg et al., 1989b) but has a pI (8.9) approximately 10 to 100 times more acidic than either human MBP or ECP, due to a relatively lower number of arginine residues in the protein, which probably accounts for its reduced cytotoxicity (Barker et al., 1989).

The human *EDN* gene (*RNS2*) maps to chromosome 14 in the same region, q24-q31, as *RNS3* (Hamann et al., 1990), while the porcine homolog is found in the p1.3-p1.2 domain of chromosome 7 (Lahbib-Mansais et al., 1995). Human *RNS2* consists of a noncoding and coding exon separated by a single intron (Tiffany et al., 1996) and produces a preprotein (Olsson et al., 1986; Rosenberg et al., 1989b; Mastrianni et al., 1992) that is subsequently processed to the stored form found within the matrix of the specific granules. This genomic structure is common among mammalian RNases and suggests that the mechanism(s) of gene regulation is conserved. The structural similarity between ECP and EDN led Spry (1988) to propose that these proteins should be renamed eosinophil RNases. Indeed, since that proposal, the cDNAs of EDN and ECP have been shown to be 88% homologous at the nucleotide level, including the 27-amino acid signal peptide (Barker et al., 1989; Rosenberg et al., 1989b) and 70% identical at the amino acid level for the "pre" form of both proteins (Gleich et al., 1986; Barker et al., 1989; Hamann et al., 1991). Moreover, there is marked similarity between the 3′- and 5′-untranslated regions and the single introns in *RNS2* and *RNS3* providing compelling evidence for gene duplication (Hamann et al., 1990). The monoclonal antibody EG2 recognizes an epitope on EDN endorsing its close similarity to ECP (Tai et al., 1984).

A functional promoter has been identified within *RNS2* that depends upon the activity of upstream enhancer elements located in the first 60 bases of the first intron (Tiffany et al., 1996; Handen and Rosenberg, 1997). Specifically, a consensus sequence for NF-AT-1 has been found (Tiffany et al., 1996; Handen and Rosenberg 1997) that differs from the corresponding site within *RNS3* by a single base (Handen and Rosenberg, 1997). A putative binding domain for AP-1 also has been identified (Handen and Rosenberg, 1997). In differentiated eosinophilic HL-60 cells, a region in the first intron contains tandem PU.1-binding sites that are apparently important for enhancer activity (van Dijk et al., 1998). Gel-shift analysis and DNA affinity precipitation have demonstrated that this enhancer domain binds multiple forms of the transcription factor PU.1. Moreover, point mutations within the PU.1-binding domain drastically attenuates intronic enhancer activity, indicating an important role for the expression of EDN by cells of the eosinophilic lineage (van Dijk et al., 1998).

Rosenberg et al. (1989b) have isolated a 725-bp cDNA clone for human EDN. The open reading frame encodes a 134-amino acid, 15.5-kDa mature polypeptide, and a 27-residue hydrophobic leader sequence at the amino-terminus akin to that found for ECP. The discrepancy (~3 kDa) between the predicted mass of EDN and the purified enzyme is due to glycosylation. The amino acid

sequence of EDN, deduced from the coding sequence of the corresponding cDNA, is identical with urinary RNase (Hamann et al., 1989; Rosenberg et al., 1989b) and shows a high degree of homology with human, nonsecretory, pancreatic RNase, and angiogenin (Gleich et al., 1986).

The mean content of EDN of a normal human eosinophil is approximately 10 pg, but marked variation exists between individuals, and the amount is considerably lower in cells harvested from patients with various forms of eosinophilia (Venge, 1993). Despite its name, EDN is not restricted to eosinophils; it has been identified in basophils, mononuclear cells, and possibly neutrophils, and is probably secreted by the liver (Rosenberg et al., 1989b; Ten et al., 1991; Wilde et al., 1992).

Although a relatively poor helminthotoxin and cytotoxin EDN, like ECP, is neurotoxic and causes the Gordon phenomenon when injected intrathecally into laboratory animals (Durack et al., 1979; Fredens et al., 1982). This deleterious effect has been linked to its marked RNase activity but it is not sufficient to account totally for its neurotoxicity (Sorrentino et al., 1992). Indeed, the RNase activity of EDN is approximately 125-fold higher than that of ECP (Rosenberg et al., 1989b), a property conferred by arginine and/or isoleucine residues adjacent to the carboxyl-terminus of the protein (Rosenberg and Dyer 1997), yet it is considerably less neurotoxic (Fredens et al., 1982). Thus, the high level of RNase activity associated with EDN suggests an additional but, as yet, undefined physiological function.

d. EOSINOPHIL PEROXIDASE. EPO (donor: H_2O_2 oxidoreductase) is a member of the family of haloperoxidases that catalyze the peroxidative oxidation of halides and pseudohalides (see *XII.G*). It is localized exclusively to the matrix of the secondary granules (Egesten et al., 1986; Enomoto and Kitani, 1986) where, in human eosinophils, it accounts for approximately 5% (~15 pg/cell) of the total granule protein (Carlson et al., 1985; Venge, 1993). Intriguingly, the expression of EPO is not uniform across mammals and in eosinophils derived from the hyena, rhinoceros, giraffe, birds, and certain cats (domestic cat, tiger, and lion), it is absent (Undritz et al., 1956; Presentey et al., 1980). However, the properties and level of expression of EPO are essentially the same in eosinophils purified from normal subjects and individuals with eosinophilia (Bos et al., 1981). A number of publications have described the purification of EPO from several species including the horse (Jorg et al., 1982a) and humans (Olsen and Little, 1983; Bolscher et al., 1984; Carlson et al., 1985; Olsson et al., 1985; Menegazzi et al., 1986; Ten et al., 1989), and, more recently, the cloning and expression of EPO was reported (Sakamaki et al., 1989; Ten et al., 1989).

Structurally, EPO is a haem-containing protein composed of two subunits: a heavy chain of some 50- to 57-kDa and a 11- to 15-kDa light chain (Olsson et al., 1985; Ten et al., 1989). Screening of a cDNA library derived from HUVECs with oligonucleotides obtained from the partial amino acid sequence of both subunits, led Ten and colleagues (1989) to identify a clone corresponding to EPO. The nucleotide sequence of the clone revealed an open reading frame of 2106 bp corresponding to a prosequence, a heavy chain, and a light chain. The deduced amino acid sequence of the proform is rich in arginine and leucine, resulting in a highly basic protein (pI = 10.9) with a molecular mass of ~79.5 kDa. Based on these and other studies (Olsson et al., 1985; Sakamaki et al., 1989), the general consensus is that EPO is produced as a 79.5-kDa prepro-protein which is cleaved twice: first, by removal of the 13.8 kDa "pro" sequence to form an intermediate, and again resulting in two highly basic fragments corresponding to a light (12.7 kDa, pI = 10.8) and a heavy (53 kDa, pI = 10.7) chain (Ten et al., 1989). These chains may be reassembled to form native EPO that is composed of a two-chain monomer or, possibly, a four-chain dimer, which would be similar to the organization of myeloperoxidase (MPO).

A comparison of EPO with neutrophil MPO and other peroxidases has led to the theory of a peroxidase multigene family that has evolved through gene duplication in an analogous fashion to ECP and EDN (Sakamaki et al., 1989; Ten et al., 1989; Hamann et al., 1991). Despite this, monensin, a proton ionophore which blocks the sequestration of MPO by the specific granules, does not inhibit the processing of EPO, indicating that different mechanisms are required to direct peroxidases into storage organelles (Olsson et al., 1985).

The gene for human EPO has been isolated using human MPO cDNA as a probe (Sakamaki et al., 1989) and maps to chromosome 17. Like MPO, it is composed of 12 exons and 11 introns spanning 12 kb and encodes for a 715-amino acid protein. The coding sequence of MPO and EPO is about 72% and 70% homologous at the nucleotide and amino acid level, respectively; however, there is little sequence similarity at the 5′-flanking region (Sakamaki et al., 1989; Ten et al., 1989) that features promoter elements, suggesting differences in peroxidase gene regulation. Information also is available on the murine *EPO* gene; it has been mapped to chromosome 11 (Denzler et al., 1997b) and shares a considerable degree of conservation with its human counterpart (Horton et al., 1996). Thus, at the nucleotide level human and murine EPOs are 86% homologous in the protein-coding region and 66% homologous in the 3′-untranslated region; at the amino acid level they show 90% identity.

In studies designed to probe the transcriptional regulation of the *EPO* gene, Yamaguchi et al. (1994a,b) cloned a 1.5-kb fragment of the human *EPO* gene upstream of the transcription start site into an eosinophil-inducible leukemic cell line, HL6-C15, and examined *cis*-acting elements required for promoter activity. Consensus sequences for the transcription factors Egr-1, H4TF-1, PuF, UBP-1, CTCF, and GaEII were identified

(Yamaguchi et al., 1994a,b). Northern blot analysis of developing cord blood-derived eosinophils has established that, in the presence of EL-4-conditioned medium, EPO mRNA transcripts rise rapidly, peak at day 8 and still are detectable at day 34, a time when the cells resemble mature eosinophils (Ten et al., 1991). Those findings are entirely consistent with the presence of very low levels of EPO mRNA in peripheral blood eosinophils from eosinophilic patients (Gruart et al., 1992). Essentially, identical data are available for MBP, whereas the number of transcripts for EDN and ECP remain relatively constant throughout maturation, implying that the genes encoding granule proteins are subject to different regulatory constraints (Gruart et al., 1992).

Once released, EPO can elicit a number of effects, some of which are protective and others potentially destructive. In particular, it inactivates the peptido-LTs (Henderson et al., 1982) and converts LTC_4 to all-*trans* isomers of LTB_4 (Goetzl, 1982). LTB_4 is similarly inactivated by EPO but at a much slower rate (Henderson et al., 1982). EPO also is bactericidal (Bujak and Root, 1974; Jong et al., 1980) and, in the presence of peroxide and bromide, can catalyze the formation of hypobromous acid and the highly reactive singlet oxygen (see *XII.G*). A number of cells including basophils, mast cells, and neutrophils will actively endocytose EPO by utilizing a vesicular transport system. That finding explains the variable detection of EPO in proinflammatory leukocytes and tempts speculation that the EPO is deliberately stored until such a time when release is deemed necessary (Dvorak et al., 1985; Zabucchi et al., 1986).

e. CHARCOT-LEYDEN CRYSTALS. The identification and description of distinct, needle-shaped crystal structures was originally reported in tissues from a patient with leukemia (Charcot and Robin 1853) and, subsequently, from the sputum of individuals with asthma (Leyden, 1872). Now known as Charcot-Leyden crystals, this protein is localized to the primary granules of mature eosinophils (Dvorak et al., 1988) and also is present in basophils in a roughly equal amount (Ackerman et al., 1982; Tanabe et al., 1993). Structurally, Charcot-Leyden crystals are colorless, hexagonal, and bi-pyramidal, 20 to 40 μm in length and 2 to 4 μm across, and are routinely found in the feces and sputum of animals with severe gastrointestinal and respiratory eosinophilia (Zucker Franklin, 1980). Molecular genetics technology has identified and sequenced a human full-length cDNA clone for Charcot-Leyden crystals and has localized the gene that encodes this protein to chromosome 19 (Mastrianni et al., 1992). Transcription of the Charcot-Leyden crystals gene ultimately yields a 17.4-kDa hydrophobic protein with inherent lysophopholipase activity which represents the sole protein component (Weller et al., 1980, 1981, 1982, 1984). Charcot-Leyden crystals account for 10% of the total eosinophil protein (Weller et al., 1982, 1984), suggesting that they are functionally important, although their precise role is far from clear.

f. OTHER GRANULE PROTEINS. In addition to Charcot-Leyden crystals and the four main cationic proteins described in the aforementioned sections, eosinophils also store a plethora of other enzymes in significantly greater amounts than are present in autologous neutrophils. Besides the proteins listed in Table 3, eosinophils express α-mannosidase, β-galactosidase, β-hexosaminidase, histaminase, collagenase, alkaline phosphatase, matrix metalloproteinase 9 (gelatinase B), MIF, the serine proteinase esp-1, inducible and endothelial nitric oxide synthase (iNOS and eNOS, respectively), NGF, and eotaxin (see Archer, 1963; Makita and Sanborn, 1970; Heyneman, 1975; Zeiger and Colten, 1977; Williams et al., 1978; Hibbs et al., 1982; Weller et al., 1983; Davis et al., 1984; Spry, 1988; Weller, 1991; Zanardo et al., 1997; Ohno et al., 1997; Rossi et al., 1998; Shlopov and Hasty, 1998; Inoue et al., 1998; Nakajima et al., 1998; Solomon et al., 1998).

2. Morphological Changes

a. SECRETION. In many secretory cells, the most common form of degranulation is regulated secretion. This is believed to be an exocytotic process involving the fusion of granules with the plasma membrane and the partial or total extrusion of secretory products. A complicating factor in studying exocytosis in secretory cells is the rapidity and transience of the response. Accordingly, evidence for fusion sites has been difficult to obtain with conventional chemical fixatives. However, Newman et al. (1996) adopted an arrest procedure, using tannic acid (Buma et al., 1984), to "trap" exocytotic events. Tannic acid, when applied to secretory cells before fixation, arrests exocytosis of granule contents, which causes an accumulation of fusion sites (Buma et al., 1984). Two main forms of exocytotic secretion have been described, simple and compound (Fig. 7), and both types have been visualized in guinea pig peritoneal eosinophils at the ultrastructural level (Henderson and Chi, 1985; Dvorak et al., 1993; Newman et al., 1996). The first and most simple method involves the fusion of single granules with the plasmalemma with full incorporation of the granule membrane. In ultrathin sections and freeze-fracture replicas of guinea pig eosinophils permeabilized with streptolysin O and stimulated with Ca^{2+} and GTPγS, large numbers of crystalloid granules have been found in a state of arrested fusion with the plasma membrane (Fig. 8, a and b) when compared to unstimulated cells (Newman et al., 1996). Figure 8, a and b, shows a single fusion event of a specific granule exhibiting the classical omega shape with retention of the central crystalloid core (see Fig. 8b). In addition to simple secretion, compound exocytosis also can occur in eosinophils (Newman et al., 1996). In this case, multiple granules first fuse intracellularly to form a large degranulation chamber or cavity (see Fig. 8c) that can occupy a substantial volume of the cytoplasm in some cells (see Fig. 8d). This structure then fuses with the cell mem-

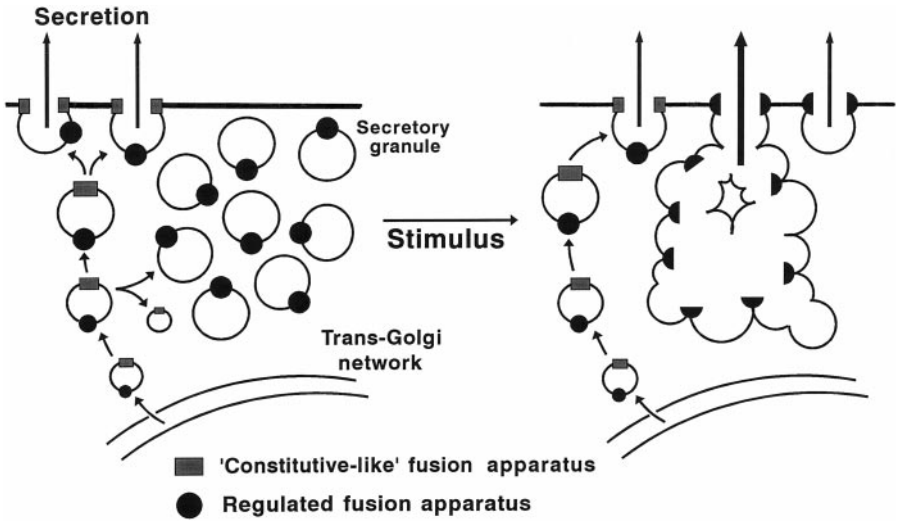

FIG. 7. Model of constitutive and regulated exocytosis. Upon synthesis from the *trans*-Golgi network, granules can either be secreted via a constitutive, or unregulated mechanism, or can enter a regulated pathway of secretion with loss or masking of the constitutive fusion apparatus. After application of a degranulation-evoking stimulus, granules can additionally be released by the regulated pathway involving either simple or compound exocytosis (see Fig. 8 for electron micrographs of these phenomena).

brane before discharging its contents in a coordinated and vectorial fashion.

Two mechanisms have been described that govern the secretion of granule contents. One of these is called constitutive or unregulated secretion and is characterized by small intracellular vesicles containing secretory products that are unpackaged and released in a stimulus-independent manner. The other process is known as regulated secretion and results from the formation of the degranulation chamber or cavity described above and the release of stored contents in response to external stimuli (see Fig. 7). Under resting conditions, the significance of the constitutive pathway in eosinophils is questionable as little, if any, granule products are secreted spontaneously.

b. PIECEMEAL DEGRANULATION. An alternative process of secretion that commonly is seen in human eosinophils is piecemeal degranulation. This differs from secretion in that small protein-containing vesicles bud off from, and gradually empty, the secondary granules (Tai and Spry 1981; Torpier et al., 1988; Dvorak et al., 1991, 1992a). Tai and Spry (1981) have studied this phenomenon in some detail and reported marked variability in granule morphology. Some secondary granules are completely empty or lack only the dense core while, in others, partial or complete loss of the matrix is evident. Thus, it seems likely that the formation of small vesicles from the specific granules permits, in a stimulus-dependent manner, the selective release of an individual secretory protein from a common storage organelle. Indeed, piecemeal degranulation provides an explanation for the ability of anti-IgE and anti-IgG selectively to release EPO and ECP respectively (Tomassini et al., 1991).

c. CYTOLYSIS. Histological analysis of tissue samples from sites of inflammation reveals that many eosinophils have undergone cytolytic degranulation, which is characterized by swollen mitochondria, cell and nuclear membrane rupture, lysis of chromatin, and the release of "clusters of free eosinophil granules" into surrounding structures that are in different stages of dissolution (Figs. 9 and 10) (Erjefalt et al., 1996, 1997a,b; 1998; Greiff et al., 1998). Many of these histological features of cytolysis have been described at the ultrastructural level and are associated with eosinophilic diseases including bullous pemphigoid (Dvorak et al., 1982), nasal polyposis (Greiff et al., 1998), eosinophilic pneumonia (McEvoy et al., 1978), atopic dermatitis (Leiferman et al., 1990), and asthma (Filley et al., 1982; Beasley et al., 1989; Jeffery et al., 1992; Ohashi et al., 1992; Laitinen et al., 1993). In addition, human eosinophil cytolysis has been produced in vitro in response to secretory IgA and IgG-bound to Sepharose beads (Weiler et al., 1996) and Ca^{2+} ionophore, A23187 (Fukuda et al., 1985a). However, in the context of eosinophilic inflammation and the killing of parasites, this phenomenon was largely ignored until the middle of the 1990s when Persson and Erjefalt (1997a,b, 1998) emphasized its potential significance. Thus, in addition to secretion and piecemeal degranulation, the ability of eosinophils to undergo nonapoptotic lysis may represent the "ultimate activation" of eosinophils in vivo (Persson and Erjefalt, 1997b).

3. Cell-Signaling Events. Although many stimuli are known to promote the release of preformed granule products, relatively little is known of the biochemical basis of this response. In human eosinophils, immobilized immunoglobulins and fMLP effectively evoke degranulation; Ca^{2+} ionophore, A23187, similarly is active on equine eosinophils (Henderson et al., 1983; Abu Ghazaleh et al., 1989; White et al., 1993; Kaneko et al., 1995a) and the cell-signaling pathways that could underlie this response have been investigated to some ex-

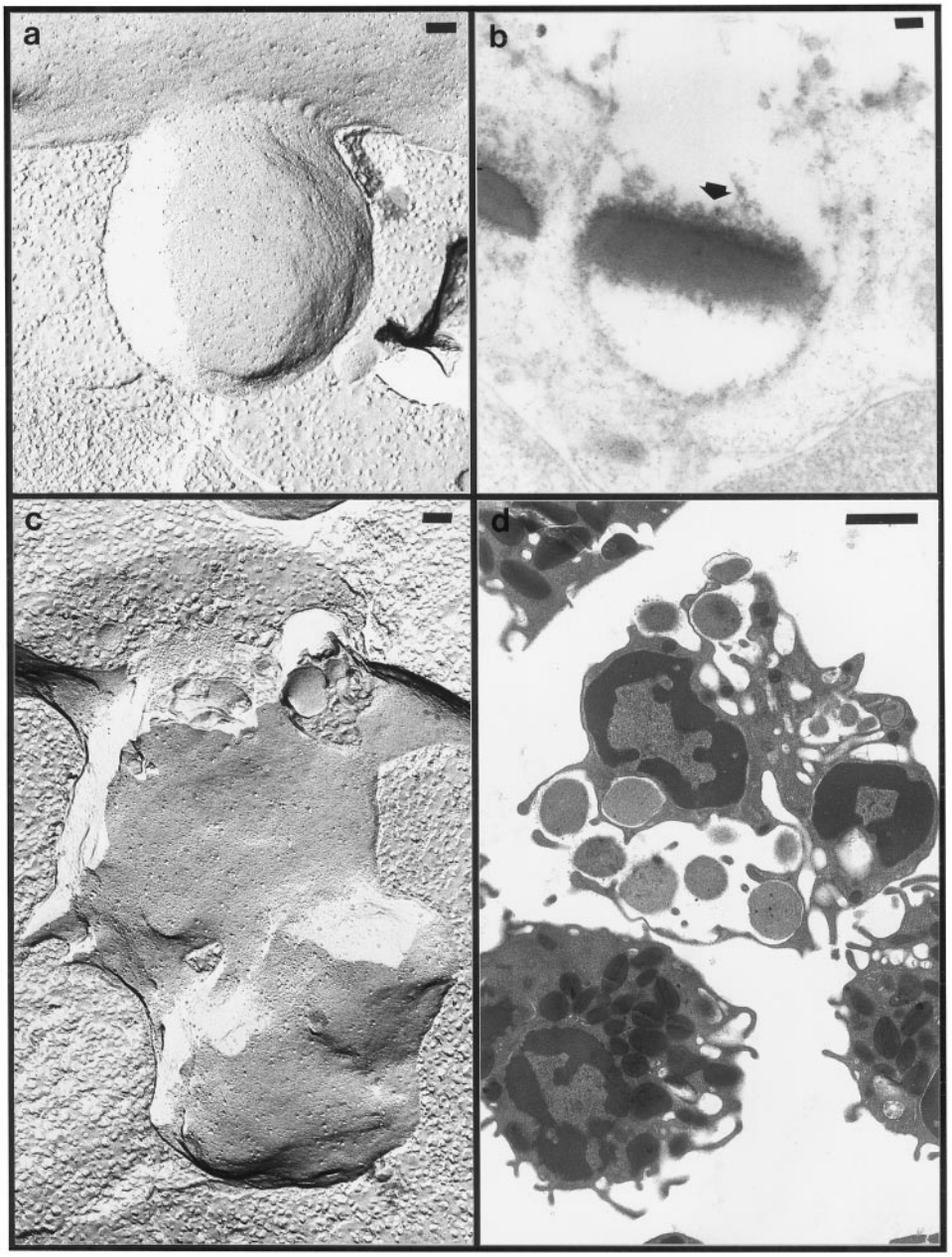

FIG. 8. GTPγS/Ca^{2+}-induced secretory granule exocytosis in streptolysin O-permeabilized guinea pig peritoneal eosinophils arrested by incubation with tannic acid. a and c, a single fusion site (giving the classical omega shape found in many secretory systems) and a compound fusion site, respectively, in cells prepared for freeze-fracture replication. b and d, the same phenomena in ultrathin sections. Note the retention of the crystalloid core in the single fusion site (b, arrow). Bars: a, 100 nm.Original magnification, 63,000×. b, 100 nm. Original magnification, 55,000×. c, 500 nm. Original magnification, 58,000×. d, 1 μm. Original magnification, 18,000×. See *XII.B.2.a* for further details.

tent. Another approach has been to study exocytosis in permeabilized eosinophils (Cromwell et al., 1991; Gomperts and Cromwell, 1991), which allows the intracellular environment to be precisely manipulated and the importance of putative messenger molecules, enzymes, cofactors, and ions to be assessed directly.

a. STUDIES WITH IMMUNOGLOBULINS, fMLP, AND A23187. Of the many stimuli that evoke degranulation of human eosinophils, IgG and secretory IgA, immobilized on to Sephadex beads, are two of the most effective and have provided a model system with which to examine the cell-signaling events that could account for the ultimate fusion of secretory granules with the plasma membrane. A limited number of studies also have used fMLP and A23187 as secretagogues. One of the earliest events that follows ligation of FcαR is the formation of inositol polyphosphates, indicating that a PLC is stimulued (Kita et al., 1994; Kato et al., 1995). The identity of the PLC isoform(s) to which FcαR couple is unknown but, because IgA-induced inositol phosphate accumulation is abolished by PTX (Kita et al., 1994; Kato et al., 1995), a good case can be made for PLC-β_1 and/or PLC-β_2 that are known to couple to one or more members of the G_i and/or G_o families of heterotrimeric GTP-

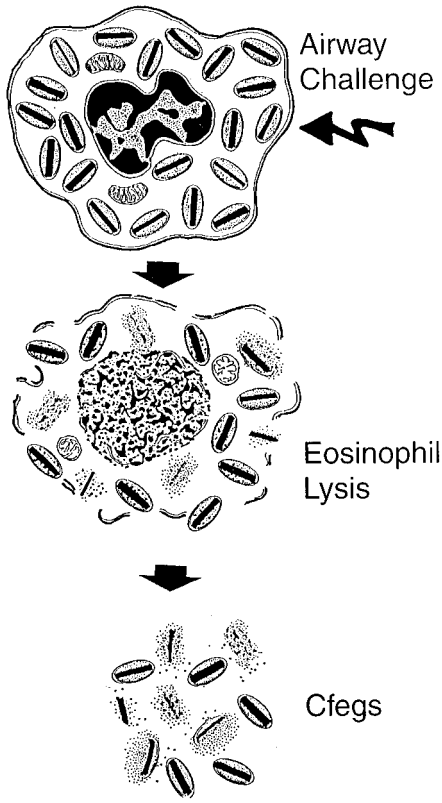

FIG. 9. Eosinophil cytolysis. Allergen provocation of a mucosal eosinophil promotes cytolysis, the release of cfegs and the subsequent deposition of their contents in intimate apposition to the target tissue. See *XII.B.2.c* for further details.

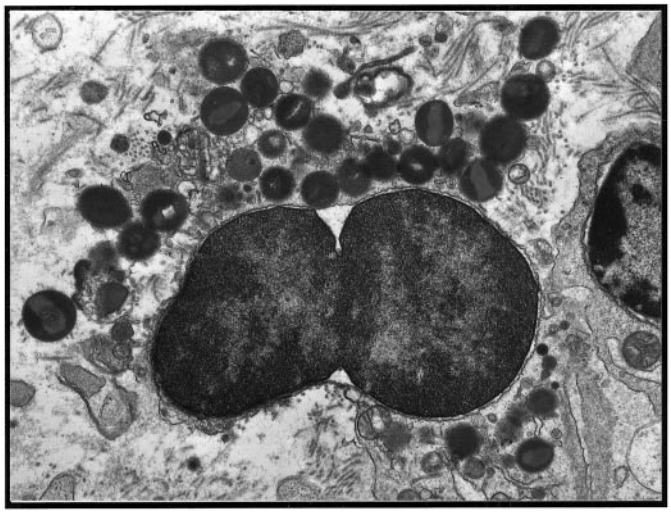

FIG. 10. Cytolysis of a human eosinophil within the airway mucosa 24 h after experimental allergen provocation. Note the chromolytic nucleus and the absence of a cell membrane. Original magnification, 4000×. See *XII.B.2.c* for further details.

binding proteins (Katz et al., 1992). Another phospholipase, PLC-γ_2, which is activated by tyrosine phosphorylation, also is present in human eosinophils but its role (if any) in Ig-induced degranulation is unclear.

Secretory IgA promotes the tyrosine phosphorylation of a number of proteins in human eosinophils with the most prominent labeling seen of bands migrating at 50, 65, 70, 100, and 115 kDa on SDS-polyacrylamide gels. The finding that pervanadate, an inhibitor of phosphotyrosine phosphatases, and genistein and herbimycin A, inhibitors of protein tyrosine kinases, promote and suppress, respectively, tyrosine phosphorylation and degranulation (Kita et al., 1994; Kato et al., 1995) provides compelling evidence that tyrosine kinase-dependent processes play a central role in regulating exocytosis. Furthermore, the general acceptance that regulated secretion in eosinophils is a Ca^{2+}-requiring process is supported by studies with tyrosine kinase inhibitors that similarly prevent secretory IgA-induced inositol phosphate accumulation (Kita et al., 1994; Kato et al., 1995).

Similar results have been obtained with immobilized IgG. Thus, ligation of Fcγ receptors results in activation of PLC and the tyrosine phosphorylation of essentially the same proteins that are phosphorylated in response to secretory IgA, albeit at a slower rate (Kita et al., 1994; Kato et al., 1995). IgG also is reported to activate rapidly the *src*-related tyrosine kinase, *fgr* (Kato et al., 1995). This is an important observation since FcγRII, the main receptor for IgG on resting eosinophils, is devoid of intrinsic tyrosine kinase activity (Ravetch and Kinet, 1991; Ravetch, 1994, 1997), suggesting that once bound by an activating ligand it must recruit directly or indirectly a nonreceptor tyrosine kinase. In this respect it is intriguing that *fgr* is known to associate specifically to FcγRII in human neutrophils (Hamada et al., 1993).

Of primary significance is the *insensitivity* of Ig-induced tyrosine phosphorylation to PTX (Kato et al., 1995). At least two explanations could account for this finding. First, two pathways might be activated in parallel that are required for degranulation but only one of these is sensitive to PTX. Alternatively, a critical tyrosine protein kinase may lie upstream of G_i/G_o and, therefore, be insensitive to PTX (Kato et al., 1995).

Although there are marked similarities between the signaling mechanisms recruited by Fcγ and Fcα receptors, differences also are apparent. A particularly pertinent observation is that PTX abolishes secretory IgA-induced EDN release, whereas the same response evoked by IgG is only affected transiently (Kita et al., 1991a). Indeed, the sensitivity of eosinophils to IgG recovers over a period of 16 h despite the continued presence of PTX (Kita et al., 1991a). Consistent with those functional results is the finding that PTX catalyzes the ADP ribosylation of two proteins in eosinophil membranes of 41 and 44 kDa. However, over time the level of unmodified 44-kDa protein gradually reappears whereas the amount of unmodified 41-kDa protein remains reduced (Kita et al., 1991a). Thus, those results suggest that the receptors for IgG and secretory IgA are coupled to the suppression of degranulation via distinct, PTX-sensitive G proteins of 44 kDa and 41 kDa, respectively. In this respect, Gomperts and his colleagues iden-

tified two PTX substrates ($G_{i\alpha 3}$ and G_{io}) in membranes prepared from guinea pig eosinophils (Lacy et al., 1995).

A central role for PLA_2, AA, and possibility lipoxygenase products in granule protein release from equine and human eosinophils has been suggested on the basis of pharmacological experiments (Henderson et al., 1983; White et al., 1993). In 1983, Henderson and coworkers reported that the secretion of EPO from equine eosinophils evoked by A23187 was inhibited by eicosatetraynoic acid, a dual inhibitor of cyclooxygenase and lipoxygenase, but not by indomethacin. Similarly, the PLA_2 inhibitor, 4-bromophenacyl bromide, attenuated noncytolytic secretion of EPO effected by exogenous porcine purified secretory PLA_2. Those two observations led to the conclusion that Ca^{2+}-dependent degranulation requires the activation of PLA_2 and the generation of lipoxygenase products (Henderson et al., 1993). A similar situation exists in human eosinophils. Thus, fMLP-induced EPO release is prevented in cells treated with the PLA_2 inhibitors 4-bromophenacyl bromide and mepacrine by a mechanism that is restored by the addition of exogenous AA (White et al., 1993).

b. STUDIES WITH STREPTOLYSIN O-PERMEABILIZED CELLS. In streptolysin O-permeabilized guinea pig eosinophils, the exocytotic response depends minimally on two effectors: Ca^{2+} and a guanine nucleotide (Cromwell et al., 1991). In cells treated with 2-deoxyglucose and antimycin D, to suppress the endogenous levels of ATP, neither Ca^{2+} nor GTPγS promote exocytosis as assessed by the release of β-hexosaminidase. However, in combination, Ca^{2+} and GTPγS evoke robust degranulation that suggests an intimate interaction between the proteins to which these two effectors bind (Cromwell et al., 1991). Since GTPγS is nonhydrolyzable and cannot function as a phosphoryl donor, the release of β-hexosaminidase from permeabilized eosinophils must occur independently of protein phosphorylation. This is contrary to the situation that prevails in intact cells, however, where ATP plays an obligatory role in exocytosis. Collectively, these data indicate that ATP-dependent phosphorylation is essential for those processes that govern the early stages of stimulus-secretion coupling. The absolute requirement for ATP in intact cells is illustrated by the fact that ATP augments β-hexosaminidase release in permeabilized cells by increasing the affinity of Ca^{2+} and GTPγS for their respective binding proteins (Cromwell et al., 1991).

The difference in requirement for ATP between intact and permeabilized eosinophils is significant because it provides compelling evidence that a G protein mediates a stage in the exocytotic process distal to the activation of PLC (Gomperts, 1990). ATP is required to maintain the concentration of PtdIns(4,5)P_2 in cells and also to provide, by nucleotide transphosphorylation, sufficient GTP for G protein activation. Thus, although GTPγS will activate all G proteins in permeabilized eosinophils, its ability, with Ca^{2+}, to release β-hexosaminidase in the absence of ATP effectively dissociates products derived from the hydrolysis of PtdIns(4,5)P_2 from exocytosis. This is so for two reasons. First, any Ins(1,4,5)P_3 produced will rapidly leak out of the cell through the permeabilization pores and, in any case, the small amount of Ca^{2+} that could be released will be readily chelated by the EGTA in the buffer. Second, any PtdIns(4,5)P_2-derived diglyceride will be unable to activate PKC owing to the absence of ATP (Gomperts, 1990). Collectively, these facts support the belief that GTP interacts with a novel, functionally distinct G protein, designated G_E, that is fundamental for the latter stages of regulated secretion of granule proteins in eosinophils. The identity of G_E is uncertain, but it has been proposed that Rac, or another Rho-related protein, might fulfill this function (Larbi and Gomperts, 1997), which would be similar to the situation that prevails in permeabilized mast cells (O'Sullivan et al., 1996). Other possibilities include $G_{\alpha i3}$ (Lacy et al., 1995), which may act as a stimulatory form of GE in mast cells (Aridor et al., 1993), and G_o (Lacy et al., 1995), which in bovine adrenal chromaffin cells has been proposed to act as an inhibitory G_E (Vitale et al., 1993).

An important consideration that arises from the preceding description of exocytisis is how ATP augments the magnitude of Ca^{2+}-/GTPγS-induced degranulation and the respective affinity of GTPγS for G_E and of Ca^{2+} for its binding protein. Studies by Cromwell et al. (1991) have proposed that this is due to the activation of PKC by diglyceride cleaved from membrane phospholipids by PLC or PLD and the subsequent phosphorylation of G_E and the Ca^{2+}-binding protein. This sequence of events is supported by the observation that GTPγS-induced β-hexosaminidase release is attenuated (but not abolished) by a peptide inhibitor of PKC (Cromwell et al., 1991).

Magnesium ions inhibit exocytosis in permeabilized eosinophils driven by Ca^{2+} and GTPγS in the presence of ATP. Logic dictates that this effect might reasonably result from competition with Ca^{2+} at a Ca^{2+}-binding site. However, studies designed to assess this possibility revealed no simple relationship between the EC_{50} value of Ca^{2+} to promote secretion and the IC_{50} value of Mg^{2+} required to suppress this response (Larbi and Gomperts, 1997). Indeed, the mechanism of inhibition is apparently complex and, to some extent, depends on the stimulus (Larbi and Gomperts, 1997). Thus, exocytosis evoked by GTPγS in the presence of 10 μM free Ca^{2+} is inhibited by Mg^{2+} in a manner consistent with the behavior of a competitor. This observation led Larbi and Gomperts (1997) to suggest that inhibition could be due to the well established ability of Mg^{2+} to retard the dissociation of GDP from most monomeric G proteins, including Ras and Rho, thereby suppressing their activation. However, when exocytosis is initiated by GTPγS in the absence of Ca^{2+}, conditions that require ATP (see above), the inhibitory effect of Mg^{2+} is seen over a very narrow con-

centration range irrespective of the concentration of either nucleotide. Based on these results, Larbi and Gomperts (1997) have concluded that in the presence of ATP, Mg^{2+} inhibits exocytosis by acting at an undefined site(s) downstream of those processes activated by G_E.

4. Electrophysiological Changes. Compelling evidence that the secretion of granule contents from human (Aizawa et al., 1992; Hartmann et al., 1995), guinea pig (Nusse et al., 1990; Lindau et al., 1993), and equine (Scepek and Lindau, 1993; Hartmann et al., 1995) eosinophils occurs by an exocytotic process has been provided from high-resolution capacitance measurements (an index of membrane area) of cells patch-clamped in the whole-cell configuration. At a Ca^{2+} concentration chemically constrained to ~1 μM, the introduction of GTPγS into a single eosinophil elicits well resolved, stepwise increases in membrane capacitance (approximating to the number of crystalloid granules in that eosinophil), each consistent with the fusion of a single granule with the plasma membrane (Nusse et al., 1990; Aizawa et al., 1992; Scepek and Lindau, 1993; Hartmann et al., 1995). The magnitude of each discrete increment in capacitance is in remarkable agreement with the size distribution of granules identified by electron microscopy, and demonstrates that an exocytotic stimulus promotes *sequential*, not random, granule-membrane fusion (Nusse et al., 1990; Lindau et al., 1993; Scepek and Lindau, 1993; Hartmann et al., 1995). Moreover, secretion is a vectorial phenomenon that allows appropriate discharge of granule contents against the target cell, tissue or invading organism (e.g., parasite) and minimizes uncontrolled diffusion that could harm the host cell (Scepek and Lindau, 1993). In some eosinophils, the clearly resolved, stepwise changes in capacitance are preceded by a gradual, less well defined increase that might represent the fusing of small vesicles with the membrane (Lindau et al., 1993). Significantly, in equine eosinophils, at relatively high concentrations (20 μM) of GTPγS, the magnitude of each stepwise increment in capacitance is markedly increased but at the expense of the total number of fusion events. This observations has been taken as evidence for compound exocytosis (Scepek and Lindau, 1993), as described in *XII.B.2.a*, where two or more granules coalesce within the eosinophil's interior to form larger "compound" structures, which then migrate to, and subsequently fuse with, the cell membrane (Scepek and Lindau, 1993). Indeed, this idea is entirely consistent with the finding that the final increase in plasma membrane area resulting from the application of high and low concentrations of GTPγS is in keeping with a cell that features a constant number of secretory granules.

The release of preformed material stored within eosinophil granules after their fusion with the plasma membrane is made possible by the formation of a distinct connection between the two structures known as a fusion pore (Lindau and Almers, 1995). In equine eosinophils and other secretory cells, the fusion pore has a mean conductance of approximately 200 pS after the initial fusion event and enlarges as the pore expands to several nanoSiemens permitting effective and rapid discharge of granule contents (Lindau and Almers, 1995). The ability of fusion pores to enlarge or expand is regulated by protein phosphorylation and dephosphorylation although the substrates are unknown (Scepek et al., 1998). PMA and micromolar Ca^{2+} accelerate the rate of fusion pore expansion approximately 2-fold although the amplitude and time course of GTPγS-induced degranulation are unaffected by PMA (Scepek et al., 1998). It is significant that staurosporine blocks only the effect of PMA and that fusion pore expansion can occur in the presence of staurosporine. Thus, Ca^{2+} and PKC regulate the rate of fusion pore expansion by different mechanisms (Scepek et al., 1998). A broad-spectrum phosphatase inhibitor, α-naphthylphosphate, inhibits granule fusion and retards pore expansion but neither protein phosphatases 1 or 2A seem to be implicated in regulating pore expansion rate since selective inhibitors of those enzymes (canthardin and okadaic acid, respectively) do not mimic the effect of α-naphthylphosphate (Scepek et al.,1998).

The stepwise increases in membrane capacitance observed in GTPγS-stimulated equine eosinophils can be about six times larger than those recorded from eosinophils of human or guinea pig origin (Hartmann et al., 1995). However, although this observation is in accordance with the size distribution of secretory granules, variable or multimodal increases in membrane capacitance are seen in response to GTPγS (Hartmann et al., 1995). The smallest of these has been attributed to the fusing of single, or unit, granules with the plasma membrane, which have dimensions (450–500 nm in diameter, 0.7 μm^2) largely invariant across species. To account for this variability, it has been proposed that GTPγS evokes the exocytosis of multiples of the unit granule (Elmalek and Hammel, 1987; Hartmann et al., 1995). In human eosinophils, approximately 2 granules are permitted to fuse with each other whereas in horses 7 to 15 is not uncommon (Hartmann et al., 1995). Fusion of unit granules to large mature granules also has been used to explain size differences between mature and immature granules in PC12 cells (Tooze et al., 1991) and mast cells (Hammel et al., 1983, 1985, 1988; Alvarez de Toledo and Fernandez, 1990). The marked discrepancy between species implies that mechanisms exist that govern or limit the maximum number of unit granules that fuse to form the mature granule. It is known that vesicular traffic and intracellular fusion events are controlled by small [e.g., rab, ARF (Balch, 1990)] and heterotrimeric (e.g., G_i/G_o) GTP-binding proteins (Leyte et al., 1992), and, in equine permeabilized eosinophils, Scepek and Lindau (1993) have reported that GTPγS promotes granule-granule fusion.

Scepek and Lindau (1993) also have monitored the in vitro maturation of human cord blood eosinophils to establish when exocytotic competence is achieved. After 10 days of culture in medium containing IL-3 and IL-5, GTPγS evokes exocytosis at a time that coincides with the appearance of granules, and after 21 days the magnitude of the exocytotic response is equivalent to that seen in mature peripheral blood eosinophils. Thus, the machinery for exocytosis is in place and can be recruited as soon as granules are formed. Time course experiments have demonstrated that eosinophil maturation from day 10 to day 35 is associated with a marked reduction in plasma membrane area (from 700–400 μm^2) consistent with endocytosis of membrane to form small secretory vesicles.

The activation of Ca^{2+}-activated K^+ channels in human eosinophils has been associated with agonist-induced degranulation (Saito et al., 1997). Submicromolar concentrations of PAF caused activation of single channels that were selectively permeable to K^+ but not to Na^+ or Cl^- (Saito et al., 1997). Agents that increased intracellular Ca^{2+} directly, such as thapsigargin and the ionophores A23187 and ionomycin, produced similar increases in K^+ channel activity, suggesting that they were regulated by Ca^{2+}. Two levels of single-channel activity were observed (10 and 24 pS) that are consistent with the gating of intermediate and small conductance, Ca^{2+}-activated K^+ channels, respectively. The finding that quinidine blocked both K^+ currents and the release of MBP suggests a causal relationship between the electrophysiological and exocytotic responses (Saito et al., 1997).

C. Generation of Lipid Mediators

Eosinophils have the capacity to synthesize an array of phospholipid-derived mediators that have widespread biological actions. Some of the more important products include PAF, LTC_4, PGE_2, TX, and LXA_4. The first stage in lipid mediator biosynthesis is the liberation of free AA from the *sn*-2 position of appropriately esterified membrane phospholipids. This reaction is catalyzed by PLA_2. Like many enzymes, PLA_2 is a generic term that describes an ever increasing family of proteins, which are immunologically distinct and differ in their mode of regulation and cofactor requirements (Dennis, 1997). The beginning of 1997 saw the classification and partial characterization of nine families of PLA_2, and two of those (the so-called type IIA and type IV enzymes, often refered to as secretory and cytosolic PLA_2, respectively) are expressed by human eosinophils (Debbaghi et al., 1992; Zhu et al., 1996; Munoz et al., 1997b; Blom et al., 1998). Secretory PLA_2's are 13- to 15-kDa secretory enzymes that require Ca^{2+} in the millimolar range for activity, are expressed at a level 20 to 100 times greater in human eosinophils relative to other circulating leukocytes, and are apparently confined to the specific granules and to phagosomes in cells challenged with opsonized particles (Blom et al., 1998). In contrast, cytosolic PLA_2 is a heavier protein (85 kDa) found exclusively in the cytosol and is catalytically active in the presence of Ca^{2+} in the low micromolar range. Despite the apparently selective localization of secretory PLA_2 to eosinophils, the cytosolic isoform is the most abundant (Munoz et al., 1997b). Although relatively little is known of the specific functions PLA_2 isoforms subserve, a common finding is that cytosolic and secretory PLA_2 both can translocate to the plasma membrane of eosinophils upon appropriate stimulation (Munoz et al., 1997b).

1. Platelet-Activating Factor. Two pathways have been delineated for the formation of PAF. One of these is believed to maintain physiological concentrations of PAF for normal cell function (Chung and Barnes, 1991) and is regulated by choline phosphotransferase that acts directly on ether-linked phospholipids (Snyder, 1987). The other biosynthetic route initially involves the release of lyso-PAF from membrane phospholipids by PLA_2 or an endogenous acyltransferase, which then is acetylated to form biologically active PAF. In human eosinophils, an acetyltransferase (1-*O*-alkyl-2-acetyl-*sn*-glycerol-3-phosphocholine:acetyl-CoA acetyltransferase) has been identified that fulfills this latter function and is transiently activated (4-fold) in a time-, concentration- and Mg^{2+}/Ca^{2+}-dependent manner in response to the Ca^{2+} ionophore A23187 and certain chemoattractants such as C5a and fMLP (Lee et al., 1984). The molecular species of PAF generated by human eosinophils are apparently dependent on the stimulus (Triggiani et al., 1992). Gas-chromatographic/mass spectrophotometric analyses of A23187-stimulated eosinophils identified three molecular species of PAF. The predominant form was 1-*O*-hexadecyl-2-acetyl-*sn*-glycerol-3-phosphocholine (16:0) followed by lower amounts of 1-*O*-octadecyl-2-acetyl-*sn*-glycerol-3-phosphocholine (18:0) and 1-*O*-octadecenyl-2-acetyl-*sn*-glycerol-3-phosphocholine (18:1) (Triggiani et al., 1992). In contrast, fMLP promotes only the generation of the 16:0 molecular species in an amount 100 times less than that evoked by the Ca^{2+} ionophore (Triggiani et al., 1992). An enzyme (1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine:acetyl CoA acetyl hydrolase) that inactivates PAF by converting it back to lyso-PAF also has been described in human eosinophils (Lee et al., 1982) but, unlike the acetyltransferase, it is not up-regulated by the same stimuli (Lee et al., 1984). Eosinophils are perhaps the richest source of PAF among leukocytes. Indeed, both normodense and hypodense eosinophils (purified from individuals with rhinitis, asthma and hypereosinophilic syndromes) produce more PAF than neutrophils (Cromwell et al., 1990).

A number of more physiological/pathophysiological stimuli have the ability to promote PAF formation in human eosinophils including unopsonized zymosan (Burke et al., 1990), C5a (Lee et al., 1984), IgG-coated Sepharose particles (Cromwell et al., 1990), ECF-A tetrapeptides (Lee et al., 1984), and, in hypodense cells, IgE

(Capron et al., 1988b), the majority of which is retained (at least initially) intracellularly (Burke et al., 1990; Cromwell et al., 1990). Equine eosinophils also generate PAF in response to the Ca^{2+} ionophores, A23187 and ionomycin (Asmis and Jorg, 1990).

Lee and colleagues (1982) have reported that the activity of the acetyltransferase that catalyzes the acetylation of lyso-PAF is greater in peripheral blood eosinophils purified from donors with eosinophilia than from normal subjects. However, PAF formation is greater from eosinophils of normal density than from those of the hypodense phenotype (Cromwell et al., 1990). To account for this apparent paradox it is likely that the activity of the acetylhydrolase which deacetylates PAF to lyso-PAF is markedly up-regulated in cells of this phenotype. In addition, Cromwell et al., (1990) noted that the incorporation of [^{3}H]PAF into phosphatidylcholine was greater in low density eosinophils which would tend to limit the detection of free bioavailable PAF.

2. Cyclooxygenase Products. The initial reactions that ultimately result in the formation of prostaglandins, TX and prostacyclin are catalyzed by the enzyme cyclooxygenase. Two molecules of oxygen are initially inserted into AA to form PGG_2 which is subsequently reduced to its 15-hydroxy analog, PGH_2, in an endoperoxidase reaction. Western blotting has established that eosinophils constitutively express cyclooxygenase 1 and cyclooxygenase 2 (Lee et al., 1997) although the relative contribution of these isoforms to prostanoid formation has not been studied. Cowburn and coworkers (1998) also have localized both cyclooxygenase isoforms to eosinophils present within biopsies taken from normal subjects and individuals with asthma.

Depending on the cell type or tissue in question, other enzymes then catalyze the biosynthesis of specific prostanoids. Of the five principle products that can be formed following the cyclooxygenation of AA, E-series prostaglandins (Hubscher, 1975a,b; Bruijnzeel and Verhagen, 1989) and TX (Foegh et al., 1986) are the only metabolites produced to any significant degree by human eosinophils. However, the absolute amounts released are relatively small compared with human macrophages (Foegh et al., 1986) which raises obvious questions of their importance in allergic reactions. The possibility that platelet contamination is responsible for much of the TX release by eosinophils was excluded by Foegh and colleagues (1986) who obtained comparable results from resting and stimulated human eosinophils obtained from the peritoneal cavity of patients undergoing dialysis. In another study, it was reported that the concentration of PGE_2 recovered from the BAL fluid of two patients with eosinophilic pneumonia was increased and that this was positively related to the degree of eosinophilia (Ogushi et al., 1987). Neither PGD_2 nor prostacyclin are primary eosinophil products although a low level of 6-keto-$PGF_{1\alpha}$, the initial metabolite of prostacyclin, is elaborated from human peritoneal eosinophils (Foegh et al., 1986). Similarly, $PGF_{2\alpha}$ is not synthesized by eosinophils to any appreciable extent (Parsons and Roberts, 1988) despite its ability to promote bronchoconstriction and the fact that the plasma concentration of $PGF_{2\alpha}$ is elevated in asthma (Skoner et al., 1988).

Guinea pig peritoneal eosinophils synthesize a similar complement of cyclooxygenase products. Thus, TX and PGE_2 are generated in response to PAF and the Ca^{2+} ionophore A23187 (Hirata et al., 1989; Sun et al., 1989; Giembycz et al., 1990). The effect of PAF is receptor-mediated and reduced, in a concentration-dependent manner by dazmegrel, a TX synthetase inhibitor, with a corresponding increase in the elaboration of PGE_2 indicating that free AA has been diverted to the formation of other prostanoids (Giembycz et al., 1990). C5a, fMLP, and PMA also promote the release of TX and PGE_2 from guinea pig eosinophils (Hirata et al., 1989; Sun et al., 1989; Giembycz et al., 1990).

3. 5-Lipoxygenase Products. Following an appropriate stimulus, 5-lipoxygenase, which resides in the euchromatin region of the nucleus, translocates to the nuclear membrane (Rouzer and Kargman, 1988; Malaviya and Jakschik, 1993; Woods et al., 1994, 1995). There it performs two roles in the early stages of leukotriene generation by a mechanism that requires an additional 18-kDa protein called FLAP (Dixon et al., 1990). First, it catalyzes the formation of 5-HPETE by inserting an oxygen atom into the 5 position of AA; it then transforms 5-HPETE into the unstable epoxide, LTA_4. Depending on species, either LTB_4 or LTC_4 then is formed under the influence of LTA_4 hydrolase or LTC_4 synthetase respectively. Early studies demonstrated that LTC_4 is the major product of the 5-lipoxygenase pathway in human (Weller et al., 1983; Ziltener et al., 1983; Borgeat et al., 1984; Henderson et al., 1984; Shaw et al., 1984; Verhagen et al., 1984; Bruynzeel et al., 1985a,b), equine (Jorg et al., 1982b), and murine (de Andres et al., 1990, 1991) eosinophils, and an 18-kDa LTC_4 synthetase has since been identified in human eosinophils by Western blotting (Penrose et al., 1995). However, guinea pig (Sun et al., 1989; Hirata et al., 1990) and bovine eosinophils (Freiburghaus and Jorg, 1990) lack LTC_4 synthetase and, instead, contain LTA_4 hydrolase, resulting in the predominant formation of LTB_4. Guinea pig and murine eosinophils also have the capacity to synthesize 5-HETE (Turk et al., 1983; Hirata et al., 1989; Sun et al., 1989).

LTC_4 synthetase-positive cells are widely distributed in the airway mucosa of normal individuals and subjects with asthma with occasional clusters colocalizing with $EG2^+$ eosinophils (Cowburn et al., 1998). Indeed, eosinophils are enriched in LTC_4 synthetase and account for approximately 70% of all LTC_4 synthetase-positive cells (Cowburn et al., 1998). Moreover, the expression of this enzyme is elevated in aspirin-intolerant asthmatic subjects in whom aspirin and other nonsteroidal anti-inflammatory agents promote bronchoconstriction (Spec-

tor et al., 1979) through the generation of cysteinyl-leukotrienes (Israel et al., 1993; Austen, 1995; Holgate et al., 1996). FLAP and 5-lipoxygenase similarly have been immunolocalized to eosinophils consistent with their central role in leukotriene biosynthesis (Cowburn et al., 1998).

The development of mature eosinophils from cord blood progenitors is associated with a differential expression of the enzymes that synthesize leukotrienes. Boyce et al. (1996) reported that immature cultured progenitors lacked mRNA and protein for LTC_4 synthetase after 7 days of culture and did not generate LTC_4 in response to A23187 despite the presence of cytosolic PLA_2, FLAP and 5-lipoxygenase. However, at day 14, 94% of cells were of the eosinophil lineage and contained mRNA and protein for LTC_4 synthetase, and responded to A23187 with LTC_4 formation (Boyce et al., 1996). Further maturation of eosinophils up to day 28 then was associated with the enhanced expression of LTC_4 synthetase, FLAP, 5-lipoxygenase, and LTC_4 (Boyce et al., 1996).

Little is known of the intracellular mechanism regulating LTC_4 synthesis and release. However, it is established that LTC_4 is initially produced intracellularly (Owen et al., 1987; Mahauthaman et al., 1988) in human eosinophils stimulated with A23187, which then is exported from the cell via a regulated and saturable mechanism (Lam et al., 1989). In addition, a preliminary report implicates cytosolic PLA_2 in leukotriene formation based on the observation that trifluoromethylketone, a selective inhibitor of this enzyme, blocks fMLP-induced LTC_4 generation (Munoz et al., 1997b).

A number of stimuli have been found to stimulate LTC_4 release from eosinophils, the most potent of these being the Ca^{2+} ionophore A23187 (Table 18). A comparison of A23187-induced LTC_4 release from eosinophils obtained from patients with asthma or related allergic diseases and normal subjects has produced conflicting results although, in general, LTC_4 release is increased in asthma (Taniguchi et al., 1985; Kauffman et al., 1987; Hodges et al., 1988; Schauer et al., 1989, 1990, 1995; Wang et al., 1989; Aizawa et al., 1990; Kohi et al., 1990; Roberge et al., 1990; Bruijnzeel et al., 1993b; Laviolette et al., 1995; Shindo et al., 1996). It is possible that differences in sensitivity to A23287 reflect changes in the density profile of eosinophil populations (Weller, 1993), suggesting that they have been exposed in vivo to priming agents. Indeed, a number of studies have reported that hypodense eosinophils secrete larger quantities of LTC_4 than normodense cells following A23187 stimulation (Hodges et al., 1988; Roberge et al., 1990; Schauer et al., 1990; Bruijnzeel et al., 1993b). In addition, A23187-induced LTC_4 generation in vitro is enhanced by PAF (Schauer et al., 1990; Shindo et al., 1996), fibronectin (Yoshida et al., 1995), and IL-3/IL-5/GM-CSF (Laviolette et al., 1995) in eosinophils obtained from asthmatic subjects but not from normal individuals.

4. 12-Lipoxygenase Products. 12-Lipoxygenase was identified in murine eosinophils in 1983 (Turk et al., 1983) and this was subsequently confirmed (Nakamura et al., 1995). It has been suggested that following the formation of 12-HPETE from AA, a glutathione peroxidase may catalyze the production of 12-HETE (Spector et al., 1988). However, although 12-HETE is produced by both murine (Turk et al., 1983; Brash et al., 1985) and porcine eosinophils (Brash et al., 1985), it is not a major metabolite (Spector et al., 1988). 12-HETE is not well studied but it may be chemotactic for eosinophils (Goetzl et al., 1977; Rand et al., 1982).

5. 15-Lipoxygenase Products. Eosinophils are unique granulocytes in that they express catalytically active amounts of 15-lipoxygenase, in addition to 5-lipoxygenase (Sigal et al., 1988b,c). It has been proposed that 15-lipoxygenase might exist in multiple forms (Izumi et al., 1991) although no differences have been found be-

TABLE 18
Stimuli causing the induction and/or enhancement of LTC_4 release from eosinophils

Stimuli	Potency	Enhancing Factor	Reference(s)
A23187	High	PAF, PMA, TNFα, IL-3, IL-5, GM-CSF, monocyte-derived factors, coculture with endothelial cells	Jorg et al. (1982b); Weller et al. (1983); Ziltener et al. (1983); Borgeat et al. (1984); Henderson et al. (1984); Shaw et al. (1984); Verhagen et al. (1984); Bruynzeel et al. (1985a,b); Dessein et al. (1986); Silberstein et al. (1986); Elsas et al. (1987); Owen et al. (1987); Rothenberg et al. (1987, 1988, 1989); Roubin et al. (1987); Mahauthaman et al. (1988); Tamura et al. (1988); Howell et al. (1989); Burke et al. (1990); Fabian et al. (1992a); Nagata et al. (1995b)
Opsonized zymosan	Low	fMLP, PAF	Bruynzeel et al. (1985a, 1987); Kauffman et al. (1987); Mahauthaman et al. (1988); Burke et al. (1990)
fMLP	Low	TNFα, IL-3, IL-5, GM-CSF	Fitzharris et al. (1986); Owen et al. (1987, 1991); Takafuji et al. (1991, 1992, 1995); White et al. (1993)
		Inhibited by NGF	Takafuji et al. (1992)
PAF	Low		Weller et al. (1983); Kajita et al. (1985); Bruynzeel et al. (1986, 1987); Tamura et al. (1988); Miyagawa et al. (1992); Dent et al. (1998)
IgG-coated particles	Low		Shaw et al. (1985)
IgG *Aspergillus fumigatus* antigen immune complex	Low		Cromwell et al. (1988)
IgG/IgE binding to schistosomula	Low		Moqbel et al. (1990a)

tween the enzyme in human lung or reticulocytes (Sigal et al., 1992). Unlike 5-lipoxygenase, which prefers free AA as substrate, mammalian 15-lipoxygenase will oxygenate, in addition, linoleic acid, polyenoic acids esterified in phospholipids, and even more elaborate lipid complexes such as lipoproteins and biomembranes (Jung et al., 1985; Kuhn et al., 1990; Ford-Hutchinson, 1991; Belkner et al., 1993). Eosinophils also have been reported to preferentially synthesize 13-HODE from linoleic acid (Engels et al., 1996). A FLAP-like docking protein is not required for 15-lipoxygenase activity (Brinckmann et al., 1998).

Human eosinophil 15-lipoxygenase is constitutively expressed by eosinophils and exists predominantly (75–90%) as a cytosolic enzyme (Sigal et al., 1988c; Brinckmann et al., 1998) expressed in close apposition to the plasma membrane (Brinckmann et al., 1998). It has been purified to electrophoretic homogeneity from human eosinophils (Sigal et al., 1988b,c) and the same enzyme from human reticulocytes has been cloned (Sigal et al., 1988a) and expressed in eucaryotic cells. The reticulocyte variant is a 70-kDa protein that is distinct from, but shares homology with, other lipoxygenases, and is now recognized as a member of the LTA_4 synthetase family of proteins (MacMillan et al., 1994). In the presence of phosphatidylcholine or Ca^{2+}, 15-lipoxygenase is stimulated whereas ATP competitively blocks enzyme activation (Sigal et al., 1988b). This mode of regulation differs from the activity of human leukocyte 5-lipoxygenase, which is enhanced by ATP (Rouzer and Samuelsson, 1985). Immunoelectron microscopy of human eosinophils has demonstrated an increase in membrane-associated 15-lipoxygenase in response to A23187. Moreover, RP-HPLC of hydrolyzed lipid membranes resolved 15-HETE in an amount that was significantly greater than that present in untreated eosinophils (Brinckmann et al., 1998). Significantly, 15*S*-HETE dominated over the corresponding 15*R* antipode, indicating that it was formed by 15-lipoxygenase and not nonenzymatically (Brinckmann et al., 1998).

A histological study of biopsies obtained by fiber-optic bronchoscopy has localized 15-lipoxygenase-immunoreactive cells to the airway submucosa of normal and asthmatic subjects (Bradding et al., 1995). The majority (85%) of the cells was eosinophils and the number was elevated in tissue taken from asthmatic individuals (Bradding et al., 1995). Those data are consistent with the localization of the 15-lipoxygenase gene to human eosinophils and confirms that the expression of this gene displays a restricted tissue/cell specificity. It is of interest that the 5′-flanking region of the 15-lipoxygenase gene contains a cluster of three binding sites for the GATA family of transcription factors (O'Prey and Harrison, 1995), providing further support for the latter in eosinophil gene regulation (see Zon et al., 1993; Yamaguchi et al., 1998).

The lipid intermediate 15-HPETE is the primary product produced following the hydroperoxidation of AA acid by 15-lipoxygenase and can be converted subsequently into a number of biologically active mediators including 15-HETE. Studies by a number of investigators have found that the level of 15-HETE produced by human eosinophils or by purified eosinophil 15-lipoxygenase is 100 to 300 times higher than that found in neutrophils, endothelial cells, fibroblasts, and HL-60 cells (Sigal et al., 1988b; Holtzman et al., 1989; Morita et al., 1990a). Those findings support the results of other experiments where 15-HETE and additional 15-lipoxygenase products were identified as the predominant eicosanoids found in human eosinophils (Maas et al., 1981; Turk et al., 1982; Henderson et al., 1984; Brash et al., 1985; Smith et al., 1987; Nadel et al., 1991). Murine eosinophils also produce 15-HETE (Turk et al., 1983).

Other AA-derived 15-lipoxygenase products that have been identified in human, porcine, and murine eosinophils include the dihydroxyeicosatetraenoic acids, 8,15-diHETE and 14,15-diHETE (Maas et al., 1981; Turk et al., 1983; Henderson et al., 1984). Additionally, 5,15-diHETE and the lipoxins also may be produced by the sequential action of 5′- and 15′-lipoxygenases (Holtzman, 1991).

6. Lipoxins. In eosinophil-rich granulocytes stimulated with A23187, the initial stages of lipoxin formation involve the sequential lipoxygenation of free AA by 15- and 5-lipoxygenase that produce 15-HPETE and 5,15-diHPETE, respectively. LXA_4, but not LXB_4, is subsequently produced via the formation of a 5(6)-epoxide tetraene intermediate (Serhan et al., 1987). Why eosinophils fail to synthesize LXB_4 is not understood but this may simply reflect the activities of the endogenous lipoxygenases; alternatively, LXB_4 may normally be formed from intercellular interactions between eosinophils and other leukocytes (Weller, 1993).

Steinhilber and Roth (1989) also have reported that AA- and HETE-treated eosinophils generate another series of lipoxins when exposed to A23187 that they named LXC_4, LXD_4, and LXE_4. These "new" members also are believed to result from sequential lipoxygenation of AA by 5- and 15-lipoxygenase and an additional enzyme, glutathione-*S*-transferase. Thus, LXC_4, like LTC_4, contains glutathione and is formed via conjugation of a 5(6)-epoxide tetraene intermediate. LXD_4 and LXE_4 are produced by sequential peptidolytic cleavage of the glutathione moiety in a manner analogous to the formation of LTD_4 and LTE_4 by reactions catalyzed by γ-glutamyltransferase and dipeptidase, respectively. It is noteworthy that A23187 preferentially promotes LTC_4 formation in human eosinophils unless AA or HETE are present, in which case the lipoxins predominate (Steinhilber and Roth, 1989).

The level of LXA_4 is elevated in the BAL fluid of asthmatic subjects (Lee et al., 1990) which tempts speculation that the eosinophil is a potential source.

7. Lipid Bodies as Sites of Eicosanoid Formation. As described in the previous sections, the nuclear envelope, plasmalemma and/or endoplasmic reticulum, are viewed as primary sites for arachidonyl-containing phospholipids that can be attacked by phospholipases to liberate free AA. However, evidence accrued over the last decade has provided increasing evidence that eicosanoid formation within eosinophils and other leukocytes is further compartmentalized to specialized intracellular cytoplasmic inclusions that have been designated lipid bodies, and it has been hypothesized that the different sites of synthesis might relate to different autocrine and paracrine functions of eicosanoids (Serhan, 1996; Smith et al., 1996a). Low numbers of lipid bodies are normally found in eosinophils. However, in inflammatory disorders including asthma and Crohn's disease and in syndromes associated with hypereosinophilia, lipid bodies increase in size and frequency (Solley et al., 1976; Weller and Dvorak, 1985; Weller et al., 1991a; Beil et al., 1995; Bozza et al., 1997a,b, 1998), providing increased substrate for the liberation of free AA.

Lipid bodies are not simply formed in response to increased availability of lipid precursors. *cis*-Unsaturated fatty acids, proinflammatory mediators, such as PAF, and pharmacological agents that activate PKC all effectively promote lipid body formation (Weller et al., 1989, 1991a; Bozza et al., 1996a,b, 1998). In neutrophils and eosinophils, PAF promotes lipid body formation in a concentration-dependent manner by a mechanism that is blocked by apafant and PTX, indicating that activation of G_i-coupled, cell surface receptors mediate this effect (Bozza et al., 1996a, 1997b). Moreover, lyso-PAF, the direct precursor and metabolite of PAF that does not have efficacy at the PAF receptor, is inactive (Bozza et al., 1996a, 1997a). Curiously, other agonists that act at G protein-linked receptors including LTB_4, C5a, fMLP, and IL-8 are without effect (Bozza et al., 1996a), implying that specific signal transduction pathways need to be recruited for effective lipid body formation. In this respect, PLC and PKC seem to play a central role as inhibitors of these enzymes suppress lipid body generation evoked by PAF and *cis*-unsaturated fatty acids (Weller et al., 1991a; Bozza et al., 1996a,b, 1997a, 1998). In addition, actinomycin D and cycloheximide abolish induced lipid body formation in eosinophils, indicating a requirement for new protein synthesis (Bozza et al., 1996a,b, 1997a). Phosphorylation on tyrosine residues also is important for PAF-induced lipid body formation in eosinophils (Bozza et al., 1998). Curiously, this mechanism is restricted to cells harvested from individuals with hypereosinophilic syndrome although sensitivity to tyrosine kinase inhibitors can be conferred to normal eosinophils after their culture in a mixture of IL-3, IL-5, and GM-CSF (Bozza et al., 1998). Thus, lipid body formation can be differentially regulated.

Additional and necessary evidence to support a role of lipid bodies in localized eicosanoid formation in eosinophils derives from several pieces of evidence. Electron microscopic autoradiography has demonstrated that [^{3}H]AA is incorporated into lipid bodies where it is esterified almost totally in glycerolipids, primarily phosphatidylcholine and phosphatidylinositol (Weller and Dvorak, 1985; Weller et al., 1991a). Furthermore, immunocytochemistry allied with immunoblotting has established that many of the enzymes required for AA release and metabolism are colocalized in lipid bodies including cytosolic PLA_2, 5-lipoxygenase, 15-lipoxygenase, cyclooxygenase, and LTC_4 synthetase (Dvorak et al., 1992b, 1994; Weller and Dvorak, 1994; Bozza et al., 1997a, 1998). In U937 promonocytic cells, a number of upstream enzymes that are known to regulate cytosolic PLA_2 including ERK-1, ERK-2, and p38 MAP kinase also have been localized to lipid bodies (Yu et al., 1998).

Significantly, studies with eosinophils and neutrophils have demonstrated a positive correlation between PAF- and *cis*-unsaturated fatty acid-induced lipid body formation and the elaboration of cyclooxygenase and 5-lipoxygenase products. Conversely, agents that suppress lipid body formation, such as actinomycin D and cycloheximide, attenuate this response (Bozza et al., 1996a,b, 1997a). The changes in eicosanoid biosynthesis appear to be specifically related to the number of lipid bodies within cells, rather than to changes in the nuclear pool of eicosanoid-forming enzymes, since identical results are obtained in enucleated eosinophil cytoplasts (Bozza et al., 1997a).

Pharmacological experiments have been performed on the regulation of lipid body formation, given their putative role in inflammation. In neutrophils, the induction of lipid bodies by PAF is blocked by the 5-lipoxygenase inhibitor, zileuton, and the FLAP antagonist MK886 (Bozza et al., 1996a). In contrast, lipid body formation evoked by *cis*-unsaturated fatty acids is independent of 5-lipoxygenase but is inhibited by nonsteroidal anti-inflammatory drugs such as indomethacin and aspirin. This effect does not involve cyclooxygenases 1 or 2 because sodium salicylate, which does not block prostanoid formation, is equally active (Bozza et al., 1996b).

D. Generation of Cytokines

mRNA transcripts and protein for a plethora of cytokines have been identified in human eosinophils (see Table 4). However, a comparison of the literature clearly highlights marked differences between independent investigations (see below). Perhaps this is not surprising given that the source (e.g., blood, BAL fluid, tissue) of eosinophils varies markedly between studies. Moreover, disease status clearly will have a major impact on the expression of certain cytokines possibly through the generation of phenotypically heterogeneous eosinophil populations akin to Th_1 and Th_2 $CD4^+$ T lymphocytes originally described in the mouse.

1. Interleukin-1α. Protein and mRNA transcripts for IL-1α have been detected in eosinophils obtained from

hypereosinophilic human donors that are up-regulated by the phorbol diester PMA (Weller et al., 1993). Studies with murine eosinophils have shown that IL-1α mRNA is detected 6 h after treatment with LPS while continued exposure (18–24 h) of the cells results in extracellular protein release (del Pozo et al., 1990).

2. Interleukin-2. Both IL-2 mRNA and protein have been detected in circulating human eosinophils (Bosse et al., 1996; Levi Schaffer et al., 1996). Using the technique of immunocytochemistry, Levi Schaffer et al. (1996) found that 6.8% of eosinophils from atopic asthmatic individuals exhibited granular staining, whereas the frequency increased to 36% in severe atopic asthmatic subjects (Bosse et al., 1996). IL-2 is localized to the crystalloid core of the secondary granules (Levi Schaffer et al., 1996) where it is seemingly stored together with other cytokines and MBP (Fig. 11). Freshly prepared, unstimulated eosinophils contain approximately 6 pg of IL-2/10^6 cells that is reported to increase to 26 pg/10^6 cells in response to serum-coated Sephadex beads; a small amount of IL-2 (2 pg/10^6 cells) also is released extracellularly (Levi Schaffer et al., 1996).

3. Interleukin-3. Freshly prepared human eosinophils from nonatopic, nonasthmatic subjects do not express IL-3 mRNA (Nakajima et al., 1996). However, prolonged exposure of the same cells to ionomycin (Kita et al., 1991d), IFN-γ (Fujisawa et al., 1994), and IL-13 (Horie et al., 1997b), as well as VLA-4-dependent binding to immobilized fibronectin (Anwar et al., 1993; G. M. Walsh et al., 1995) all have been shown to stimulate the release of IL-3 indicative of gene induction. In each investigation, IL-3 was identified indirectly by the demonstration that neutralizing anti-IL-3 antibodies blocked the enhancement of eosinophil survival. TNFα and immobilized immunoglobulins also promote IL-3 mRNA expression by a mechanism that is enhanced by IL-5 (Nakajima et al., 1996).

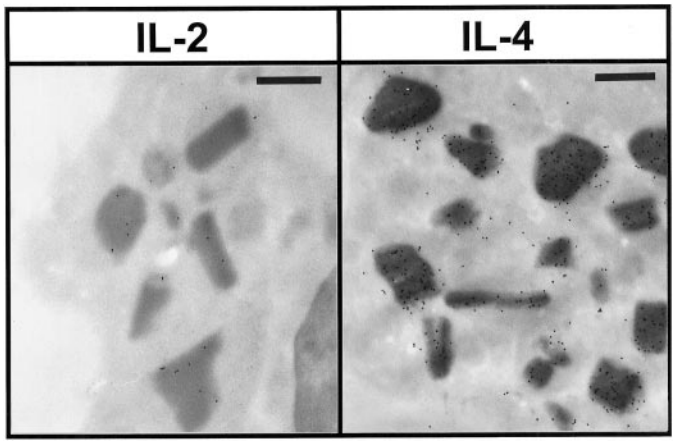

FIG. 11. Immunogold labeling of embedded intact purified human eosinophils of IL-2- and IL-4-like immunoreactivity. Note the preferential distribution of gold particles over the specific granules. Bars: IL-2, 500 nm. Original magnification, 38,000×; IL-4, 500 nm. Original magnification, 35,000×. See *XII.D* for further details.

4. Interleukin-4. A number of independent investigators have reported that IL-4 mRNA and protein are expressed in peripheral blood and tissue eosinophils (Moqbel et al., 1995; Nonaka et al., 1995; Bjerke et al., 1996; Moller et al., 1996a; Nakajima et al., 1996; Ying et al., 1997). Experiments aimed at localizing IL-4 protein have established that it is costored within the electron-dense crystalloid core of the secondary granules (Moqbel et al., 1995; Moller et al., 1996a) with IL-2 and GM-CSF (Fig. 11). In one study, 22% of eosinophils taken from atopic asthmatic subjects were IL-4^+ and contained approximately 108 pg/10^6 cells (Moqbel et al., 1995). An examination of skin biopsies obtained from atopic individuals during the LPR (Moqbel et al., 1995) and from nasal polyp tissue (Nonaka et al., 1995) suggests that the inflammation is associated with the infiltration of IL-4-expressing eosinophils. Thus, Moqbel et al. (1995) and Nonaka et al. (1995), respectively, demonstrated that 85% of eosinophils obtained from skin biopsies and up to 44% of the eosinophils in nasal polyps were IL-4^+. Freshly isolated circulating eosinophils purified from normal donors also constitutively express IL-4 albeit at a lower level (20–25 pg/10^6 cells). However, culture of those cells for 24 h in medium alone, or in the presence of stimuli (secretory IgA, IgG) that can evoke degranulation, results in the loss of stored IL-4 without a commensurate release into the extracellular medium (Nakajima et al., 1996). In contrast, secretory IgA immune complexes and serum-coated particles increase both IL-4 mRNA expression in (Nonaka et al., 1995) and release from (Moqbel et al., 1995; Nonaka et al., 1995) eosinophils obtained from allergic individuals. The reason for this discrepancy is unclear although it is possible that the secreted IL-4 from "normal" eosinophils is, for some reason, more rapidly degraded by eosinophil-derived proteinases than the IL-4 released from "allergic" cells (Nakajima et al., 1996). Intriguingly, IL-5 attenuates the reduction in IL-4 seen in cells cultured in medium alone, suggesting that it enhances the biosynthesis and/or the storage of IL-4 (Nakajima et al., 1996).

The magnitude of IL-4 release from human eosinophils clearly suggests that it is of physiological relevance (Moqbel et al., 1995; Nonaka et al., 1995). It is well established that IL-4 can enhance the local production of IgE, up-regulate VCAM-1 expression upon endothelial cells and induce the local chemotaxis of eosinophils (see *XII.A.3*). In addition, a study using mice infected i.p. with *Schistosoma mansoni* eggs showed that eosinophil-derived IL-4 rises rapidly which has lead to the suggestion that it may be required for the priming of T lymphocytes and the expression of the Th2 phenotype (Sabin et al., 1996).

5. Interleukin-5. The hematopoeitic cytotokine IL-5 has been detected at the mRNA and protein level in blood and tissue eosinophils obtained from patients with a range of inflammatory diseases. Initial studies were able to demonstrate IL-5 mRNA in the eosinophils infil-

trating the mucosa of four patients with coeliac disease as well as in the circulating cells of three of those individuals (Desreumaux et al., 1992). Similarly, Broide et al. (1992) found that 68% of eosinophils recovered from the BAL fluid of asthmatic subjects following allergen challenge contained IL-5 mRNA. In other investigations, IL-5 mRNA and protein have been localized to eosinophils found in heart sections from patients with eosinophilic endomyocarditis (Desreumaux et al., 1993) and in blood and tissue eosinophils obtained from individuals with eosinophilic cystitis and hypereosinophilic disease (Dubucquoi et al., 1994). In contrast to the aforementioned studies, IL-5 mRNA was not detected in circulating eosinophils obtained from normal individuals (Nakajima et al., 1996) or, surprisingly, from subjects with Crohn's disease (Dubucquoi et al., 1994). Thus, subpopulations of eosinophils may exists (Lamkhioued et al., 1995b) that can be distinguished on the basis of their cytokine profiles.

Cell localization studies have established that IL-5 is stored within the crystalloid core of the secondary granules (Dubucquoi et al., 1994; Moller et al., 1996b) and can be released following incubation of eosinophils with IgA, IgE, and IgG immune complexes, or following VLA-4-mediated adherence to fibronectin-coated plates (Dubucquoi et al., 1994; G. M. Walsh et al., 1995).

6. Interleukin-6. IL-6 mRNA is constitutively expressed in circulating eosinophils obtained from normal and hypereosinophilic individuals (Hamid et al., 1992; Melani et al., 1993) where it is stored within the matrix of the specific granules (Lacy et al., 1998). Hamid et al., (1992) have reported that culture of human eosinophils for 24 h with IFNγ significantly increases the elaboration of IL-6 into the surrounding medium; presumably this effect is the result of the associated increase in the number of eosinophils expressing IL-6 mRNA and stored protein. Interestingly, up-regulation of IL-6-like immunoreactivity in human eosinophils occurs very rapidly (within minutes) which might point to an important action of IFNγ in the translation of preexisting IL-6 mRNA (Lacy et al., 1998).

7. Interleukin-10. The inhibitory cytokine, IL-10, is constitutively expressed in some, but not all, human circulating eosinophils at the mRNA (Lamkhioued et al., 1995b) and protein level (Lamkhioued et al., 1995b; Nakajima et al., 1996). However, consistent with data obtained for IL-4 (Nakajima et al., 1996), culture of eosinophils for 24 h in medium alone or medium supplemented with immobilized secretory IgA or IgG resulted in a loss of intracellular IL-10 in the absence of extracellular release. Similar experiments performed in the presence of IL-5 prevented the loss of IL-10 in eosinophils incubated with media and TNFα [but not immobilized secretory IgA or IgG (Nakajima et al., 1996)].

8. Interleukin-12. Neither atopic nor nonatopic individuals constitutively express mRNA for the p35 or p40 subunits of IL-12 and this is reflected by little, if any, secreted IL-12 protein (Grewe et al., 1998). However, significant mRNA and biologically active IL-12 expression has been observed following culture of eosinophils with IL-4, GM-SCF, and, to a lesser extent, TNFα and IL-1 (Grewe et al., 1998). IL-5 also is active in this respect in some individuals (Grewe et al., 1998). In contrast, RANTES selectively up-regulates mRNA for the p40 subunit of IL-12 without having an effect on p35 message or protein (Grewe et al., 1998). Based on these data it is logically speculated that eosinophil-derived IL-12 may switch the immune response from a Th1 to a Th2-like state (Grewe et al., 1998) although this outcome is not inevitable since IL-12 can enhance chemokine generation in certain circumstances (Pearlman et al., 1997). See *XI.E* for further details.

9. Interleukin-16. In 1996 a "lymphocyte chemotactic factor" was identified in the supernatants of cultured eosinophils and was shown, by use of neutralizing antibodies, to be IL-16 (Lim et al., 1996). Constitutive expression of mRNA transcripts and protein was subsequently found in human eosinophils (Lim et al., 1996) and a more contemporary investigation established that IL-16 is present in the airways of asthmatic subjects after histamine challenge, indicating a possible role in the early infiltration of $CD4^+$ T cells (Mashikian et al., 1998). Similar data have been reported in a murine model of allergic asthma where IL-16 appears to be involved in up-regulating IgE production and in airways hyperresponsiveness (Hessel et al., 1998).

10. Interferon-γ. Lamkhioued and colleagues (1995b) have demonstrated that some eosinophils express mRNA and protein for IFN-γ. By use of double in situ hybdridization or double immunostaining, the same investigators reported that eosinophils never coexpressed IL-5 and IFNγ, tempting speculation of different eosinophil phenotypes secreting distinct patterns of cytokines.

11. Tumor Necrosis Factor α. mRNA for TNFα has been detected in eosinophils present within nasal polyps (Costa et al., 1993; Finotto et al., 1994), the intestinal mucosa of patients with necrotizing enterocolitis (Tan et al., 1993), and the circulation of normal and hypereosinophilic subjects (Costa et al., 1993; Nakajima et al., 1996). Consistent with these data is the demonstration of TNFα in circulating eosinophils obtained from hypereosinophilic patients (Costa et al., 1993), where it has been immunolocalized to the matrix of the secondary granules (Beil et al., 1993). Moreover, TNFα is spontaneously secreted by human eosinophils in a cycloheximide-sensitive manner, indicating de novo translation of mRNA (Costa et al., 1993), and this effect is enhanced by LPS (Takanaski et al., 1994). Curiously, TNFα has not been found in circulating eosinophils purified from "normal" individuals (Beil et al., 1993), suggesting that the blood of subjects with hypereosinophilia contains stimuli that promote translation of TNFα mRNA transcripts.

12. Granulocyte/Macrophage Colony-Stimulating Factor. mRNA transcripts for GM-CSF have been detected in circulating eosinophils of both normal (Nakajima et al., 1996) and atopic asthmatic individuals (Moqbel et al., 1991), although the corresponding immunoreactive protein has not always been found even after allergen challenge (O'Sullivan et al., 1996). However, it is reported that a significant number (45%) of eosinophils recovered from the BAL fluid of allergen-challenged asthmatic subjects give a positive signal for GM-CSF mRNA (Broide et al., 1992; Sullivan and Broide, 1996). Similarly, a related study found that 30% of eosinophils in tissue sections prepared from individuals with nasal polyposis expressed GM-CSF mRNA (Ohno et al., 1991). On balance, those data might indicate that relatively few eosinophils are GM-CSF$^+$ in the blood of normal volunteers and subjects with allergy but that expression increases upon their migration into tissue where the autocrine production of GM-CSF is required to prolong longevity.

Eosinophils obtained from atopic asthmatics contain about 15 pg/10^6 of GM-CSF that is localized to, and apparently stored within, the core of secretory granules (Levi Schaffer et al., 1995). Moreover, the release of GM-CSF from eosinophils can be effected by ionomycin (Kita et al., 1991d), LPS (Takanaski et al., 1994), IFNγ (Moqbel et al., 1991), ligation of CD40 (Ohkawara et al., 1996), and CD9 (Kim et al., 1997) or following adherence of eosinophils to immobilized fibronectin (Anwar et al., 1993; G. M. Walsh et al., 1995).

13. Macrophage Migration Inhibitory Factor. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine secreted by a variety of cells including human eosinophils (Rossi et al., 1998). Unstimulated eosinophils do not constitutively secrete MIF but the phorbol diester PMA and more physiological stimuli (IL-5 and C5a) are effective secretogogues. PMA-induced MIF secretion is significantly attenuated by the protein synthesis inhibitor cycloheximide, and an inhibitor of PKC, Ro 31-8220. In the context of airways inflammation, it is significant that the BAL fluid of asthmatic subjects contains elevated levels of MIF when compared to that of nonatopic normal volunteers. The possibility that MIF plays a pathogenic role in eosinophil-based inflammatory disorders merits investigation.

E. Generation of Chemokines

1. Interleukin-8. Attempts to demonstrate the presence of IL-8 in human eosinophils have yielded inconsistent data. For example, Yousefi et al. (1995) found that IL-8 mRNA and protein were constitutively expressed in eosinophils purified from the blood of normal subjects and that the latter was elevated in eosinophils obtained from patients with bronchial asthma and atopic dermatitis. However, a subsequent investigation failed to verify the presence of IL-8 mRNA in normal eosinophils and reported only very low levels of immunoreactive protein (Nakajima et al., 1996). Despite these marked discrepancies, eosinophils are capable of synthesizing IL-8 in response to appropriate stimuli. Thus, immobilized secretory IgA or IgG, soluble secretory IgA, and TNFα all increase IL-8 mRNA copy number and evoke secretion which is augmented by IL-5 and suppressed by actinomycin D (Nakajima et al., 1996). Similarly, ionomycin promotes a robust secretion of IL-8 from human eosinophils, in an amount significantly greater than IL-3 and GM-CSF, and is accompanied by a cycloheximide-sensitive appearance of IL-8 mRNA (Braun et al., 1993). A number of other stimuli promote IL-8 release, including LPS (Takanaski et al., 1994), MBP (Kita et al., 1995) and, in the presence of cytochalasin, B, C5a, and fMLP (Miyamasu et al., 1995). C5a-induced IL-8 secretion is enhanced in eosinophils primed with IL-3 and IL-5 (Miyamasu et al., 1997).

2. Macrophage Inflammatory Protein 1α. Macrophage inflammatory protein-1α mRNA has been identified in 39 to 91% of circulating eosinophils obtained from patients with hypereosinophilic syndrome as well as in most eosinophils found in nasal polyp tissue (Costa et al., 1993). However, these results contrast with the very low expression of MIP-1α mRNA in circulating eosinophils from normal patients (Costa et al., 1993).

3. RANTES. Human eosinophils have the capacity to synthesize, store, and secrete biologically active concentrations of RANTES. Constitutive expression of mRNA (and/or protein) has been reported by a number of investigators (Lim et al., 1996; Ying et al., 1996, Nakajima et al., 1996), and it has been estimated that atopic individuals contain approximately 7 ng of RANTES/10^6 blood eosinophils and that approximately 24% of that is released in response to serum-coated particles (Ying et al., 1996). In the context of allergic reactions, it is significant that a study by Ying and colleagues (1996) found that 7 to 10% and 4 to 17% of eosinophils expressed RANTES mRNA and protein, respectively, and that this was increased to 22 to 30% and 11 to 20% following in vitro stimulation (16 h) with IFNγ. The expression of RANTES mRNA also has been observed in sensitized human subjects. Sequential immunocytochemistry and in situ hybridization on biopsies from allergen-induced cutaneous LPRs showed that 55 to 75% of the infiltrating RANTES$^+$ cells were EG2$^+$ eosinophils (Ying et al., 1996). Eosinophils might, therefore, represent an important source of RANTES with relevance to allergic inflammation.

4. Eotaxin. Nakajima et al. (1998) have reported that human eosinophils from normal, nonatopic individuals express granule-associated eotaxin that is released by a number of secretogogues including C5a and ionomycin. The finding that eosinophils also store IL-5 strongly supports the idea that eosinophil-derived eotaxin and IL-5 could contribute to the local accumulation of eosinophils to inflammatory loci and to their enhanced survival.

F. Generation of Growth Factors

1. Transforming Growth Factor α. Transforming growth factor α was first identified in eosinophils within the interstitial tissue adjacent to colonic adenocarcinomas and oral squamous cell carcinomas, and subsequent experiments, measuring mRNA, extended that finding to 80% of circulating eosinophils in patients with idiopathic hypereosinophilic syndrome (Wong et al., 1990). However, evidence both for (Walz et al., 1993; Brach et al., 1994) and against (Wong et al., 1990) constitutive expression of TGFα in eosinophils from normal healthy individuals has been published, suggesting that factors other than disease can govern its biosynthesis. In vivo, TGFα mRNA-expressing eosinophils have been found in eosinophil-associated wound healing in rabbit (Todd et al., 1991) and hamster (Wong et al., 1993) skin and in the hamster cheek pouch mucosa during malignant transformation (Elovic et al., 1990; Ghiabi et al., 1992). Similarly, the majority of eosinophils in tissue sections of nasal polyps express both TGFα mRNA and immunoreactive protein (Elovic et al., 1994). Little is known of the regulation of TGFα synthesis and secretion although Brach et al. (1994) and Walz et al. (1994) have found that certain cytokines (IL-3, IL-5, GM-CSF) relevant to the pathogenesis of Th2-driven immune responses enhance TGFα mRNA and protein expression.

2. Transforming Growth Factor β_1. mRNA transcripts and protein for the multifunctional cytokine TGF-β_1 have been found in peripheral blood eosinophils of patients with idiopathic hypereosinophilic syndrome and asthma (Wong et al., 1991; Ohno et al., 1992, 1996). In addition, the complementary techniques of in situ hydridization and immunocytochemistry have been used to localize TGFβ_1 mRNA and protein to tissue eosinophils associated with nasal polyposis (Elovic et al., 1994) and nodular sclerosing Hodgkin's disease (Kadin et al., 1993). Those observations have led to the proposal that eosinophil-derived TGFβ_1 may contribute to some of the pathological alterations (thickening of epithelial basement membrame, glandular hyperplasia, stromal fibrosis, angiogenesis) observed in nasal polyposis and in the histogenesis of nodular sclerosing Hodgkin's disease. Other abnormalities where eosinophil-derived TGFβ_1 is seen include severe asthma, where it is overexpressed in bronchial biopsies, and in a hamster model of eosinophil-associated wound healing (Wong et al., 1993). Circulating eosinophils from normal healthy subjects do not express TGFβ protein (Wong et al., 1991).

3. Platelet-Derived Growth Factor. Asthma and nasal polyposis can be associated with tissue remodeling, which include stromal deposition of extracellular matrix proteins, airways smooth muscle hypertrophy and hyperplasia, and subbasement deposition of collagen. Of the varied mediators that can contribute to this metamorphosis, PDGF is believed to be pivotal, and the eosinophil is likely to be a rich source of this growth factor given the high number present within these tissues. Indeed, almost all resident eosinophils in nasal polyps and lung biopsies taken from severe asthmatic sufferers express mRNA for the B chain of PDGF (Ohno et al., 1995). Regulation of the *PDGF* gene is little studied although the Ca^{2+} ionophore A23187 significantly increases PDGF-B mRNA transcripts and immunoreactivity in peripheral blood eosinophils (Ohno et al., 1995).

4. Heparin-Binding, Epidermal Growth Factor-Like Growth Factor. Heparin-binding, epidermal growth factor-like growth factor (HB-EGF), a member of the EGF family of growth factors, is a mitogen for fibroblasts and smooth muscle cells. Powell and colleagues (1993) have shown by histochemistry that following the induction of pulmonary hypertension in rats exposed to high oxygen and normobaric pressure there is eosinophil accumulation and clustering around lung microvessels and a 100-fold increase in the expression of HB-EGF mRNA transcripts. The possibility that eosinophil-derived HB-EGF contributes to the thickening of lung microvessels by stimulating vascular smooth muscle hypertrophy and hyperplasia has been suggested (Powell et al., 1993).

5. Vascular Endothelial Growth Factor. Horiuchi and Weller (1997) have demonstrated that freshly prepared eosinophils from healthy volunteers constitutively express mRNA and protein for VEGF, which also is known as vascular permeability factor. VEGF is a multifunctional cytokine existing as four splice variants that exert a variety of important actions on the vasculature and endothelium. In particular, VEGF is a mitogen for endothelial cells and a highly potent angiogenic agent involved in tumor and normal physiological angiogenesis. Exposure of eosinophils to GM-CSF and IL-5 increases mRNA expression and the extracellular release of VEGF by a mechanism that is attenuated by the inhibitor of transcription, actinomycin D. Several pharmacological agents including a glucocorticoid (dexamethasone), a protein-tyrosine kinase inhibitor (genistein), and a protein kinase C inhibitor (chelerythrine) also have been shown to attenuate gene induction and secretion of VEGF (Horiuchi and Weller, 1997). Of the four splice variants of VEGF identified, eosinophils contain transcripts mainly for the 121- and 165-amino acid isoforms. It has been suggested that cytokine-activated eosinophils are an important source of VEGF and might contribute to edema formation at sites of inflammation and to airways remodeling due to its angiogenic properties (Horiuchi and Weller, 1997).

6. Nerve Growth Factor. Recent studies have shown that NGF, a polypeptide originally discovered in connection with its neurotrophic activity, is found in various immune organs and leukocytes including eosinophils where it is stored (see Aloe et al., 1997; Solomon et al., 1998). Moreover, circulating levels of NGF are elevated in parasitic infections and in various autoimmune and allergic diseases (for review, see Aloe et al., 1997).

G. Activation of the NADPH Oxidase

The NADPH oxidase (EC 1.23.45.3) catalyzes the single electron reduction of molecular O_2 to superoxide, a powerful oxidizing and reducing agent (Babior et al., 1973). In the presence of superoxide dismutase, superoxide anions dismutate to H_2O_2 which subsequently can be converted into hypobromous acid in the presence of EPO and bromide (Weiss et al., 1986; Mayeno et al., 1989; Thomas et al., 1995) (Fig. 12). Alternatively, in the presence of ferrous ions, superoxide anions and H_2O_2 interact to form the membrane-perturbing hydroxyl radical, one of the most unstable oxidizing species known (see Fig. 12). Other pathways of free radical formation also have been described including the reaction of superoxide anions with nitric oxide to form peroxynitrite which provides an additional, iron-independent route of hydroxyl radical formation along with nitrogen dioxide radicals (see Fig. 12). Hypobromous acid is able to interact with H_2O_2 to form singlet oxygen (see Fig. 12), the biological significance of which is currently unclear (Kanofsky et al., 1988). Activation of the NADPH oxidase and the subsequent production of toxic oxygen radicals are thought to be relevant to eosinophils in host defense (Butterworth and Thorne, 1993). However, it is now appreciated that activation of the NADPH oxidase may be cytotoxic to many mammalian cells, particularly those of the gut, skin, and lung, and has implicated eosinophils in the pathogenesis of a number of nonparasitic inflammatory disorders, including Crohn's disease, atopic dermatitis, and allergic asthma (Butterworth and Thorne, 1993). Indeed, the activity of the NADPH oxidase is significantly higher in eosinophils than in other phagocytes (Learn and Brestel, 1982; Shult et al., 1985; Yamashita et al., 1985; Petreccia et al., 1987; Yazdanbakhsh et al., 1987a; Sedgwick et al., 1988; Yagisawa et al., 1996).

Human and guinea pig eosinophils in suspension undergo a rapid and transient activation of the NADPH oxidase in response to a range of soluble and particulate stimuli (Table 19). Preincubation of eosinophils with subthreshold concentrations of PAF, IL-3, IL-5, and GM-CSF primes the NADPH oxidase to activation by opsonized particles (Mabuchi et al., 1992; Tool et al., 1992; van der Bruggen et al., 1993a) and fMLP (Zoratti et al., 1992; Nagata et al., 1995b). Recent studies have demonstrated a similar priming of human eosinophils adherent to tissue culture plates coated with a range of extracellular matrix proteins (e.g., fibronectin, fibrinogen, collagen, laminin) and fetal calf serum. Under those conditions, the cytokines TNFα and GM-CSF, which are unable to stimulate the NADPH oxidase in nonadherent cells, produce a slowly developing and sustained generation of superoxide anions (Dri et al., 1991; Horie and Kita, 1994). Moreover, antibodies directed against CD18 and CD11b block this effect (Horie and Kita, 1994), suggesting a central role for CR3. That conclusion was confirmed in subsequent studies following the observation that CD18-mediated adhesion primed eosinophils for fMLP-induced respiratory burst (Nagata et al., 1995a) and that antibody ligation of β_1 and β_2 integrins promoted the generation of oxygen-derived free radicals (Laudanna et al., 1993). In addition to CR3-mediated adhesion, anti-VCAM-1-coated plates were shown to induce eosinophil adherence and NADPH oxidase activation although the response to fMLP was dependent upon CD18 (Nagata et al., 1995a). There are no studies concerning the biochemical mechanism of NADPH oxidase activation in adherent eosinophils and the data in this

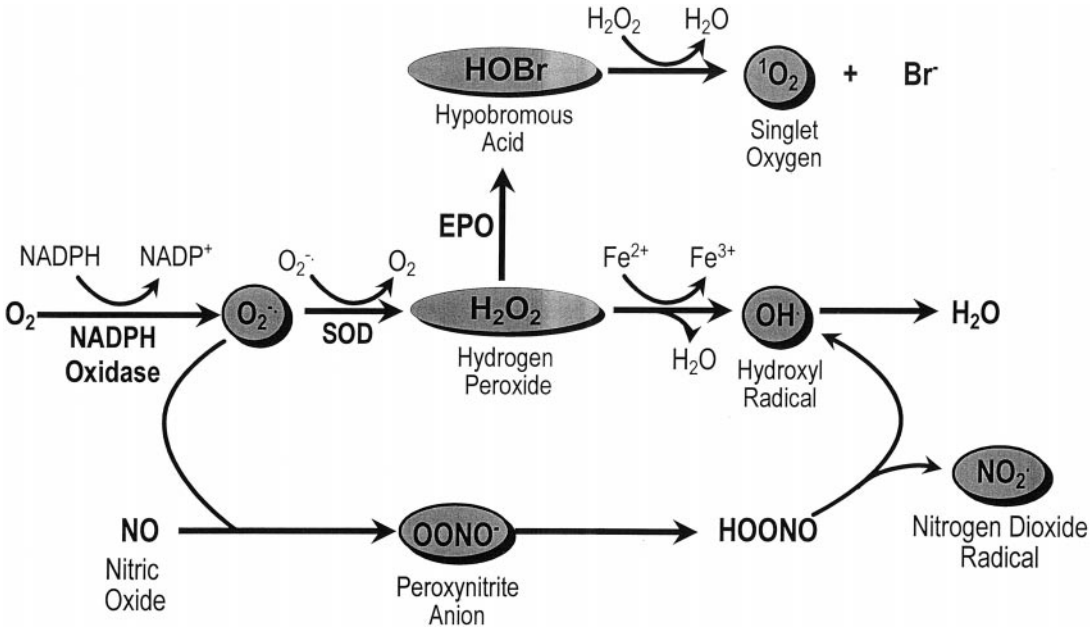

FIG. 12. Route of formation of free radicals in eosinophils and other phagocytes following the activation of the NADPH oxidase and subsequent generation of superoxide anions. See *XII.G* for further details.

TABLE 19
Stimuli that activate the NADPH oxidase in eosinophils

Stimulus	Species	Reference(s)
LTB_4	Human	Palmblad et al. (1984)
	Guinea pig	Maghni et al. (1991); Ng et al. (1991); Rabe et al. (1992); Subramanian (1992); Perkins et al. (1995); Lindsay et al. (1998a,b,c); Perkins et al. (1995)
PAF	Human	Kroegel et al. (1989c); Chanez et al. (1990); Mabuchi et al. (1992); Elsner et al. (1995); Zeck Kapp and Kapp (1995); Wymann et al. (1995); Dent et al. (1998)
	Guinea pig	Kroegel et al. (1989a); Shute et al. (1990)
	Rat	Cypcar et al. (1996)
fMLP	Human	Beswick and Kay (1981); Palmblad et al. (1984); Wymann et al. (1995)
	Rat	Cypcar et al. (1996)
	Guinea pig	Kroegel et al. (1990)
C5a	Human	De Simone et al. (1986b); Elsner et al. (1995, 1996d); Wymann et al. (1995); Zeck Kapp and Kapp (1995)
RANTES	Human	Rot et al. (1992); Chihara et al. (1994); Kapp et al. (1994); Elsner et al. (1995)
5-ETE/5-HETE	Human	Czech et al. (1997)
MCP-4	Human	Petering et al. (1998)
IL-8	Human	Wymann et al. (1995)
Eotaxin	Human	Elsner et al. (1996b); Tenscher et al. (1996)
Eotaxin-2	Human	Elsner et al. (1998)
Opsonized particles	Human	Petreccia et al. (1987); Koenderman et al. (1990); Shute et al. (1990)
	Rat	Cypcar et al. (1996)

section focuses predominantly on studies conducted with nonadherent cells.

In neutrophils, an active NADPH oxidase complex assembles at the phagocytic and plasma membranes following activation (Segal and Abo, 1993). At least five proteins are required for the formation of an active oxidase complex: the membrane-bound cytochrome b_{558} (consisting of two subunits gp91^{phox} and p22^{phox}) and the cytosolic proteins p47^{phox}, p67^{phox}, and a small GTP-binding protein, Rac-1 or Rac-2 (Bokoch, 1994). Recently, two additional components have been identified, these being the cytosolic protein, p40 ^{phox}, which appears to be associated with p67^{phox} (Wientjes et al., 1993; Tsunawaki et al., 1994), and a membrane-associated small GTP-binding protein, Rap1a (Gabig et al., 1995). Under resting conditions, the cytosolic components exist as a 240- to 300-kDa oligomer (Park et al., 1992,1994). Following activation, translocation of these components to the membrane-bound cytochrome b_{558} and assembly of the active oxidase complex is thought to be mediated by a mechanism involving both protein binding through SH3 domains and phosphorylation of p47^{phox} (Rodaway et al., 1990; McPhail, 1994; Park and Ahn, 1995; de Mendez et al., 1996). In eosinophils, evidence for a similar, if not identical, mechanism of oxidase assembly and activation also is available. Thus, the cytosolic components p47^{phox}, p67^{phox}, p40^{phox}, and membrane components p22^{phox} and gp91^{phox} have been identified (Segal et al., 1981; Kuribayashi et al., 1995; Yagisawa et al., 1996; Zhan et al., 1996), whereas p47^{phox} and p67^{phox} have been shown to reconstitute NADPH oxidase activity in cell-free systems prepared from both neutrophil and eosinophil fractions (Bolscher et al., 1990; Someya et al., 1991).

At present, little is known of the intracellular mechanisms responsible for NADPH oxidase activation in eosinophils. Previous studies have demonstrated a rapid and transient increase in both Ins(1,4,5)P_3 and $[Ca^{2+}]_i$ following exposure of guinea pig and human eosinophils to LTB_4, PAF and fMLP (Kroegel et al., 1991; Perkins et al., 1995; Wymann et al., 1995). Furthermore, human eosinophils release DAG following stimulation with opsonized particles (Koenderman et al., 1990). However, the generation of oxygen-derived free radicals is only marginally suppressed in Ca^{2+}-depleted cells, suggesting that an increase in $[Ca^{2+}]_i$ does not play a major role (Subramanian, 1992; Perkins et al., 1995; Wymann et al., 1995). This conclusion is supported by studies showing that LTB_4- and PAF-induced NADPH oxidase activation in guinea pig eosinophils occurs at concentrations greater than 100-fold higher than those required for Ca^{2+} mobilization (Kroegel et al., 1989a; Lindsay et al., 1998c). Similarly, although phorbol diesters are potent and robust stimulants of oxidase activation (Petreccia et al., 1987; Rossi et al., 1989; Yoshie et al., 1989; Perkins et al., 1995), the PKC inhibitors, Ro-31 8220 (Perkins et al., 1995) and 1-*O*-hexadecyl-2-*O*-methylglycerol (Rabe et al., 1992), only partially inhibit (by 20–30%) agonist-induced H_2O_2 release in guinea pig eosinophils, suggesting that PKC is not central to this response. Indeed, in human eosinophils exposed to opsonised particles, the rate of oxygen consumption is *augmented* in the presence of inhibitors of PKC (van der Bruggen et al., 1993b), implying that one of more of these enzymes can negatively regulate oxidase activation. Collectively, therefore, these data provide persuasive evidence that agonist-induced activation of the NADPH oxidase in eosinophils is mediated by mechanisms that are largely independent of intracellular Ca^{2+} and PKC.

The role of PLD in NADPH oxidase activation has so far proved elusive. Although C5a stimulates PLD in human eosinophils (Minnicozzi et al., 1990), this is not observed in guinea pig eosinophils exposed to LTB_4 (Perkins et al., 1995). Unusually, the latter study found that butan-1-ol, an inhibitor of PLD, abrogated NADPH oxidase activation. However, it is likely that the action of

butan-1-ol is due to its ability to elevate intracellular cAMP, which is known to inhibit the activation of the NADPH oxidase in eosinophils (see below) (Perkins et al., 1995).

Experiments with wortmannin suggest that the conversion of PtdIns(4,5)P_2 to PtdIns(3,4,5)P_3 by PtdIns 3-kinase is implicated in SOZ-evoked respiratory burst in IL-5- and PAF-primed eosinophils (Coffer et al., 1998). It has been proposed that priming is mediated by the serine/threonine kinase PKB/c-Akt, a downstream substrate for PtdIns 3-kinase (Coffer et al., 1998). However, the role of these signal transduction elements in oxidant production is likely to be species and/or stimulus-specific since wortmannin attenuates eotaxin-induced NADPH oxidase activation in human eosinophils (Elsner et al., 1996b), but has no affect upon LTB_4-induced H_2O_2 generation in guinea pig eosinophils (Perkins et al., 1995).

A possible role for PLA_2 in the stimulation of the respiratory burst has been advanced based on the demonstration that exogenous AA stimulates H_2O_2 generation in guinea pig eosinophils (Lindsay et al., 1998c). This response is unaffected by inhibitors of cyclooxygenase and 5-lipoxygenase, indicating that AA per se, rather than TX, a PG(s) or a LT(s), is the causative mediator. However, the functions of PLA_2 and AA in receptor-mediated NADPH oxidase activation in eosinophils is open to conjecture. Pharmacological studies using the PLA_2 inhibitors mepacrine and 4-bromophenacyl bromide have implicated PLA_2 in fMLP- (White et al., 1993) and SOZ-stimulated (Shute et al., 1990) superoxide anion generation. However, both inhibitors are nonselective and no measurement of AA release was made. Indeed, a more contemporary investigation (Lindsay et al., 1998c) found that although the liberation of [^{3}H]AA from guinea pig eosinophils occurred with a time- and concentration-dependence consistent with a causal role in the generation of H_2O_2, the nonselective PLA_2 inhibitor mepacrine caused only a small inhibition of H_2O_2 generation at a concentration that abolished [^{3}H]AA release. Thus, based on those data, PLA_2 activation does not seem to be central to the mechanism of LTB_4-induced NADPH oxidase activation (Lindsay et al., 1998c). Again, it is important to appreciate that differences in species and/or stimulus could profoundly influence the signaling pathways recruited for effective oxidant generation.

LTB_4 can activate the raf1/ERK pathway in guinea pig eosinophils by a PKC-independent mechanism (Araki et al., 1995). Since p47^{phox} features putative MAP kinase phosphorylation sites, this finding tempts speculation that MAP kinases could play a role in the activation of the NADPH oxidase. However, the results of experiments designed to test this hypothesis have proved negative. Thus, the MEK-1 inhibitor PD098059 does not suppress LTB_4-induced oxidant production from guinea pig eosinophils at concentrations that abolish ERK-1/2 activation (Lindsay et al., 1998b). Similarly, PD098059 is without effect on SOZ-induced respiratory burst in PAF- and IL-5-primed human eosinophils despite clear activation of ERK-2 (Coffer et al., 1998).

Pharmacological experiments suggest that protein tyrosine kinases play a role in oxidative metabolism evoked by eotaxin (Elsner et al., 1996b), LTB_4 (Lindsay et al., 1998b), and following adherence of eosinophils to VCAM-1 (Nagata et al., 1995a). Herbimycin A, an inhibitor of nonreceptor tyrosine kinases, attenuates SOZ-induced respiratory burst in IL-5-, IL-3-, and GM-CSF- but not PAF-primed human eosinophils (van der Bruggen et al., 1998). The identity of the tyrosine kinase(s) is presently unknown although the LTB_4-induced response in guinea pig eosinophils appears to be mediated independently of the activation of members of the *src*-related family of tyrosine kinases (Lindsay et al., 1998a).

It has been proposed that the opening of voltage-sensitive K^+ channels might be related to the generation of superoxide anions by Ca^{2+}-mobilizing stimuli based on the finding that quinidine, a potent inhibitor of voltage-sensitive K^+ channels, attenuated the activation of the NADPH oxidase in eosinophils obtained from patients with hypereosinophilic syndrome in response to ionophore A23187 (Saito et al., 1995).

H. Apoptosis

Of great importance in determining the number of eosinophils found in the blood and tissues is the balance between cell production and cell death (for reviews, see Simon and Blaser, 1995; Simon, 1997; Simon et al., 1997b, Walsh, 1997a; Yousefi et al., 1997). In the absence of cytokines, eosinophil death is thought to occur in a controlled or programmed manner by a mechanism known as apoptosis (Yamaguchi et al., 1991; Stern et al., 1992). This process is distinct from necrosis in which cell lysis and the uncontrolled release of cellular contents occurs that may produce harmful actions. Apoptosis is characterized by specific biochemical and morphological changes including cell shrinkage, which may involve K^+ efflux (Beauvais et al., 1995a), surface blebbing, chromatin condensation, and endonuclease-catalyzed DNA fragmentation. The cell then fragments into discrete apoptotic bodies that are recognized and engulfed by monocytes/macrophages following the coordinate binding, by thrombospondin (expressed by the apoptotic eosinophil), to CD36 and the vitronectin receptor that are expressed by the phagocyte (Stern et al., 1992, 1996).

Studies conducted primarily in the 1980s convincingly demonstrated that the survival of human eosinophils in vitro is greatly enhanced (>10 days) by GM-CSF (Begley et al., 1986; Lopez et al., 1986; Tai et al., 1991), IL-3 (Rothenberg et al., 1988; Tai et al., 1991), and IL-5 (Begley et al., 1986; Tai et al., 1991) by inhibiting the natural processes that govern apoptosis (Yamaguchi et al., 1991). Although the longevity-enhancing cytokines are predominately T cell-derived, eosinophils provide an

effective autocrine and/or paracrine source. Thus, the increased survival of eosinophils adherent to laminin (Tourkin et al., 1993) and fibronectin (Anwar et al., 1993, G. M. Walsh et al., 1995) results from VLA4-mediated release of hematopoeitic cytokines (Anwar et al., 1993; G. M. Walsh et al., 1995). Similarly, suppression of the constitutive rate of apoptosis by IFNγ (Fujisawa et al., 1994), CD40 (Ohkawara et al., 1996), IL-13 (Luttmann et al., 1996), LPS (Takanaski et al., 1994), TNFα (Levi-Schaffer et al., 1998), and CD9 (Kim et al., 1997) is mediated indirectly through the autocrine release of GM-CSF and, in the case of IL-13, IL-3. In contrast, TGF-β abrogates the actions of IL-3, IL-5, and GM-CSF through the induction of apoptosis although this can be overcome by increasing the concentration of IL-5 (Alam et al., 1994; Atsuta et al., 1995). It seems likely that cytokines and, arguably, the processes of adherence also are likely to affect eosinophil viability in vivo in certain inflammatory diseases. Indeed, IL-5 mRNA expression is elevated in eosinophils recovered from the BAL fluid of asthmatic subjects after allergen challenge (Broide et al., 1992), infiltrating the mucosa in coeliac disease (Desreumaux et al., 1992) and in the dermis of individual with atopic dermatitis (Tanaka et al., 1994).

Evidence is available that CD95 (Matsumoto et al., 1995; Tsuyuki et al., 1995; Druilhe et al., 1996) and CD69 (Walsh et al., 1996b) are physiological death receptors. Freshly purified blood eosinophils express little if any mRNA or functional protein for CD95 but marked time-dependent induction is observed following their culture in the absence of cytokines (Druilhe et al., 1996). Ligation of CD95, with an activating antibody, induces apoptosis in both cytokine-deprived and IL-5-stimulated eosinophils (Matsumoto et al., 1995; Tsuyuki et al., 1995; Druilhe et al., 1996). However, unlike TGFβ, CD95 seemingly recruits different signal transduction elements since its apoptotic effect cannot be overcome by IL-5 (Matsumoto et al., 1995). In vivo studies utilizing sensitized mice have established that CD95 expression on, and CD95-mediated apoptosis of, eosinophils in the BAL fluid is increased following allergen challenge (Tsuyuki et al., 1995). Furthermore, inhalation of an anti-CD95 antibody is associated with a reduction in airway eosinophils, an increase in peroxidase-positive macrophages, and the subsequent resolution of eosinophilic airway inflammation (Tsuyuki et al., 1995). Interestingly, apoptosis is induced in murine eosinophils by neutralizing antibodies against FcγRII (CD32) by a mechanism dependent upon the expression of CD95 (de Andres et al., 1997a).

CD69 is not constitutively expressed on freshly purified eosinophils but is present on BAL fluid eosinophils obtained from patients with asthma and pulmonary eosinophilia (Nishikawa et al., 1992; Hartnell et al., 1993). It is likely that the in vivo elaboration of cytokines from a number of resident lung cells is responsible for the appearance of this death receptor since CD69 can be induced in vitro in a time- and concentration-dependent manner by IL-3, IL-5, and GM-CSF (Walsh et al., 1996b). In GM-CSF-activated eosinophils cultured on fibronectin, ligation of CD69, by an activating antibody, induces apoptosis (Walsh et al., 1996b). This effect is not secondary to the autocrine release of the apoptotic cytokine TGFβ_1 despite the knowledge that it is released from GM-CSF-exposed eosinophils (Walsh et al., 1996b).

Of clinical relevance is the demonstration that glucocorticoids, such as dexamethasone, can promote eosinophil apoptosis (Meagher et al., 1996) and reduce cytokine-induced survival (Cox et al., 1991; Lamas et al., 1991; Wallen et al., 1991; Hallsworth et al., 1992). Although these effects can be overcome by low concentrations of hematopoeitic cytokines, the fact remains that this might represent a mechanism by which glucocorticoids resolve eosinophilia in a number of inflammatory diseases such as asthma (Schleimer and Bochner, 1994).

The intracellular second messenger pathways responsible for spontaneous and CD95-mediated apoptosis and the enhanced cell survival effected by IL-3-, IL-5-, and GM-CSF are unclear. Investigations conducted in the early 1990s established that neither actinomycin D nor cycloheximide affected spontaneous apoptosis of human cultured eosinophils but effectively inhibited the survival-promoting actions of hematopoeitic cytokines, suggesting that their antiapoptotic effect is dependent upon RNA and protein synthesis (Tai et al., 1991; Yamaguchi et al., 1991). A central role for tyrosine phosphorylation is suggested from pharmacological experiments with tyrosine kinase and tyrosine phosphatase inhibitors (Yousefi et al., 1994; Simon et al., 1995a, 1998). Thus, GM-CSF-induced eosinophil survival is inhibited by genistein, while phenylarsine oxide-induced tyrosine phosphorylation prevents apoptosis in the absence of GM-CSF. The finding that IL-5 signals through *lyn*/*syk*-ras-raf1-MEK-ERK (Pazdrak et al., 1995a; Bates et al., 1996), Jak2-STAT1 (Pazdrak et al., 1995b; van der Bruggen et al., 1995; Bates et al., 1996), and SHPTP-2 (Pazdrak et al., 1997) strongly implicates tyrosine phosphorylation in cytokine-enhanced eosinophil survival. Further support for that contention was the additional observations that 1) the tyrosine phosphorylation of *lyn*, Jak-2, and ERK by IL-5 was reversed by the apoptotic cytokine TGFβ (Pazdrak et al., 1995c); 2) antisense oligonucleotides to *lyn*, *syk*, raf-1, and SHPTP-2 abrogated the survival-enhancing effect of IL-5 and GM-CSF (Yousefi et al., 1996; Pazdrak et al., 1997, 1998); and 3) the ability of IL-5 and GM-CSF to augment the life span of human eosinophils was prevented by tyrphostin AG490 and tyrphostin B42, respectively, selective inhibitors of Jak-2 but not *lyn* kinase (Simon et al., 1997; Pazdrak et al., 1998). Studies aimed at identifying downstream effectors of spontaneous apoptosis or the survival-enhancing effects of hematopoietic cytokines have excluded ERK-1/2 on the basis that PD 098059, a

highly selective inhibitor of MEK-1, modifies neither response (Kankaanranta et al., 1998). In contrast, p38 MAP kinase does seem to play a role in spontaneous apoptosis. Time course experiments have shown that activation of p38 MAP kinase is positively correlated with the degree of eosinophil apoptosis, and that SB203580, which selectively inhibits the α and β isoforms of this enzyme family, enhances the rate of cell death (Kankaanranta et al., 1998). Intriguingly, both of these affects are reversed by IL-5 (Kankaanranta et al., 1998), implying that this pathway is not responsible for IL-5-induced survival but is central to prolonging viability in cytokine-deprived cells.

Tyrosine phosphorylation apparently plays a pivotal role in CD95-mediated apoptosis. The degradation of the proteinase lamin B_1 following exposure of human eosinophils to CD95L is antagonized by the broad-spectrum tyrosine kinase inhibitor lavendustin A (Simon et al., 1998). Experiments with antisense oligonucleotides have implicated *lyn* in the regulation of apoptosis (Simon et al., 1998). This is an unexpected finding given that *lyn* also is implicated in IL-5-induced eosinophil survival (see *VI.B*). Whether the different isoforms of this kinase subserve distinct functions or extracellular stimuli can dictate the role *lyn* plays in eosinophil longevity remains to be established.

Evidence is available that constitutive eosinophil apoptosis and the survival-enhancing activity of IL-5 and GM-CSF involve the Bcl-2 family of proteins which include both anti-apoptotic (Bcl-2, Bcl-x_L, Mcl-1, A1) and proapoptotic (Bcl-x_S, Bax) members. In freshly prepared blood eosinophils, high amounts of Bax and Bcl-x_L (but see Druilhe et al., 1998) have been detected by Western blotting but little, if any, Mcl-1, Bcl-2, and Bcl-x_S (Druilhe et al., 1998, Dibbert et al., 1998). Using the complementary techniques of reverse transcription (RT)-PCR, Western blotting, flow cytometry, and immunohistochemistry, Dibbert and colleagues (1998) reported that the mRNA and protein levels for Bcl-x_L decreased during spontaneous eosinophil apoptosis although this was not confirmed in an independent investigation (Druilhe et al., 1998). In contrast, IL-5 significantly increased the expression of Bcl-2 mRNA transcripts and protein content in cultured eosinophils which coincided with rescue of cells from apoptosis (Ochiai et al., 1997). Significantly, introduction of a phosphothioate antisense oligonucleotide targeted at the ATG start codon of Bcl-2 mRNA into the cultures blocked the antiapoptotic action of IL-5 when compared with the sense cDNA. Similar results also have been described by Dibbert et al. (1998). Thus, IL-5 and GM-CSF either maintained or up-regulated Bcl-x_L mRNA and protein levels in human cultured eosinophils. Furthermore, Bcl-x_L antisense but not scrambled oligonucleotides attenuated the survival-enhancing activity of IL-5. It is noteworthy that identical experiments focusing on Bcl-2 failed to detect any change in expression in response to IL-5 which contrasts with the data published by Ochiai et al. (1997). The reason for this discrepancy is unclear.

XIII. Eosinophil Heterogeneity

Human peripheral blood eosinophils are physically, morphologically, and, above all, functionally heterogeneous (Connell, 1968; Tai and Spry, 1976). Variations in oxidative metabolism and antibody-dependent cytotoxicity were initially reported in the early 1980s even among eosinophils of the same buoyant density (Bass et al., 1980; David et al., 1980; Pincus et al., 1981) and were described before the formal recognition of physical heterogeneity. It is now believed that heterogeneity of eosinophils has physiological/pathophysiological significance, and this has prompted many investigations aimed at understanding the mechanisms responsible for inducing a hypodense phenotype and how these distinct populations of cell could be manipulated therapeutically.

A. Physical Heterogeneity

Essentially three distinct populations of eosinophils are now recognized that are characterized on the basis of density and responsiveness to activating stimuli. Compared to the majority (~90%) of eosinophils purified from the blood of normal individuals, which are of the resting normodense phenotype, increased numbers of "hypodense" cells are present in the blood of patients with eosinophilia associated with parasitosis (De Simone et al., 1982b; Prin et al., 1983, 1984), asthma (Fukuda et al., 1985b; Kajita et al., 1985; Hodges et al., 1988; Kloprogge et al., 1989a), allergic rhinitis (Frick et al., 1988), idiopathic eosinophilic syndrome (Winqvist et al., 1982; Prin et al., 1983; Peters et al., 1988), and malignancies (Winqvist et al., 1982; Prin et al., 1983, 1984). It has been suggested that the number of hypodense cells is positively related to the degree of blood eosinophilia (Fukuda et al., 1985b; Shult et al., 1988) although this is not always seen (Frick et al., 1988; Sedgwick et al., 1990a). Thus, the mechanisms that govern the induction of hypodensity are likely to be different from those that promote eosinopoiesis. In addition, a third population of eosinophils that is neither hypodense nor resting has been unequivocally identified. Cells in this category are said to be "primed" in that they respond to stimuli that are ordinarily inactive or relatively weakly active on eosinophils that are normodense. Although the precise numerical definition of hypodensity is unclear, there is persuasive evidence that eosinophils of low buoyant density are activated both metabolically and functionally, and that their number positively correlates to severity of symptoms (Frick et al., 1988). Moreover, the vast majority (60–100%) of eosinophils isolated from the BAL fluid and from the pleural cavity of patients with diseases associated with eosinophilia

are invariably hypodense (Winqvist et al., 1982; Prin et al., 1984, 1986).

B. Functional Heterogeneity

The enhanced activity of hypodense eosinophils is manifest in a variety of ways. Resting and stimulated low-density cells demonstrate increased oxidative metabolism (Winqvist et al., 1982) and hexose transport (Prin et al., 1983), and generate significantly more LTC_4 that autologous normodense eosinophils. This is not obviously related to the stimulus and is observed in response to diverse agents that include SOZ (Kauffman et al., 1987), IgG-coated particles (Shaw et al., 1985), the Ca^{2+} ionophore A23187, and *S. mansoni* coated with parasite-specific IgE (Moqbel et al., 1990a). Other functional responses that are up-regulated include PAF generation (Ojima Uchiyama et al., 1991), antibody-dependent, cell-mediated cytotoxicity (Prin et al., 1983; Capron et al., 1984), degranulation (Khalife et al., 1986), chemotaxis (Wardlaw et al., 1986), and adherence (Kimani et al., 1988).

Two possibilities that could account for the heightened functional responsiveness of hypodense eosinophils have been considered but neither of those is entirely satisfactory:

1. Cell-Cell Interactions. Before the advent of immunomagnetic beads to remove unwanted $CD16^+$ cells, hypodense eosinophils were difficult to separate from neutrophils. Thus, it was suggested that interactions between these two cell types could enhance the sensitivity and responsiveness of eosinophils to activating stimuli. However, carefully designed studies to assess this possibility have proved negative (Kajita et al., 1985; Kauffman et al., 1987; Hodges et al., 1988) with one exception (Kloprogge et al., 1989b) in which the generation LTC_4 from a mixture of human eosinophils and neutrophils was greater than the sum of LTC_4 produced by either cell type alone.

2. Up-Regulation of Cell Surface Receptors. Hypodense eosinophils express a greater number of receptors for IgG, IgE, CD44, complement, and the p55 subunit of the IL-2 receptor when compared to their normodense counterparts (Winqvist et al., 1982; Capron et al., 1985; Rand et al., 1991a; Matsumoto et al., 1998). Therefore, it is possible that this contributes to their enhanced activation status. However, the density of many more receptors is not different between eosinophil populations (Hartnell et al., 1990), and the expression of some cell surface epitopes (e.g., CD18) is even decreased in the hypodense phenotype (Hartnell et al., 1990). Moreover, the lack of consistency in the magnitude of various responses elicited by Ca^{2+} ionophores and phorbol esters (that act independent of cell surface receptors) in hypoand normodense eosinophils suggests that up-regulation of receptor expression is not sufficient to account totally for differences in eosinophil behavior.

C. Morphological Heterogeneity

Low-density eosinophils from the blood of patients with idiopathic eosinophilic syndromes are typically vacuolated, contain more lipid bodies, and express less MBP than their normodense counterparts. Moreover, the morphology of the intracellular granules is markedly altered; they are significantly smaller than those present in cells of normal density (although the absolute number is the same), slightly more lucent, and occupy considerably less cell volume (Henderson et al., 1988; Peters et al., 1988; Caulfield et al., 1990). In one study, the percentage of the cytoplasm occupied by eosinophil granules fell from 33.1% in normodense eosinophils to 19.7% in low-density cells (Caulfield et al., 1990). These structural abnormalities, in particular the hypogranularity and loss of MBP, provide a rational morphological basis for hypodensity in vivo. However, the morphology of low density eosinophils resident in BAL fluid differs from autologous blood eosinophils. Significantly, the granules in those cells generally are completely lucent indicative of degranulation (Metzger et al., 1986) which equally could explain the hypodense phenotype.

Eosinophil density also can be modulated in vitro in response to various naturally occurring substances and pharmacological stimuli. A hypodense phenotype can rapidly (after 15–60 min) be formed from normodense cells by agents such as fMLP, PAF, SOZ, and Ca^{2+} ionophores (Fukuda and Gleich, 1989; Kloprogge et al., 1989a; Agrawal et al., 1996). Significantly, these altered cells behave similarly to those found in the peripheral blood of eosinophilic subjects in that they generate more superoxide and have higher peroxidase activity than unstimulated cells. Thus, an interaction of normodense eosinophils with mediators involved in immediate hypersensitivity reactions may represent one means of generating the hypodense phenotype. This contrived method of altering eosinophil density is not apparently dependent on degranulation or limited cytolysis, for there is no concomitant release of EPO, arylsulphatase B, or lactate dehydrogenase. However, morphologically, the density transition is associated with multiple structural alterations. In particular, low-density cells phagocytose their granules and display an increase in the surface area to cell volume ratio (Owen, 1993).

IL-3, IL-5, and GM-CSF also can reduce the buoyant density of eosinophils (Owen et al., 1987; Rothenberg et al., 1988, 1989) but their characteristics similarly can differ depending on the stimulus and experimental conditions in much the same way as hypodense cells found in vivo. Thus, there is no morphological consistency that explains the physical transition of normodense eosinophils to a hypodense phenotype.

D. Acquisition of a Hypodense Phenotype

It is clear from the above discussion that it is not possible to identify a single unifying hypothesis that

adequately explains the generation of low-density eosinophils and, in vivo, it is likely that multiple, possibly related processes, are involved. At least four possibilities have been considered and all of them are entirely plausible.

1. Low-density eosinophils arise when normodense circulating cells enter the extravascular compartment where upon they become activated;

2. Eosinophils are rendered hypodense in the circulation and then migrate into tissue;

3. Low-density eosinophils reflect an increase in the number of immature cells that have been released from the bone marrow;

4. Hypodense eosinophils appear solely as a function of disease severity.

The knowledge that certain cytokines (e.g., IL-3, IL-5, GM-CSF) and/or mediators (e.g., PAF) can enhance eosinophil survival and/or promote hypodensity in vitro, and that they are present at biologically active concentrations in blood, in tissue fluids, and at sites of inflammatory reactions adds further support to the belief that eosinophil hypodensity can be effected by multiple mechanisms.

For additional information on eosinophil heterogeneity, interested readers should consult the following excellent articles (Sorice and De Simone, 1986; Fukuda and Gleich, 1989; Owen, 1993; Wardlaw, 1995).

XIV. Pharmacological Modulation of Eosinophil Function

A. Phosphodiesterase Inhibitors

Cyclic nucleotide PDEs are a heterogeneous group of immunologically distinct enzymes whose sole function is to metabolize the second messenger purine nucleotides, cAMP and cyclic GMP, to their biologically inactive nucleotide 5′-monophosphates. Currently, PDEs are categorized into nine broad families (PDEs 1–9; see Beavo et al., 1994; Fisher et al., 1998a,b; Soderling et al., 1998) that are distinguished by a number of criteria including substrate specificity, kinetic properties, sensitivity to allosteric modulators and synthetic inhibitors, and primary amino acid sequence (Beavo, 1988; Giembycz and Souness, 1994). In many cases these families comprise multiple subtypes which suggests that the degradation of cAMP and cyclic GMP is a highly complex and tightly regulated process.

1. Enzymology. The predominant PDE isoenzyme expressed by guinea pig peritoneal (Dent et al., 1991; Souness et al., 1991) and human peripheral blood eosinophils (Dent et al., 1994; Hatzelmann et al., 1995; Aloui et al., 1996) preferentially hydrolyzes cAMP over cyclic GMP. In fact, little cyclic GMP hydrolysis is detected in eosinophil lysates from either species. The metabolism of cAMP by PDE in eosinophils is insensitive to Ca^{2+}/calmodulin, cyclic GMP, and inhibitors of PDE3, but is potently inhibited by rolipram, denbufylline, RP 73401, Org 20241, and a number of related compounds (Dent et al., 1991; Souness et al., 1991; Dent et al., 1994; Barnette et al., 1995a; Hatzelmann et al., 1995; Nicholson et al., 1995; Souness et al., 1995). Thus, based upon criteria established previously (Beavo, 1988; Beavo et al., 1994), the PDE in eosinophils can be ascribed to the cAMP-specific, or PDE4, family of isoenzymes. Despite a lack of biochemical data, a small amount of PDE3 may be present in guinea pig eosinophils given that milrinone is reported to suppress agonist-induced chemotaxis at concentrations considered selective for inhibiting PDE3 (Cohan et al., 1992).

Currently, four genes (*PDE4A*, *PDE4B*, *PDE4C*, *PDE4D*) have been identified in rat (Colicelli et al., 1989; Davis et al., 1989; Swinnen et al., 1989a,b), mouse (Cherry and Davis, 1995), and humans (Livi et al., 1990; Bolger et al., 1993; McLaughlin et al., 1993; Obernolte et al., 1993; Baecker et al., 1994; Sullivan et al., 1994a; Engels et al., 1995) that can encode multiple, distinct PDE4 isoenzymes. In human eosinophils, mRNA transcripts for PDE4A, PDE4B, and PDE4D have been detected by RT-PCR (Engels et al., 1994), whereas only PDE4D gene products have been found in guinea pig cells (Souness et al., 1995).

Greater than 90% of the cAMP hydrolytic activity in guinea pig and human eosinophils is confined to the particulate fraction (Dent et al., 1991, 1994; Souness et al., 1991). Treatment of eosinophil membranes with Triton X-100 or high ionic strength buffers does not dislodge PDE4 activity (Dent et al., 1991, 1994; Souness et al., 1991), indicating that the enzyme is an integral membrane component. However, almost total solubilization of eosinophil PDE4 can be achieved with the bile acid, deoxycholate, in the presence of millimolar concentrations of NaCl (Souness et al., 1991). Although yet to be formally investigated in eosinophils, it is highly likely that membrane localization is conferred by specific amino acid residues at the extreme amino-terminus of the protein (Shakur et al., 1993; Lobban et al., 1994; Houslay et al., 1995; Scotland and Houslay, 1995; Houslay, 1996; Smith et al., 1996b).

Complex kinetics of cAMP hydrolysis are exhibited by eosinophil PDE4 (Dent et al., 1991, 1994; Souness et al., 1991, 1992; Souness and Scott, 1993; Giembycz and Souness, 1994), a feature shared with other membrane-bound PDEs (Souness et al., 1985; Wright et al., 1990). The explanation for this phenomenon is uncertain but it is unlikely to be due to the expression of multiple PDE4 subtypes since the K_m of cAMP for PDE4 gene products is similar (Wang et al., 1997). This assertion is strengthened by the finding that anion exchange chromatography of solubilized membrane-bound PDE4 from guinea pig eosinophils resolves a single peak of catalytic activity (Souness et al., 1992). Moreover, the marked nonlinear kinetics of cAMP hydrolysis is largely lost upon solubilization, which has led to the now widely accepted belief that PDE4 isoenzymes can adopt at least two distinct

and noninterconvertible conformations, $PDE4_H$ and $PDE4_L$, for which rolipram has high and low affinity, respectively (Barnette et al., 1995a,b, 1996; Kelly et al., 1996; Souness et al., 1996, 1997; Souness and Rao, 1997). In guinea pig eosinophils, the inhibition of fMLP-induced superoxide generation suggests that PDE4 adopts a conformation at which rolipram and related molecules interact with low affinity (Barnette et al., 1995a). Whether this relationship holds true for other functional responses in the eosinophil is currently unknown.

2. Activation of the NADPH Oxidase. Nonselective PDE inhibitors (theophylline, IBMX) and drugs which have been categorized as inhibitors of PDE4 (e.g., rolipram, denbufylline, Ro 20-1724, RP 73401, CP-80,663, D-22888) and hybrid inhibitors of PDE3 and PDE4 (e.g., zardaverine, Org 20241, Org 30029, benafentrine) attenuate the activation of the NADPH oxidase in eosinophils isolated from the guinea pig peritoneal cavity and from human venous blood in response to a variety of stimuli including fMLP, PAF, LTB_4, C5a, SOZ, IL-5, and C3b-opsonized zymosan (Dent et al., 1991, 1994, 1998b; Souness et al., 1991, 1995; Maruo et al., 1994; Torphy et al., 1994; Barnette et al., 1995a; Nicholson et al., 1995; Cohan et al., 1996; Ezeamuzie and Al-Hage, 1998). Suppression of the respiratory burst is associated with the inhibition of PDE4, an increase in the cAMP content and the activation of PKA (Souness et al., 1991). However differences and anomalies are apparent between investigations. In a study conducted by Hatzelmann et al. (1995) PDE4 inhibitors failed to suppress C5a-induced activation of the NADPH oxidase unless a β_2 adrenoceptor agonist was present. Although this might simply reflect low basal adenylyl cyclase activity in those cells, it is strange that the IC_{50} value derived for RP 73401 and tolafentrine was at least two orders of magnitude lower than would be predicted from the inhibition of PDE4 in a cell-free system, yet the potency of two other inhibitors, rolipram and zardaverine, was consistent with their corresponding IC_{50} values for the inhibition of PDE4. The reason for this discrepancy is not clear, but the ability of PDE4 isoforms to apparently adopt different conformations for which some inhibitors interact with different affinities provides a possible explanation. See Souness and Rao (1997) for additional details.

3. Degranulation. The nonselective PDE inhibitor IBMX prevents the release of EDN from human normodense eosinophils challenged with IgG- and secretory IgA-coated Sephadex beads (Kita et al., 1991b). The mechanism of this effect probably relates to inhibition of cAMP hydrolysis since it potentiates the same effect elicited by PGE_2 and the β adrenoceptor agonists salbutamol and isoprenaline (Kita et al., 1991b). Moreover, similar results have been obtained with selective inhibitors of PDE4 including rolipram, zardaverine, RP 73401, and tolafentrine for the inhibition of C5a-induced ECP and EDN release (Hatzelmann et al., 1995). However, in those studies degranulation was suppressed only in the presence of salbutamol, which might relate to the selective nature of the inhibitors examined (cf. IBMX) or, alternatively, the possibility that the degranulation-evoking stimulus influences the sensitivity of human eosinophils to cAMP. The ability of PDE4 inhibitors to suppress eosinophil degranulation is not confined to cells of human origin; indeed rolipram and RP 73401 inhibit the release of ECP and MBP from guinea pig cells stimulated with LTB_4 (Souness et al., 1995).

4. Adhesion and Adhesion Molecule Expression. Torphy et al. (1994) have reported that *R*-rolipram reduces the adhesion of guinea pig eosinophils to HUVECs stimulated with PMA and TNFα. That effect was modest (25–40%) with maximum inhibition of adhesion observed when both cell types were exposed concurrently to the PDE4 inhibitor (Torphy et al., 1994). In contrast, neither siguazodan (PDE3 inhibitor) nor zaprinast (PDE5 inhibitor) demonstrated activity in this experimental system (Torphy et al., 1994), which is consistent with PDE4 being the primary cyclic nucleotide PDE isoenzymes expressed by eosinophil (Dent et al., 1991, 1994; Souness et al., 1991; Hatzelmann et al., 1995; Aloui et al., 1996) and vascular endothelial cells (Blease et al., 1998). Those data are supported by studies performed with human eosinophils in which the expression of CD11b and shedding of L-selectin by PAF and eotaxin were inhibited, albeit modestly, by rolipram (Berends et al., 1997; Santamaria et al., 1997). Thus, part of the potential anti-inflammatory action of PDE4 inhibitor might be a direct effect on eosinophils to reduce their propensity to adhere to the vascular endothelium and ability to migrate out of the blood vessels.

5. Chemotaxis and Chemokinesis. The migration of rat and guinea pig eosinophils evoked by a number of stimuli including PAF, LTB_4, and C5a is inhibited by the PDE4 inhibitor rolipram when studied in Böyden microchemotaxis chambers (Cohan et al., 1992; Alves et al., 1996) and is potentiated in the presence of forskolin (Alves et al., 1996). Siguazodan and zaprinast are inactive under comparable experimental conditions. Identical results are available for human eosinophils. Thus, eotaxin-, PAF-, LTB_4-, and C5a-induced migration is partially prevented by PDE4 inhibitors and theophylline (Kaneko et al., 1995c; Tenor et al., 1996; Santamaria et al., 1997). Although C5a- and PAF-induced chemotaxis is enhanced in eosinophils purified from the peripheral blood of atopic subjects when compared to that of normal individuals, the potency (IC_{50}) of theophylline and rolipram as inhibitors of migration is not significantly different.

6. Synthesis of Lipid Mediators. Of the limited studies conducted to date, PDE4 inhibitors prevent or significantly attenuate the elaboration of lipid mediators from activated eosinophils. Tenor and colleagues (1996) demonstrated that little if any LTC_4 is released from human eosinophils by PAF and C5a unless indomethacin is

present. It was reasoned that under normal conditions, inhibitory prostaglandins (e.g., PGE_2) are generated preferentially in response to activating stimuli and act in an autocrine manner to suppress eosinophil activation. Indeed, in the presence of indomethacin, substantial amounts of LTC_4 were release in response to PAF and C5a. Furthermore, this lipolytic response was inhibited by rolipram with a potency in the high nanomolar range and could be prevented by the PKA inhibitor Rp-8-Br-cAMPS. The site of action of cAMP appears to be at the level of PLA_2 since exogenous AA reversed the inhibitory effect of rolipram on LTC_4 formation (Tenor et al., 1996). In guinea pig eosinophils, LTB_4-induced TX generation is prevented by rolipram, ibudilast, and RP 73401 (Souness et al., 1994).

7. Apoptosis. Little is published of the effect of PDE inhibitors on eosinophil longevity although IBMX has been reported to rescue eosinophils from apoptosis, resulting from activation of the CD95 receptor by an activating antibody. The mechanism of this effect is unclear since neither rolipram nor denbufylline affected eosinophil viability in GM-CSF-treated human eosinophils (Hallsworth et al., 1996). See *XII.H*, *XIV.C*, *XIV.D,* and *XIV.N.1* for further discussion.

8. In Vivo Effects. Comparatively little is known of the actions of isoenzyme-selective PDE inhibitors upon either the acute (IgE-mediated) or chronic (proinflammatory/immunocompetent cell-mediated) consequences of allergen provocation in vivo. However, there are many reports describing the effect of PDE inhibitors on passive cutaneous anaphylaxis and cell infiltration into sites of inflammation. In particular, the effect of PDE inhibitors on the infiltration of proinflammatory cells into the airway lumen, skin, and eye of guinea pigs, rats, mice, rabbits, and monkeys in response to various mediators and allergen has been extensively documented (Table 20). Schudt et al. (1991) reported that pretreatment of sensitized guinea pigs with zardaverine, a mixed PDE3/4 inhibitor, markedly suppressed allergen-induced infiltration of eosinophils, macrophages, and neutrophils into the BAL fluid to a level achieved with dexamethasone. Comparable data have been reported for the PDE3/4 inhibitor, benafentrine, on PAF- (Sanjar et al., 1989; Sanjar et al., 1990b) and allergen-induced (Sanjar et al., 1990c) pulmonary eosinophil recruitment in guinea pigs after chronic (6 days) dosing. More contemporary experiments conducted with rolipram have corroborated those data. Thus, intragastric administration of rolipram to conscious guinea pigs selectively attenuated allergen-induced pulmonary eosinophil influx into the BAL fluid and tissue (Underwood et al., 1993). Similarly, the introduction of rolipram directly into the airways of guinea pigs as a micronised dry powder almost completely prevented the appearance of proinflammatory leukocytes into the BAL fluid in response to allergen provocation (Raeburn et al., 1993). Essentially identical results have been reported in a guinea pig model of cutaneous inflammation (Teixeira et al., 1994b). Systemic administration of rolipram, but not zaprinast or SK&F 94120, suppressed the accumulation of ^{111}In-labeled eosinophils into the skin sites that had been challenged with zymosan-activated plasma, PAF, and histamine (Teixeira et al., 1994b).

Treatment of sensitized cynomolgous monkeys with rolipram and CP 80663 does not block the immediate increase in airways resistance that follows acute antigen provocation (Turner et al., 1994, 1996) but abrogates the pulmonary eosinophilia and airways hyperresponsiveness after multiple exposures to the antigen. Thus, these data are consistent with the findings of Howell et al. (1993) that PDE4 inhibitors may be anti-inflammatory and act primarily to prevent the activation of immune cells in the lung rather than exerting an antispasmogenic or spasmolytic effect at the level of airway smooth muscle.

Other models of inflammation are also sensitive to PDE4 inhibitors. For example, allergen-induced lung eosinophilia in Brown Norway rats, an IgE-producing, steroid-sensitive species that exhibits both early and LPRs, is suppressed by PDE3 (milrinone, CI-930), PDE4 (rolipram, denbufylline), and the hybrid PDE3/4 inhibitor, Org 20241 (Elwood et al., 1995; Howell et al., 1995), when administered to the animals acutely and before allergen challenge. The results of Howell et al. (1995) are particularly intriguing as they demonstrate an anti-inflammatory effect of PDE3 inhibitors in an in vivo model of eosinophilia. An interpretation of those data is that the eosinophil is not the cellular target of milrinone and CI-930 since PDE3 probably is not expressed by these cells (Dent et al., 1991, 1994; Souness et al., 1991; Hatzelmann et al., 1995; Aloui et al., 1996). Curiously, in guinea pigs given PDE inhibitors chronically (daily for 7 days), a different profile of activity has been reported (Banner et al., 1995). Thus, rolipram and benafentrine failed to lower eosinophil numbers in the BAL fluid of allergen-challenged animals but significantly reduced the amount of EPO-like immunoreactivity indicating that eosinophil degranulation but not trafficking was inhibited (Banner et al., 1995). It is not immediately clear why eosinophil recruitment was resistant to rolipram and benafentrine in that study given that piclamilast (RP 73401) effectively reduced the cell number and EPO content in BAL fluid in the same study (Banner et al., 1995).

In a guinea pig eye model of tissue eosinophilia, rolipram when administered by gavage, significantly inhibited the number of eosinophils that appeared in the conjunctival epithelium in response to histamine, and a combination of LTB_4 and LTD_4 (Newsholme and Schwartz 1993). In another study, Griswold et al. (1993) reported that oral administration to mice with rolipram inhibited AA-induced inflammatory cell accumulation and activation assessed by myeloperoxidase activity in the inflammatory exudate.

TABLE 20
In vivo effects of PDE inhibitors on eosinophil recruitment and activation

Species	Parameter(s) Measured	PDE Inhibitor Used	Route of Administration	Effect	Reference(s)
Guinea pig	Antigen-induced BAL fluid EPO content	CP 80,633	p.o.	Inhibition	Turner et al. (1996)
Guinea pig	Antigen-induced eosinophil number in BAL fluid and histological evaluation	Rolipram	p.o.	Inhibition	Underwood et al. (1993)
Guinea pig	Antigen-induced eosinophil number in BAL fluid	Org 20241	i.p.	Inhibition	Santing et al. (1995)
		Theophylline	i.p.	Inhibition	Santing et al. (1995)
		Rolipram	i.p. (low dose)	No effect	Santing et al. (1995)
		Rolipram	i.p.	Inhibition	Ortiz et al. (1996)
		Rolipram	p.o.	Marginal inhibition	Underwood et al. (1994)
		Zardaverine	p.o.	Marginal inhibition	Underwood et al. (1994)
		Rolipram	i.p.	Inhibition after chronic dosing	Banner and Page (1995b)
		Zardaverine	i.p.	Inhibition after chronic dosing	Banner and Page (1995b)
		Benafentrine	s.c. (minipump)	Inhibition	Sanjar et al. (1990c)
		Aminophylline	s.c. (minipump)	Inhibition	Sanjar et al. (1990c)
		Rolipram	i.t.	Inhibition	Raeburn et al. (1994)
		RP 73401	i.t.	Inhibition	Raeburn et al. (1994)
		Zardaverine	p.o.	Inhibition	Schudt et al. (1991)
		Ro 20-1724	i.p.	Inhibition	Danahay and Broadley (1998)
Guinea pig	Antigen-induced eosinophil number and EPO content in BAL fluid	Rolipram	i.p. (7 days)	No effect	Banner et al. (1995)
		RP 73401	i.p. (7 days)	Inhibition	Banner et al. (1995)
		Rolipram	p.o.	Inhibition	Lagente et al. (1994a)
		Ro 20-1724	p.o.	Inhibition	Lagente et al. (1994a)
Guinea pig	IL-5 and IL-8-induced eosinophil number in BAL fluid	Rolipram	p.o.	Inhibition	Lagente et al. (1995)
		Ro 20,1724	p.o.	Inhibition	Lagente et al. (1995)
		Theophylline	p.o.	Inhibition	Lagente et al. (1995)
Guinea pig	Cytokine-induced pulmonary eosinophilia	Benafentrine	p.o./s.c. (minipump)	Inhibition	Kings et al. (1990)
Guinea pig	PAF-induced eosinophil number in BAL fluid	Benafentrine	s.c. (minipump)	Inhibition	Sanjar et al. (1989, 1990b)
		Aminophylline	s.c. (minipump)	Inhibition	Sanjar et al. (1989, 1990b)
Guinea pig	Leukotriene- and histamine-induced eosinophilia in the eye	Rolipram	p.o.	Inhibition	Newsholme and Schwartz (1993)
		Zardaverine	p.o.	Inhibition	Newsholme and Schwartz (1993)
Guinea pig	Antigen-induced lung eosinophilia/IL-5-induced pleural eosinophilia	Rolipram	p.o./i.p.	Inhibition	Hughes et al. (1996)
		RP 73401	p.o./i.p.	Inhibition	Hughes et al. (1996)
		CDP 840	p.o./i.p.	Inhibition	Hughes et al. (1996)
Guinea pig	Antigen- and mediator-induced cutaneous eosinophilia	Rolipram	i.p.	Inhibition	Teixeira et al. (1994b)
Guinea pig	Antigen-induced eosinophil number in BAL fluid	D-22888	p.o.	Inhibition	Dent et al. (1998)
		RP 73401	p.o.	Inhibition	Dent et al. (1998)
		SB 207499	p.o.	Inhibition	Dent et al. (1998)
		RS 25344	p.o.	Inhibition	Dent et al. (1998)
Guinea pig	Antigen-induced pulmonary eosinophilia	Rolipram	i.p.	Inhibition	Danahay and Broadley (1997)
		Ro-20,1724	i.p.	Inhibition	Danahay and Broadley (1997)
		Theophylline	i.p.	Inhibition	Danahay and Broadley (1997)

Rat	Antigen-induced lung eosinophilia	Rolipram	i.p.	Inhibition	Elwood et al. (1995)
		Org 20241	i.p.	Inhibition	Elwood et al. (1995)
		Aminophylline	p.o.	Inhibition	Howell et al. (1995)
		Denbufylline	p.o.	Inhibition	Howell et al. (1995)
		Milrinone	p.o.	Inhibition	Howell et al. (1995)
Rat	Antigen-induced lung eosinophilia/ IL-5-induced pleural eosinophilia	Rolipram	i.p.	Inhibition	Hughes et al. (1996)
		RP 73401	i.p.	Inhibition	Hughes et al. (1996)
		CDP 840	i.p.	Inhibition	Hughes et al. (1996)
Rat	Antigen-induced eosinophil number in BAL fluid	Rolipram	i.t.	Inhibition at high doses only	Raeburn et al. (1994)
		RP 73401	i.t.	Inhibition at high doses only	Raeburn et al. (1994)
Monkey	Antigen-induced eosinophil number in BAL fluid	Rolipram	s.c.	Inhibition	Turner et al. (1994)
		CP 80,633	s.c.	Inhibition	Turner et al. (1996)
Rabbit	Antigen-induced pulmonary eosinophilia	CDP 840	i.p.	Inhibition	Gozzard et al. (1996b)
		Rolipram	i.p.	Inhibition	Gozzard et al. (1996b)
		Theophylline	i.p.	Inhibition	Gozzard et al. (1996b)

Although proinflammatory mediators such as PAF, histamine and LTB_4 elicit pulmonary eosinophil recruitment, the eosinophil count in the airways lumen is considerably less than that seen following antigen provocation (Aoki et al., 1988). This observation, along with the finding that selective antagonists of these mediators do not abrogate eosinophil recruitment following antigen challenge, implicates mediators other than PAF, histamine, and LTB_4 in eosinophil accumulation in the lung. It is now recognized that chronic proinflammatory cytokines/chemokines including IL-3, IL-5, GM-CSF, RANTES, TNFα, eotaxin, and MIP-1α can elicit the pulmonary accumulation and activation of eosinophils. Indeed, Kings et al. (1990) documented the ability of human recombinant IL-3 and GM-CSF, and mouse TNFα, to selectively attract eosinophils into the lungs of guinea pigs. Significantly, pretreatment of the animals with the PDE3/4 inhibitor benafentrine effectively suppressed that response (Kings et al., 1990). Collectively, these are important observations since they imply that selective PDE inhibitors are effective at blocking the deleterious actions of both acute and chronic mediators of allergic inflammation. Although it is unclear how PDE inhibitors prevent pulmonary eosinophilia, it is likely that part of their action is to suppress directly the activation of eosinophils. This is suggested from studies where the injection of salmeterol-treated, ^{111}In-labeled guinea pig eosinophils into the systemic circulation of recipient guinea pigs is associated with a marked reduction in the their ability to infiltrate skin sites exposed to an inflammatory insult (Teixeira and Hellewell, 1997b).

There are no reports of the effects of PDE4 inhibitors upon eosinophil numbers in humans. However, it has been reported that ibudilast, a nonselective PDE inhibitor (Souness et al., 1994), failed to reduce the circulating eosinophil count in asthmatic subjects (Kawasaki et al., 1992). However, in another study, oral administration of the PDE4-selective inhibitor CDP 840 to 54 asthmatic patients in a placebo-controlled, double-blind clinical trial produced a marginal, but significant, inhibition of the LPR following antigen provocation, which is believed to involve the influx and activation of eosinophils from the circulation (Harbinson et al., 1997).

B. Theophylline

Theophylline is an adenosine antagonist and weak inhibitor of cyclic nucleotide PDEs and provides a mainstay in the treatment of asthma in the western world. However, the beneficial effects of theophylline on those cell types implicated in the pathogenesis of asthma, such as the eosinophil, and their pharmacological and biochemical basis essentially are unknown.

1. In Vitro Effects. Theophylline exhibits multiple pharmacological actions and its effects on leukocyte function accordingly are complex. In guinea pig and human eosinophils, theophylline attenuates PAF-, IL-5-, and SOZ-induced superoxide anion generation (Yukawa

et al., 1989b; Dent et al., 1994; Hatzelmann et al., 1995; Ezeamuzie and Al-Hage, 1998) at concentrations (>100 μM) that inhibit PDE4 in washed membranes and elevate the cAMP content in intact cells (Dent et al., 1991; Souness et al., 1991). The finding that the 8-phenyl analog of theophylline, which is not a PDE inhibitor, fails to reduce superoxide anion generation, supports the belief that theophylline inhibits the activation of the NADPH oxidase in eosinophils through PDE inhibition (Yukawa et al., 1989b). At lower concentrations of theophylline, that approximate to what is achieved clinically, a paradoxical increase in superoxide anion generation has been reported which probably is attributable to the antagonism of endogenously released adenosine. Indeed, the available data suggest that adenosine synthesized endogenously by eosinophils normally exerts a negative autocrine effect on oxidative metabolism and that theophylline, at therapeutic doses, blocks this protection. However in asthma, adenosine might act as a proinflammatory mediator through the A_1 receptor subtype. Potentially, this has important therapeutic implications in cell-mediated inflammatory diseases where theophylline is prescribed since blocking the A_1 receptor could, on balance [and despite the ability of adenosine to enhance the elaboration of superoxide anions in leukocytes (Schrier and Imre 1986)], impart an anti-inflammatory influence which might contribute to its efficacy in the treatment of asthma.

Theophylline prevents degranulation of human eosinophils (assessed as secreted EDN or ECP) in response to IgG, secretory IgA, fMLP, and C5a (Kita et al., 1991b; Hatzelmann et al., 1995; Ezeamuzie and Al-Hage 1998). A consistent finding is that IgG-induced EDN release is significantly more sensitive to theophylline than the same response evoked by secretory IgA (Kita et al., 1991b) which agrees completely with results of other experiments where β adrenoceptor agonists were studied. In another investigation, Eda et al. (1993a) reported that theophylline enhanced the ability of the β_2 adrenoceptor agonist eformoterol to suppress PAF-evoked ECP release, implying that the negative effect on degranulation is due to the inhibition of PDE and is therefore governed by a cAMP-dependent mechanism(s).

Theophylline reduces the enhanced survival of guinea pig (Adachi et al., 1996) and human eosinophils (Hossain et al., 1994a; Ohta et al., 1996) afforded by IL-5. Significantly, the morphological changes seen at the electron microscopic level and the fragmentation of DNA visualized on agarose gels show the characteristics of apoptosis rather than necrosis (Adachi et al., 1996). Although it is established that cAMP effectively promotes the apoptosis of human eosinophils (see *XIV.C* and *XIV.D*), the molecular mechanism of action of theophylline is not clear since the PDE4 inhibitors rolipram and denbufylline are inactive (Hallsworth et al., 1996).

Other in vitro functional responses that are negatively regulated by theophylline include agonist-stimulated chemotaxis (Clark et al., 1977; Eda et al., 1993a; Tenor et al., 1996), LTC_4 and TX biosynthesis (Souness et al., 1994; Tenor et al., 1996), the up-regulation of the surface adhesion molecule Mac-1 by PAF (Sagara et al., 1996), and antibody-dependent, eosinophil-mediated damage of ^{51}Cr-labeled schistosomula of *S. mansoni* (David et al., 1977).

2. In Vivo Effects. The administration of theophylline to sensitized laboratory animals before antigen provocation or in response to proinflammatory spasmogens such as PAF, histamine, or Sephadex beads has a variable effect on bronchial hyperreactivity and, in the case of allergen, on the early and LPR (Perruchoud et al., 1984; Sanjar et al., 1989; 1990b,c; Ali et al., 1992; Gozzard et al., 1996b). These are curious findings since in the majority of cases (but see Banner et al., 1995; Namovic et al., 1996), experiments conducted with sensitized rats and guinea pigs have demonstrated that theophylline suppresses pulmonary eosinophil recruitment, which is believed to be intimately associated with the development of the LPR (Gristwood et al., 1991; Tarayre et al., 1991a, b, 1992; Manzini et al., 1993; Lagente et al., 1994b, 1995; Howell et al., 1995; Gozzard et al., 1996b). It is important to appreciate, however, that in many of these studies theophylline was administered acutely, as a single dose in excess of that given therapeutically and may, therefore, have little clinical relevance. In contrast, chronic dosing of guinea pigs with theophylline for 7 days effectively prevents PAF- and allergen-induced pulmonary eosinophilia at doses that are achieved clinically (Sanjar et al., 1989, 1990b,c; but see Banner et al., 1995).

A newer xanthine PDE inhibitor, isbufylline, that has negligible affinity for adenosine receptors (Manzini et al., 1990), similarly reduces eosinophil recruitment into the airways of sensitized guinea pigs following antigen inhalation and suppresses PAF-induced bronchial responsiveness effected by i.v. histamine (Manzini et al., 1993).

Theophylline also prevents the influx of eosinophils into the pleural cavity of carrageenan-challenged mice (Saleh et al., 1996) and the cutaneous eosinophilia in sensitized guinea pigs that results following allergen challenge (Teixeira et al., 1994a). In the latter study, a substantial reduction in the accumulation of ^{111}In-labeled eosinophils into the skin of sensitized guinea pigs injected intradermally with zymosan-activated plasma (source of C5a), PAF or antigen was noted.

Although the clinical pharmacology of theophylline is well documented, it was only recently appreciated that the efficacy of this drug in asthma is attributable to actions other than bronchodilatation. Significantly, there is increasing evidence that theophylline exerts an immunomodulatory action in clinical asthma (Pardi et al., 1984; Ward et al., 1993; Jaffar et al., 1994; Sullivan et al., 1994b; Banner and Page, 1995a, 1996; Kidney et al., 1995; Chung, 1996; Finnerty et al., 1996; Kraft et al.,

1996; MacLeod and Djukanovic, 1996) at plasma concentrations that do not affect airway smooth muscle tone (Ward et al., 1993; Jaffar et al., 1994; Sullivan et al., 1994b; Finnerty et al., 1996; Tohda et al., 1998). Several lines of investigation have led to this conclusion. In particular, evidence from the majority of studies has demonstrated that theophylline protects against the LPR following allergen provocation, implying that the emigration of proinflammatory and immunocompetent cells from the circulation into the lung and/or their subsequent activation is suppressed. With respect to the eosinophil, experiments conducted by Costello and colleagues (Jaffar et al., 1994; Sullivan et al., 1994b) have demonstrated that the numbers of $EG2^+$ (marker for secreted MBP) eosinophils and $CD4^+$ T lymphocytes were reduced in allergic subjects given low-dose theophylline (mean plasma concentration 6.6 μg/ml) for 6 weeks. In another in vivo study, treatment of asthmatic subjects for 11 months with oral theophylline (600 mg/day) reduced the circulating concentration of ECP and EDN indicative of a negative effect on eosinophil degranulation (Pedersen et al., 1996). However, lung function, assessed by the measurement of FEV_1 (% predicted) and histamine PC_{20}, was not improved (Pedersen et al., 1996).

The mechanism by which theophylline could exert an immunomodulatory action is far from clear. It has been reported that oral administration of theophylline (resulting in a mean plasma concentration of 10.9 μg/ml) to moderately severe asthmatic subjects reduced the number of cells staining for IL-4 and IL-5 (Djukanovic et al., 1995; Finnerty et al., 1996), implying that theophylline may act, at least in part, to repress transcription of the *IL-4* and *IL-5* genes or reduce the translation of preexisting mRNAs. In this respect, mRNA transcripts and protein for IL-4 and IL-5 have been identified in human eosinophils (Moqbel et al., 1995; Moller et al., 1996a,b; Nakajima et al., 1996). See *XII.D.4* and *XII.D.5* for details.

C. Cholera Toxin and Forskolin

Exposure of human eosinophils to cholera toxin (CTX), which ADP ribosylates $G_{s\alpha}$ and, thereby, irreversibly activates adenylyl cyclase, results in a significant increase in the cAMP content (Kita et al., 1991b; Hallsworth et al., 1996). Functionally, secretory IgA and IgG-induced EDN release is prevented in CTX-treated cells, providing further evidence that eosinophils can be modulated by cAMP-dependent mechanisms (Kita et al., 1991b). In agreement with the inhibitory influence of β adrenoceptor agonists and IBMX on EDN secretion, IgG-induced degranulation is significantly more sensitive to CTX than the same response evoked by secretory IgA (Kita et al., 1991b). Attenuation of the secretory response is observed after a latency of approximately 1 h, which is consistent with the lag time required for the activation of $G_{s\alpha}$ by CTX.

Hallsworth et al. (1996) have reported that CTX reduces the survival of human eosinophils cultured in the presence of GM-CSF. Eosinophil DNA, extracted from CTX-treated cells, and run on an agarose gels was completely fragmented, showing a distinctive "ladder pattern" of approximately 200 bp, indicating the activation of an endonuclease characteristic of apoptosis (Hallsworth et al., 1996). The ability of CTX to reduce eosinophil survival was prevented by the PKA inhibitor H-89, implicating a cAMP-dependent mechanism(s) in apoptosis (Hallsworth et al., 1996). Taken together, these are intriguing results given that cAMP *enhances* the survival of human neutrophils (Rossi et al., 1995) and implies that fundamental differences exist in the regulation of programmed cell death between eosinophils and neutrophils. This contention is supported further by the finding that in the absence of GM-CSF, the survival of human eosinophils is significantly enhanced by CTX by processes that are not sensitive to H-89 and, therefore, may be independent of PKA (Hallsworth et al., 1996). Thus, cAMP-elevating drugs can exert opposite effects on the longevity of human eosinophils that depend upon whether or not the cells are exposed to GM-CSF.

The naturally occurring diterpene, forskolin, which in many cell types promotes a robust activation of adenylyl cyclase, has little if any effect on the cAMP content of eosinophils at concentrations up to 10 μM (Alves et al., 1996; Hallsworth et al., 1996). Increasing the concentration of forskolin further is associated with a detectable increment in cAMP mass (Alves et al., 1996; Hallsworth et al., 1996) but this effect is modest which tempts speculation that eosinophils might express a significant amount of adenylyl cyclase IX which is forskolin-insensitive (see Iyengar, 1993a,b; Pieroni et al., 1993). The limited ability of forskolin to elevate eosinophil cAMP content presumably explains, at least in part, its variable effects in functional assays. Thus, although forskolin does not promote apoptosis of human eosinophils (Hallsworth et al., 1996) or inhibit LTB_4-induced TX biosynthesis or H_2O_2 generation in guinea pig eosinophils, it nevertheless effectively suppresses chemotaxis of rat and human eosinophils in response to PAF, C5a, and LTB_4 (Kaneko et al., 1995c; Alves et al., 1996). Moreover, incubating rat eosinophils with rolipram and forskolin, at concentrations that exert no effect when examined individually, markedly attenuates chemotaxis in the absence of a detectable change in cAMP mass. These results clearly implicate cAMP in the regulation of chemotaxis and suggest that forskolin evokes a subtle increase in cAMP in rat eosinophils such that it cannot be detected when total cell cAMP is measured.

D. Cyclic Nucleotide Analogs

Almost all indices of eosinophil activation studied, that are considered to be proinflammatory or tissue preserving, are suppressed by lipophilic cAMP analogs (Clark et al., 1977; Sher and Wadee, 1981; Dent et al.,

1991; Kita et al., 1991b; 1995; Alves et al., 1996; Hallsworth et al., 1996; Berends et al., 1997; Hebestreit et al., 1998). Agonist-induced AA mobilization, activation of the NADPH oxidase, chemotaxis, expression of CD11b, and the shedding of L-selectin are reduced by dibutyryl cAMP (Clark et al., 1977; Dent et al., 1991; Alves et al., 1996; Berends et al., 1997). Similarly, pretreatment of human eosinophils with a range of analogs including dibutyryl cAMP, 8-Br-cAMP, and N^6-benzoyl-cAMP prevents IgG-, secretory IgA- and MBP-induced degranulation (Kita et al., 1991b), MBP-induced IL-8 production (Kita et al., 1995), and chemotaxis in response to endotoxin-activated serum (Clark et al., 1977). In addition, flow cytometric analyses have shown that dibutyryl cAMP enhances FcγRII (CD32) receptor expression on human peripheral blood eosinophils and on eosinophils differentiated in vitro from mononuclear cells by IL-5 (Akutagawa et al., 1994). In contrast, dibutyryl cAMP reduces the ability of IFNγ to up-regulate FcγRIII (CD16) receptor number (Akutagawa et al., 1994).

Dibutyryl cAMP also promotes apoptosis of human eosinophils cultured with GM-CSF (Hallsworth et al., 1996) but, paradoxically, reverses spontaneous and CD95-mediated death in cytokine-deprived cells (Hallsworth et al., 1996; Hebestreit et al., 1998). These results are entirely consistent with the effect of CTX on cell survival (*XIV.C*) and endorses the idea that activation of the cAMP-dependent protein kinase cascade can produce pro- and antiapoptotic effects, which are determined by the absence or presence of other eosinophil stimuli.

Dibutyryl cyclic GMP is generally inactive on eosinophils (Sher and Wadee, 1981; Dent et al., 1991). However, one exception seems to be the regulation of cell survival where it inhibits spontaneous and CD95-mediated apoptosis of eosinophils purified from human peripheral blood (Beauvais et al., 1995a; Hebestreit et al., 1998).

E. Glucocorticosteroids

Glucocorticosteroids are the most effective anti-inflammatory drugs currently available for the treatment of a number of disorders including asthma, atopic dermatitis, rhinitis, and various syndromes associated with hypereosinophilia. The clinical efficacy of these drugs is attributable to a number of distinct actions. In particular they: 1) reduce circulating eosinophil numbers (Saunders and Adams, 1950; Dahl and Venge, 1978; Hallgren et al., 1979; Baigelman et al., 1983; Gleich et al., 1984; Zora et al., 1984; Bochner et al., 1991b; Butterfield et al., 1992; Evans et al., 1993; Laviolette et al., 1994) and the percentage exhibiting a hypodense phenotype (Evans et al., 1993; Kuo et al., 1994); 2) prevent or attenuate the recruitment of eosinophils to sites of inflammation following allergen provocation (Rebuck and Mellinger, 1953; Pipkorn et al., 1987a,b; Schleimer, 1988; Bascom et al., 1989; Charlesworth et al., 1991; Varney et al., 1992; Taborda Barata et al., 1996); 3) reduce the number of eosinophils and the concentration of eosinophil secretory products in the blood, BAL, nasal fluid (Hallgren et al., 1979; Andersson et al., 1989; Bascom et al., 1989; Bisgaard et al., 1990; Janson Bjerklie et al., 1993), and the airway mucosa (Adelroth et al., 1990; Djukanovic et al., 1992; Jeffrey et al., 1992; Laitinen et al., 1992; Wang et al., 1994); 4) control symptoms in asthma patients of all ages and severity (Konig, 1988; Barnes, 1993, 1995; Barnes and Pedersen, 1993); and 5) reduce bronchial hyperresponsiveness (Barnes, 1990; O'Connor et al., 1992; Kuo et al., 1994). Similar effects have been documented in a variety of animal models of allergic and parasitic diseases (Sabag et al., 1978; Etienne et al., 1989b; Sanjar et al., 1989, 1990b,c; Kawabori et al., 1991; Parker et al., 1991; Elwood et al., 1992; Gundel et al., 1992; Kung et al., 1994; Lagente et al., 1994b; Woolley et al., 1994a; Teixeira et al., 1996c; Danahay and Broadley, 1998; Lawrence et al., 1998). Given the diverse activities of steroids, knowledge of how they affect eosinophil function in vitro and in vivo, and an understanding of their molecular pharmacology is clearly desirable if advances in anti-inflammatory therapies are to be achieved.

1. Receptors. Many of the anti-inflammatory properties of steroids are thought to be mediated via the GR, which have been identified and, to some extent, characterized in eosinophils (Peterson et al., 1981). The structure of the GR has been elucidated using site-directed mutagenesis (Muller and Renkawitz, 1991) but, unlike almost every other receptor, there is no evidence for GR heterogeneity within or between tissues (Barnes, 1997). Using [^{3}H]dexamethasone, Peterson et al. (1981) described the labeling of a population of high-affinity (K_d = 15 nM), low-capacity (B_{max} = 11,000 per cells) binding sites on human eosinophils. The proposal that glucocorticoids act through GR is supported by the finding that the rank order of potency of various steroids at inhibiting GM-CSF-enhanced eosinophil survival correlates closely with their affinity for the GR (Lamas et al., 1991). Significantly, the rank order of potency is similar to that found for steroid-induced inhibition of AA metabolism from human lung (Schleimer et al., 1986) and histamine release from human basophils (Schleimer et al., 1981). In contrast, the steroid sex hormones β-estradiol and testosterone are not anti-inflammatory in eosinophils and, as would be predicted, have very weak affinity for the GR (Lamas et al., 1991). It is notable that eosinophils purified from the peripheral blood of steroid-insensitive individuals with hypereosinophilia fail to bind [^{3}H]dexamethasone (Prin et al., 1989).

2. Maturation. It is well established that glucocorticoids inhibit the formation of eosinophil-rich colonies in in vitro bone marrow colony assays (Bjornson et al., 1985; Slovick et al., 1985; Butterfield et al., 1986), but interpretation of the data is often difficult as cells, in

addition to eosinophil progenitors, are usually present in the cultures (Gleich et al., 1996). The possibility that steroids inhibit eosinophil colony growth directly was initially suggested by results published by Bjornson and colleagues (1985) who showed that the proliferation of eosinophil progenitors obtained from the peripheral blood and bone marrow was significantly inhibited (from 49 to 4 colonies) by hydrocortisone. Significantly, the reduction in colony frequency was not prevented when adherent or E-rossetting cells were removed, or when cyclosporin A, which suppresses the activity of T lymphocytes, was included in the cultures. However, in contrast to that study, Slovick et al. (1985) reported that glucocorticoids had no effect on eosinophil colony growth when purified progenitors that were devoid of mononuclear cells and T lymphocytes were used. The finding that steroids suppressed the release of a colony-stimulating factor(s) from human monocytes led Slovick and colleagues to conclude that eosinophil maturation is blocked indirectly through the suppression of cytokine release from accessory cells present in the cultures. Persuasive evidence for this latter contention was provided 10 years later in a study where Shalit et al. (1995) were unable to demonstrate an inhibitory action of steroids on the formation of eosinophil colonies when purified $CD34^+$ primitive hematopoietic cells were stimulated with IL-3, IL-5, and GM-CSF. Thus, on balance, glucocorticoids probably suppress eosinophil maturation by tempering the production and/or release of IL-5 and other eosinopoietic factors from cells within the bone marrow rather than by directly affecting $CD34^+$ progenitors (Sanderson et al., 1985; Yamaguchi et al., 1988b; Sher et al., 1990; Rolfe et al., 1992).

3. Adhesion and Adhesion Molecule Expression. Studies performed in the late 1970s and early 1980s demonstrated that oral administration of prednisolone to human volunteers resulted in a partial inhibition of serum-induced adherence of whole blood eosinophils studied ex vivo (Clark et al., 1979; Altman et al., 1981). In those investigations, the effect was transient and a partial recovery was seen 24 to 48 h after steroid administration. Paradoxically, eosinophils purified from whole blood were insensitive to the inhibitory effect of the steroid, an effect shared by purified eosinophils studied in vitro (Altman et al., 1981). Those latter findings are entirely consistent with a more recent investigation which failed to demonstrate an inhibitory effect of budesonide on the adhesion of human eosinophils to HUVECs stimulated with PAF, fMLP, IL-1β, and IL-4 (Kaiser et al., 1993). Collectively, these results lend support to the idea that steroids suppress the accumulation of eosinophils at sites of inflammation by acting indirectly, possibly by inhibiting the generation of chemoattractants.

Despite their apparent inability to attenuate adhesion per se, glucocorticoids are reported to block the up-regulation of certain adhesion molecules on eosinophils. Thus, the increased expression of ICAM-1, promoted by IL-3, IL-5, and GM-CSF, is partially reduced by dexamethasone over the concentration range (1–100 nM) predicted for a GR-mediated effect (Guida et al., 1994). Hartnell et al. (1992a) also have documented that dexamethasone effectively suppresses IL-3-induced CR3 receptor expression although this was not confirmed in a subsequent study where the expression of CD11b by PAF and fMLP was examined (Tomioka et al., 1993). The reason for this discrepancy is unclear, but it is possible that steroids preferentially prevent the transcription of the gene encoding CD18 (the common β chain of the CD11 family) rather than the α chain of CR3 (i.e., CD11b).

4. Cell Survival and Apoptosis. As described in *XII.H*, certain cytokines, in particular IL-3, IL-5, GM-CSF, and IFNγ, can markedly prolong the survival of eosinophils in vitro for days or even weeks. However, eosinophils undergo apoptosis if they are exposed to glucocorticoids even in the continued presence of cytokine (Lamas et al., 1989, 1991; Cox et al., 1991; Wallen et al., 1991; Hallsworth et al., 1992; Mullol et al., 1995; Adachi et al., 1996; Druilhe et al., 1996; Kitagaki et al., 1996; Meagher et al., 1996; Nittoh et al., 1998; Hagan et al., 1998). This fundamental finding has led to the proposal that survival-enhancing cytokines maintain eosinophil viability by suppressing apoptosis (Williams et al., 1990). Pharmacological evidence suggests that steroid-induced apoptosis is a GR-mediated process because it is prevented by the GR antagonist RU 38486 (Meagher et al., 1996; Nittoh et al., 1998). With the exception of IFNγ (Her et al., 1991), the ability of glucocorticoids and cytokines to modulate eosinophil survival is mutually antagonistic (Her et al., 1991) where the effect of a steroid can be overcome in the presence of higher concentrations of IL-3, IL-5, or GM-CSF. It is curious that although steroids promote the apoptosis of human eosinophils, they enhance the survival of human neutrophils under similar experimental conditions (Cox, 1995; Meagher et al., 1996; Nittoh et al., 1998); the reason for this divergent effect is unknown.

The precise molecular details of how steroids regulate eosinophil survival are unknown and multiple possibilities exist. In addition to inhibiting the biosynthesis of cytokines from other "supporting" cells, steroids clearly act directly on eosinophils to modulate their longevity. Some of the more attractive processes that are under intense investigation include antagonism of the action of survival-enhancing cytokines, which could be at the level of suppressing the de novo generation and/or release of stored cytokines such as GM-CSF (Levi Schaffer et al., 1995), and down-regulating the expression of certain cytokine receptors. It is likely that glucocorticoids also induce or repress certain genes that regulate apoptosis, independent of their ability to modulate the action of survival-enhancing cytokines. Indeed, in contrast to certain T lymphocytes, protein synthesis inhibitors

such as actinomycin D and cycloheximide promote apoptosis of eosinophils (Yamaguchi et al., 1991). Although a variety of effector molecules have been implicated in the final common pathway leading to programmed cell death including Bax (Oltvai et al., 1993), Bcl-2 (Nunez et al., 1990; Sentman et al., 1991), Bcl-$_{xS}$ (Boise et al., 1993), Bcl-$_{xL}$ (Boise et al., 1993), cyclins (Sherr, 1993; Shi et al., 1994), p53 (Yonish-Rouach et al., 1991; Lowe et al., 1993), c-*myc* (Shi et al., 1992), and ICE (Barinaga, 1994; Vaux et al., 1994), little information is available for the eosinophil. However, possible clues to the mechanism of action of steroids can be derived from the observation that the enhanced survival of human eosinophils effected by IL-5 is associated with increased expression of Bcl-2 and Bcl-x$_L$ (Ochiai et al., 1997; Dibbert et al., 1998). Therefore, an action of steroids on the expression of these proteins is possible. An additional site of action is the pathway that promotes CD95L-induced apoptosis. Druilhe and coworkers (1996) reported that cross-linking CD95 in cytokine-deprived eosinophils with an "agonistic" CD95L monoclonal antibody increased the number of apoptotic nuclei by a mechanism that was significantly enhanced by dexamethasone.

Although much is to be learned of eosinophil apoptosis and its control, it is clearly a highly regulated process with unique characteristics (Adachi et al., 1996; Druilhe et al., 1996; Meagher et al., 1996). It is tempting to speculate that the ability of steroids to resolve eosinophilic inflammation might be due, in part, to the induction of apoptosis.

5. Degranulation. The effect of steroids on granule protein release from eosinophils is unclear. A study by Hallam and coworkers (1982) demonstrated that relative high concentrations (100 nM to 1 mM) of methyl prednisolone suppressed the ability of eosinophils, harvested from the peritoneal lavage fluid of *N. brasiliensis*-infected rats, to kill chicken erythrocytes in an antibody-dependent manner. That effect was abolished by cycloheximide, suggesting that the steroid was promoting new protein synthesis. Similarly, pretreatment of human eosinophils for 24 h with hydrocortisone effectively inhibited the secretion of ECP in response to serum-coated Sephadex beads (Winqvist et al., 1984). Again, evidence that the effect of hydrocortisone was transcriptional is suggested by an earlier study of Venge and Dahl (1989) who reported that acute exposure of eosinophils to steroids was poorly effective under comparable experimental conditions. Although the above results suggest that steroids can modulate the secretory event of eosinophil leukocytes, other data accrued more recently have not been able to confirm those findings. Indeed, Kita et al. (1991c), using a panel of steroids (hydrocortisone, dexamethasone, methyl prednisolone), did not detect any inhibition of IgG-induced EDN release from human eosinophils after a prolonged period of incubation or when the exocytotic response was enhanced by IL-5 (Kita et al., 1991c). Their results are entirely consistent with other studies performed with human neutrophils where dexamethasone was inactive in blocking the release of MPO (Schleimer et al., 1989). The explanation for this inconsistency is unresolved but could reflect the nature of the degranulation-evoking stimulus and/or the identity of product secreted. If steroids are generally unable to prevent eosinophil degranulation, then their activity reported in vivo implies that they act indirectly on other elements that can affect the secretory response. In this respect, Sensi and colleagues (1997) noted that the steroid flunisolide significantly reduced the level of ECP in nasal secretions taken from children with chronic allergic rhinitis.

6. Chemotaxis. The ability of corticosteroids to inhibit directed and random migration of eosinophils was first documented in 1975 using cells harvested from a patient with rheumatoid arthritis (Goetzl et al., 1975). Shortly afterward, it was reported that the chemotaxis of guinea pig eosinophils was similarly suppressed by corticosteroids (Gauderer and Gleich, 1978). Experiments performed 20 years later with a murine model of cutaneous eosinophilia have confirmed those original observations and established, using ^{111}I-labeled eosinophils from a donor mouse, that antigen-, PAF-, MIP-1α-, and LTB$_4$-induced eosinophil accumulation does not require an action on the bone marrow (Teixeira et al., 1998). However, despite these aforementioned data, the direct effect of steroids on eosinophil chemotaxis is equivocal. For example, in vivo treatment of normal subjects with prednisolone for 24 h inhibited subsequently the ability of endotoxin-activated serum to stimulate the migration of eosinophils in vitro (Clark et al., 1979). In a comparable study, eosinophil migration effected by SOZ was similarly inhibited by hydrocortisone and methylprednisolone (Altman et al., 1981). However, in vitro studies have provided conflicting results. Thus, although Kurihara et al., (1989) failed to detect any inhibitory effect of dexamethasone on PAF-induced eosinophil migration after a 6-h pretreatment period, Prin and colleagues (1989) noted a modest suppression of migration although the concentration of dexamethasone used in that study was approximately 1000-fold higher than its affinity for the GR receptor.

7. Effects on Transcription of Genes Relevant to Eosinophil Function. Steroids can effectively increase and decrease transcription of many genes that produce proteins relevant to normal eosinophil function and the aberrant behavior noted in asthma and allergy. However, with the exception of IL-8 and MCP-1, whose secretion from human eosinophils is suppressed by dexamethasone with an IC$_{50}$ (10 nM) consistent with a direct interaction at the GR (Miyamasu et al., 1998), there are no GREs in the upstream promoter region of many genes that encode cytokines. In contrast, steroids have a profound ability to up-regulate cytokine receptor expression on many cell types which presumably could enhance

their sensitivity to endogenous activating ligands (see Wiegers and Reul, 1998).

8. Activation of the NADPH Oxidase. Exposure of adherent eosinophils to a variety of stimuli (e.g., IL-5, fMLP, GM-CSF) that activate the NADPH oxidase results in a slow and protracted generation of oxygen-derived free radicals that can last for several hours. The possibility that new protein synthesis is required for this response is suggested by the finding that high concentrations of dexamethasone inhibit the release of superoxide anions in response to SOZ (Evans et al., 1990). A GR-mediated mechanism is apparently responsible for this effect because it is prevented in cells pretreated with the steroid receptor antagonist, RU 38486 (Evans et al., 1990).

9. Antigen Presentation. In a study by Guida et al. (1994), the steroids dexamethasone and hydrocortisone markedly enhanced the expression of the MHC class II antigens HLA-DR and HLA-DP on the surface of human peripheral blood eosinophils in response to IL-3, IL-5, and GM-CSF. That effect was observed at concentrations (10 nM) of the steroids approximately equal to their affinity for the GR and, significantly, was associated with an increased ability of the cells to present antigen (Guida et al., 1994). A third MHC class II molecule, HLA-DQ, generally, was not induced although in one donor, exposure of eosinophils to dexamethasone and IL-3 resulted in high levels of expression (Guida et al., 1994).

F. Estrogen

Uterine eosinophilia is induced in rats following the administration of estrogen (Tchernitchin et al., 1974; Katayama et al., 1998). This observation, which was first made in the mid 1970s, led to the proposal that eosinophil activation is responsible for a number of early oestrogenic responses (Tchernitchin et al., 1974). It was further hypothesized that eosinophils express estrogen receptors (A. Tchernitchin et al., 1976; Tchernitchin et al., 1979) of the uterine type II subtype (Lyttle et al., 1984, 1989), which were responsible for the localization of eosinophils to small blood vessels of the uterine wall (X. Tchernitchin et al., 1976). However, it is now believed that eosinophil migration is elicited by chemotactic factors released from the uterus in response to estrogen (Lee et al., 1989) that include complement C3 (Leiva et al., 1991) and cyclophilin (Xu et al., 1992). Furthermore, the preferential accumulation of eosinophils in the uterus may be due to interactions between eosinophil integrins (principally α_6 and β_2), which are up-regulated by estrogen, and uterine extracellular matrix proteins such as laminin (Katayama et al., 1998).

Although there are reports showing increased estrogen binding to eosinophils after stimulation with C5a (Klebanoff et al., 1977) and during phagocytosis (Klebanoff et al., 1977), and that 17β-estradiol induces eosinophil degranulation both in vivo and in vitro (Tchernitchin et al., 1985; Silva et al., 1997), the existence of bone fide estrogen receptors is questionable. Indeed, although estrogen binding correlates with eosinophil numbers in the uterus (Lyttle et al., 1984, 1989), in vitro culture of rat uterine cells (which lack eosinophils) with 17β-estradiol stimulates the expression of nuclear type II-binding sites (Markaverich et al., 1986), suggesting that the smooth muscle itself is the primary source of estrogen receptors.

G. Lazaroids

Lazaroid is a generic term that describes a series of 21 amino steroids that are devoid of glucocorticoid and mineralocorticoid activity. These molecules are based on the methylprednisolone structure but lack the critical 11β-hydroxyl moiety present in glucocorticosteroids. Although initially designed as inhibitors of iron-dependent lipid peroxidation, they have been examined in a number of in vitro and in vivo models for a variety of disorders. One of these, U-75412E, given by aerosol to sensitized rats, markedly attenuated the number of eosinophils that appeared in the BAL fluid after allergen challenge (Richards et al., 1991a, 1992). The effect was dose-dependent and not associated with a reduction in the weight of the thymus or adrenal glands that are typical glucocorticoid side effects. Similar results were reported by Johnson et al. (1992) where topical administration of antigen to the airways of beagle dogs over a period of 6 weeks resulted in a pulmonary eosinophilia that was significantly attenuated by the lazaroid U-78517F. The efficacy of lazaroids also has been confirmed in a sensitized primate model of allergic inflammation with the demonstration that they suppress antigen-induced pulmonary eosinophilia (Johnson and Stout, 1993). The mechanism of action of these novel steroids has not been elucidated but in vitro experiments suggest that it does not apparently involve an interaction with the GR (Richards et al., 1991a) and it is reasonable to conclude, therefore, that they act in vivo also by a GR-independent mechanism. Richards et al. (1992) have proposed that the T lymphocyte could be a primary target for lazaroids due to their intrinsic antioxidant activity (Braughler et al., 1988) but this is yet to be confirmed.

H. Retinoids

Derivatives of vitamin A, or retinoids, evoke a variety of fundamental biological processes through interacting with distinct nuclear receptors that belong to a superfamily of proteins that include steroid, vitamin D, and thyroid hormone receptors, the peroxisome proliferator-activated receptor, the insect edysteroid receptor, and a number of "orphan" receptors whose ligands are currently unknown (Pfahl, 1993; Pemrick et al., 1994). Two families of retinoid receptors have been identified: the retinoic acid receptors (RARα, RARβ, and RARγ) and the retinoid X receptors (RXRα, RXRβ, and RXRγ).

Moreover, current data are consistent with the idea that there are RAR- and RXR-dependent pathways of gene regulation and that individual receptors influence the expression of specific genes. This diversity of gene regulation has permitted the use of naturally occurring and synthetic retinoids clinically where they have been shown to be particularly effective in cutaneous inflammatory diseases including psoriasis and acne (Pfahl, 1993; Pemrick et al., 1994).

One potential reason for their therapeutic activity might relate to their ability to inhibit the synthesis and/or release of proinflammatory mediators. Lehman and Henderson (1990) compared the ability of eight retinoids (tretinoin, isotretinoin, retinol, retinal, acitretin, retinyl palmitate, atretinate, Ro 15-0778) to suppress the elaboration of LTC_4 from A23187-stimulated equine eosinophils and reported that five of them (tretinoin, isotretinoin, retinol, retinal, and acitretin) were, indeed, active, although the arotinoid, Ro 15-0778, potentiated LTC_4 release. Although the molecular mechanism underlying this effect was not investigated, it was speculated, based on those limited data, that certain retinoids might be of benefit in the treatment of diseases, such as bronchial asthma, where peptido-leukotrienes play a pathogenic role (Lehman and Henderson, 1990).

I. Cromones

Cromones, including sodium cromoglycate and nedocromil sodium, are in widespread use for the prophylaxis of asthma and have been shown to inhibit the LPR following allergen provocation and control many of the symptoms of chronic asthma (Crimi et al., 1989; Brogden and Sorkin, 1993; Twentyman et al., 1993). Although the precise cellular targets through which these drugs exert their beneficial effects are unknown, they modulate directly a number of functional responses in eosinophils that could account or contribute to their clinical efficacy. In the context of host defense and inflammation, both of the aforementioned cromones reduce the number of complement and IgG rosettes on eosinophils and attenuate their propensity to kill schistosomula of *S. mansoni* in response to fMLP (Moqbel et al., 1986, 1988, 1989; Kay et al., 1987). Serum-opsonised zymosan-, C5a-, PAF-, and LTB_4-induced chemotaxis of human eosinophils under in vitro conditions is similarly suppressed by sodium cromoglycate and in some cases nedocromil sodium (Bruijnzeel et al., 1989, 1990; Warringa et al., 1993a). Moreover, in human eosinophils primed with GM-CSF and IL-3, nedocromil sodium at low nanomolar concentrations is extremely effective at preventing fMLP- and IL-8-induced chemotaxis and similarly attenuates the chemotactic response of fMLP on circulating eosinophils harvested from patients with allergic asthma 3 h after allergen provocation (Warringa et al., 1993a). In another study, Abdelaziz and colleagues (1997) reported that nedocromil sodium inhibited the enhancement of eosinophil chemotaxis, adherence to human endothelial cells, and release of soluble ICAM-1 provoked by conditioned medium obtained from human bronchial epithelial cells. Thus, epithelium-derived mediators can potentiate eosinophil activity in a nedocromil-sensitive manner which may represent a potential site where cromones exert their anti-inflammatory activity in vivo.

A number of in vitro and in vivo studies have studied the effect of cromones on eosinophil degranulation in response to diverse stimuli with mixed results. Palczynski et al. (1989) reported that disodium cromoglycate suppressed effectively eosinophil degranulation in 26 of 30 asthmatic patients that were sensitive to deuterium oxide (a stimulus that promotes degranulation in many asthma sufferers but not in normal individuals). Similarly, Spry and coworkers (1986) found that nedocromil sodium inhibited the exocytosis of preformed granule-associated proteins. In contrast, the ECP content of lachrymal secretions from 30 patients with vernal keratoconjunctivitis was poorly suppressed by disodium cromoglycate (4% solution for 10 days) which was in keeping with its limited effect on clinical symptoms (Leonardi et al., 1997).

The effect of cromones on the production of lipid mediators from human eosinophils is similarly unclear. Nedocromil sodium has been reported to be active (Bruijnzeel et al., 1989; Moqbel et al., 1989; Sedgwick et al., 1992a) and inactive (Burke et al., 1990) in suppressing the production of LTC_4 in response to A23187, fMLP, and SOZ. Again, the reason(s) for this difference is not established but might reflect, to some extent, differences in degranulation-evoking stimulus or functional antagonism.

Other potentially beneficial actions of cromones include their ability to inhibit the enhanced survival of human eosinophils afforded by IL-5 (Resler et al., 1992) and epithelial cell-derived cytokines (Mullol et al., 1997), the development of a hypodense phenotype in eosinophils cultured in 50% conditioned medium from bovine pulmonary endothelial cells (Sedgwick et al., 1992a) and the restoration (toward normal) of the eosinophil count in rectal inflammatory exudates from patients with ulcerative colitis (Rampton et al., 1982). Devalia and coworkers (1992) reported that the incubation of human eosinophils with nedocromil sodium along with an activating stimulus, such as a phorbol diester or opsonized latex beads, prevented their ability to impair the normal ciliary beat frequency of human cultured epithelial cells under in vitro conditions. This is an interesting observation given that the clearance of particulate matter from the airways is critically dependent upon uncompromised ciliary beat activity. Therefore, it might be predicted that nedocromil sodium would maintain this key function when the airways are inflamed. Given the wide spectrum of anti-inflammatory effects produced by nedocromil sodium, it is curious that cromones do not

inhibit superoxide anion generation from human eosinophils (Sedgwick et al., 1992a).

Relatively few studies have formally examined the effect of cromones in vivo. Nevertheless, cromones have been shown to reduce pulmonary eosinophilia in laboratory animals in response to diverse stimuli including carrageenan, PAF, and allergen (Hutson et al., 1988; Abraham, 1989; Church et al., 1989; Sanjar et al., 1989, 1990b,c; Kings et al., 1990; Pretolani et al., 1990; Schellenberg et al., 1991; Saleh et al., 1996). Likewise, nedocromil has been reported to suppress allergen-induced eosinophilia in asthmatic subjects (Calhoun et al., 1993) and to reduce bronchial hyperresponsiveness (Aalbers et al., 1991).

The mechanism of action of cromones is unknown but their molecular target is likely to be extracellular since both nedocromil sodium and sodium cromoglycate are extremely polar molecules and highly ionized at physiological pH; thus, they are unlikely to gain access to the cells' interior. A number of mechanisms have been proposed to account for their therapeutic activities in asthma but none are entirely satisfactorily. Interested readers should consult Eady and Norris (1997) for a detailed evaluation of current theories.

J. Loop Diuretics

Lung function measurements in normal subjects and in individuals with asthma have demonstrated that the loop diuretic frusemide effectively attenuates bronchoconstriction induced by a variety of indirect airway challenges including ultrasonically nebulized distilled water (Robuschi et al., 1988), exercise (Bianco et al., 1988), allergen (Bianco et al., 1989), metabisulphite (Nichol et al., 1990), and adenosine 5′-monophosphate (O'Connor et al., 1991), yet it is generally inactive in attenuating the bronchoconstriction elicited by histamine and methacholine which act directly on airway smooth muscle to increase bronchomotor tone (Nichol et al., 1990; O'Connor et al., 1991, but see Polosa et al., 1995). The biochemical basis for the protective action of frusemide is apparently unrelated to its ability to inhibit the $Na^+/K^+/2Cl^-$ cotransporter, which underlies its diuretic mechanism of action, since bumetanide, a more potent inhibitor of the cotransporter in the loop of Henlé, is inactive in the same indirect challenges (O'Connor et al., 1991). To account for this anomaly, an alternative mechanism has been proposed that involves the inhibition of transmembrane Cl^- fluxes (Chung and Barnes, 1992). The finding that bronchoconstriction evoked by stimuli such as adenosine 5′-monophosphate is due to the release of spasmogens from proinflammatory cells prompted studies to assess the effect of frusemide, bumetanide, and various putative Cl^- channel blocking drugs on indices of eosinophil activation.

In guinea pig peritoneal eosinophils, frusemide, but not bumetanide, inhibits LTB_4-evoked H_2O_2 generation in a concentration-dependent manner with a potency in the low micromolar range (Perkins et al., 1992). A similar result was evoked by DIDS and NPPB (Perkins et al., 1992) which are known to block small and large conductance Cl^- channels in smooth muscle and epithelial cells (Hanrahan et al., 1985; Wangemann et al., 1986). Despite those data, LTB_4-induced activation of the NADPH oxidase is it not dependent upon Cl^- influx since H_2O_2 is still formed when Cl^- are omitted from the extracellular media (Perkins et al., 1992). Based on the aforementioned data the inhibitory mode of action of frusemide, DIDS and NPPB do not seem to involve an inhibition of Cl^- influx. Although the mechanism(s) of action of these drugs is currently obscure, their effects are agonist-dependent. Thus, in contrast to LTB_4, the H_2O_2 generated by SOZ has an absolute requirement for extracellular Cl^- (Perkins et al., 1992), indicating that SOZ and LTB_4 promote the assembly and subsequent activation of the NADPH oxidase complex by recruiting different cellular signaling pathways. This conclusion is supported by pharmacological studies that show that frusemide, DIDS, and NPPB are inactive when SOZ is used as the stimulus (Perkins et al., 1992).

K. Sodium Channel-Blocking Drugs

Ohnishi and colleagues (1996) reported that the introduction of BAL fluid from 25 of 40 patients with symptomatic asthma to human peripheral blood eosinophils maintained in culture inhibited their survival. The causative factor(s) was of low molecular weight, heat stable, and its effect was overcome by an excess of exogenous cytokines (IL-5, IL-3, GM-CSF). Moreover, there was a highly significant positive correlation between the rate of enhanced death and the concentration of lidocaine in the BAL fluid, indicating that the local anesthetic used for bronchoscopy might represent the inhibitory factor. This possibility is strongly suggested by the ability of exogenous lidocaine and other local anesthetics including tetracaine, dibucane, benoxinate, proparacaine, procaine, and bupivacaine to inhibit human eosinophil survival (Ohnishi et al., 1996; Okada et al., 1998). The interaction of low concentrations of lidocaine with human eosinophils is seemingly specific because its effects are overcome by IL-3, IL-5, and GM-CSF; however, higher concentrations of the anesthetic are not overcome by hematopoietic cytokines indicative of functional antagonism (Ohnishi et al., 1996; Okada et al., 1998). Curiously, the enhanced survival of human eosinophils afforded by IL-5 is significantly more sensitive to lidocaine than cells treated identically with IL-3 or GM-CSF (Okada et al., 1998). Thus, despite the common β chain shared by the GM-CSF, IL-3, and IL-5 receptor (see *VI.A*), difference in the responsiveness of eosinophils to these cytokines is clearly apparent and corroborates the results obtained from binding studied with ^{125}I-labeled IL-5 (Lopez et al., 1991). Lidocaine also antagonizes the ability of IFNγ to enhance eosinophil survival (Ohnishi et al., 1996).

The inhibitory action of lidocaine on eosinophils is not limited to survival but seems to be restricted to functional responses effected by IL-5 and related cytokines. Thus, the generation of superoxide by human eosinophils adherent to culture plastic via a β_2 integrin-dependent process and stimulated by IL-5 also is attenuated by lidocaine, whereas activation of the NADPH oxidase by PAF and immobilized IgG is insensitive (Okada et al., 1998).

Collectively, the limited results described above suggest that lidocaine and its analogs behave as glucocorticomimetics although the mechanism of action is almost certainly different (Gleich et al., 1996; Okada et al., 1998). Studies performed by Okada and coworkers (1998) have shown that lidocaine does not inhibit the binding of ^{125}I-labeled IL-5 to human eosinophils or the subsequent changes in protein tyrosine phosphorylation. Furthermore, lidocaine and its analogs do not modify eosinophil survival by preventing Na^+ influx or by inhibiting the Na^+/H^+ antiporter (Okada et al., 1998). Instead, pharmacological evidence points to an inhibitory action of lidocaine at ATP-sensitive K^+ channels (Bankers-Fulbright et al., 1998).

Preliminary clinical investigations have shown lidocaine to be steroid-sparing (Gleich et al., 1996; Hunt et al., 1996) although indices of inflammation, like eosinophil numbers in BAL fluid, have not been reported.

L. Ketotifen

Ketotifen has a pharmacology that supports its utility in the treatment of allergic inflammatory diseases such as asthma (Grant et al., 1990). In vitro studies have demonstrated that several markers of eosinophil activation are suppressed by ketotifen. Using eosinophils purified from the peripheral blood of 25 patients with asthma, Kishimoto and colleagues (1990) found that ketotifen inhibited the release of LTC_4 and the morphological changes evoked by anti-IgG (Kishimoto et al., 1990). Chemotaxis, degranulation, actin polymerization, induction of a hypodense phenotype, and, at high concentrations, the enhanced survival of eosinophils effected by IL-5 are also reported to be suppressed by ketotifen (Podleski et al., 1984; Miyasato et al., 1988; Morita et al., 1990b; Nabe et al., 1991; Hossain et al., 1994a).

Comparable data are available from in vivo investigations. Prophylactic administration of ketotifen to baboons inhibits the accumulation of eosinophils into the BAL fluid evoked by the intratracheal administration of PAF (Arnoux et al., 1988). Identical results have been described in guinea pigs (Sanjar et al., 1989; 1990b,c; Kings et al., 1990) and mice (Nagai et al., 1996) where PAF-, allergen-, and cytokine/lymphokine-induced pulmonary eosinophilia and the increase in IL-5 levels in BAL fluid are significantly attenuated. Ketotifen also reduces the number of eosinophils located in the endometria of estrogen-challenged rats and inhibits degranulation without affecting migration per se (Soto et al., 1989).

Few studies have evaluated the effect of ketotifen on direct or indirect indices of inflammation in humans. However, the limited data available suggest some degree of efficacy. In children presenting with asthma or atopic dermatitis, an apparent correlation exist between the clinical efficacy of ketotifen and normalization of peripheral blood eosinophil numbers during treatment (Uehara et al., 1988; Ikari and Matsunaga, 1989; Grant et al., 1990). Similarly, ketotifen inhibits the cutaneous eosinophilia in response to allergen (Snyman et al., 1992) and prophylactic treatment of patients with pollinosis over the period of natural allergen provocation with ketotifen significantly inhibits the increase in serum ECP levels and eosinophil count (Kato et al., 1994).

Evidence for a direct effect of ketotifen on eosinophil function is suggested from two clinical investigations with comparable results (Hoshino, 1994; Hoshino et al., 1997). In one of those, 25 patients with atopic asthma were given ketotifen (1 mg twice daily for 8 weeks) or placebo in a double-blind parallel group study and eosinophil activation assessed in biopsies taken by fiberoptic bronchoscopy (Hoshino et al., 1997). Relative to placebo, ketotifen reduced the number of $EG2^+$ cells in the lamina propria, indicating eosinophil stabilization, which was associated with an improvement in asthma symptoms and a reduction in bronchial hyperreactivity (Hoshino et al., 1997).

M. Cyclosporin A, Tacrolimus, and Rapamycin

Evidence now exists that asthma represents a specialized chronic inflammatory condition that may represent a form of cell-mediated immunity where cytokines released from T lymphocytes promote pulmonary eosinophilia. This possibility has prompted several groups of investigators to assess the efficacy of drugs, other than steroids, in the treatment of chronic severe asthma (Corrigan and Kay, 1996). One group of potential therapeutic agents are the immunosuppressants, exemplified by cyclosporin A, tacrolimus (FK 506), and rapamycin (sirolimus) (Bonham and Thompson, 1997).

Cyclosporin A is an undecapeptide isolated from the fungus *Tolypocladium inflatum gams* and tacrolimus is a macrolide produced from the fermentation of a strain of *Streptomyces tsukubaensis*. Although these two molecules are structurally dissimilar, they have closely related biological activities, in particular their ability to alter gene expression. In contrast rapamycin, a macrolide antibiotic produced as a fermentation product of *Streptomyces hygroscopicus*, is believed to exert its biological effects by interfering with cytokine/growth factor receptor signaling, despite its close structurally similarity to tacrolimus (Bonham and Thompson, 1997).

A current dogma is that cyclosporin A and tacrolimus exert their effects by interacting specifically with intracellular proteins (collectively known as immunophilins)

termed cyclophilin and FK506-binding protein, respectively (Schreiber, 1991; Thompson, 1993; Wiederrecht et al., 1993; Fruman et al., 1994; Bonham and Thompson, 1997). The drug/immunophilin heterodimer then forms a tertiary complex with a number of other proteins including calcineurin (protein phosphatase 2B) which modulates the activity of transcription factors such as the cytoplasmic component of NF-AT (Flanagan et al., 1991; Liu et al., 1991; Clipstone and Crabtree, 1993; Wiederrecht et al., 1993; Fruman et al., 1994; Bonham and Thompson, 1997).

1. In Vitro Effects. Cyclosporin A promotes apoptosis of rat eosinophils (Kitagaki et al., 1996) and, in high concentrations, attenuates the enhanced survival of human eosinophils afforded by IL-5; this activity also is shared by rapamycin and tacrolimus (Hom and Estridge, 1993; Hossain et al., 1994a). Similar data have been obtained with eosinophils whose viability was enhanced by cytokines (IL-3, IL-5, GM-CSF) released from peripheral blood mononuclear cells harvested from asthmatic subjects in response to house dust mite (Hossain et al., 1994b). The finding that the generation of GM-CSF, IL-3, and IL-8 from A23187-stimulated human eosinophils is suppressed by tacrolimus and cyclosporin is consistent with the ability of these drugs to repress gene transcription (Kita et al., 1991d; Braun et al., 1993; Hom and Estridge, 1993; Kohyama et al., 1997). Some specificity of action is suggested by the inability of cyclosporin A to block the expression of IL-8 mRNA and protein from human eosinophils exposed to MBP (Kita et al., 1995). Interestingly, although rapamycin does not prevent cytokine generation under identical experimental conditions, it can antagonize the effect of tacrolimus when present in a large molar excess which presumably relates to its close structurally similarity (Hom and Estridge, 1993). On balance, the spectrum of activities of FK 506, cyclosporin, and rapamycin on eosinophils is comparable to that established in T lymphocytes and mast cells. Thus, although eosinophilia is T cell dependent and highly sensitive to tacrolimus and cyclosporin A (Thompson, 1993; Fruman et al., 1994; Thompson et al., 1994), similarities in certain signal transduction pathways between T lymphocytes and eosinophils would permit immunosuppressants to target the latter directly in vivo which may prove potentially useful in the treatment of eosinophil-based inflammatory conditions that do not respond to less aggressive, conventional therapies.

It is noteworthy that the ability of cyclosporin A to promote apoptosis of murine eosinophils does not seem to require binding to cyclophilin (Kitagaki et al., 1997). In fact cyclosporin H, an analog which demonstrates little affinity toward immunophilins, is as effective as cyclosporin A at enhancing apoptosis of eosinophils harvested from BAL fluid of allergen-challenged mice. A role for calcineurin and the cytoplasmic component of NF-AT does not seem to be required for the apoptotic effect of these drugs.

2. In Vivo Effects. The effect of immunosuppressant drugs in laboratory models of asthma or eosinophilia has been widely studied since the late 1980s (Etienne et al., 1989a; Akutsu et al., 1990; Arima et al., 1991; Elwood et al., 1992; Boichot et al., 1993; Francischi et al., 1993a,b,c; Bozza et al., 1994b; Fukuda, 1994; Lagente et al., 1994a; Tominaga et al., 1995; Teixeira et al., 1996c; Eum et al., 1997; Williams et al., 1997). One of the first reports was published by Etienne and colleagues (1989a) who demonstrated that cyclosporin A abolished the increase in eosinophils in the blood and peritoneal fluid of rats rendered hypereosinophilic by Sephadex beads or cyclophosphamide. Those results were essentially confirmed in a model of pleural eosinophilia elicited by intrathoracic administration of LPS (Bozza et al., 1994b); however, in that study it was concluded, by use of the monoclonal antibody, Thy-1.0, and dichloromethylene diphosphonate which deplete T lymphocytes and macrophages, respectively, that cyclosporin A was not directly affecting eosinophil trafficking.

Comparable results have been obtained with rapamycin, tacrolimus, and cyclosporin A in rat and guinea pig models of pulmonary eosinophilia (Arima et al., 1991; Elwood et al., 1992; Francischi et al., 1993a,b,c; Tominaga et al., 1995; Teixeira et al., 1996c; Eum et al., 1997; Williams et al., 1997). In a detailed histological study, Lagente et al. (1994a) measured the accumulation of eosinophils in the peribronchial area of sensitized, challenged guinea pigs and in naïve animals exposed to PAF and LTB_4. Using Luna's reagent, which labels eosinophil granule contents, they reported that cyclosporin A (10 mg/kg orally 3 times a day for 2 days and then 1 h before allergen exposure) reduced the appearance of eosinophil degranulation products. Identical results were described for tacrolimus in allergen-challenged mice where the number of eosinophils in the BAL fluid was significantly attenuated; however, this effect is likely to be due to the suppression of IL-5 release (Eum et al., 1997). In contrast, an in vivo model of allergen-induced cutaneous eosinophilia in guinea pigs was not sensitive to cyclosporin A, given systemically, under conditions where dexamethasone was effective. Similar results were obtained in naïve animals where eosinophil influx into the skin was achieved by the local administration of PAF, SOZ, or LPS (Teixeira et al., 1996c). Thus, the eosinophil might not be an important target for cyclosporin A in that model of allergic inflammation.

Clinical evidence points toward a potential therapeutic activity of immunosuppressants. Cyclosporin A has been reported to reduced the amount of ECP present in the serum of patients with severe atopic dermatitis (Caproni et al., 1996) and in the lachrymal fluid of individuals with vernal keratoconjunctivitis after chronic (7–14 days) treatment (Leonardi et al., 1995). Moreover, Shu-

pack et al. (1992) have documented that low-dose cyclosporin A decreases the peripheral blood eosinophil count in patients with severe psoriasis. Thus, those data would suggest that cyclosporin A is able to reduce the release of mediators which promote hematopoiesis, degranulation and/or, more directly, block those processes which govern the exocytotic response in eosinophils (Caproni et al., 1996).

A number of clinical trials have been conducted with oral cyclosporin A in patients with steroid-dependent, chronic severe asthma with modest beneficial effects (Szeczelik et al., 1991; Alexander et al., 1992; Fukuda, 1994). Similarly, cyclosporin A, in low doses (2.5 mg/kg/day), demonstrates significant clinical efficacy in individuals with atopic dermatitis (Ross and Camp, 1990; Wahlgren et al., 1990; Sowden et al., 1991; Salek et al., 1993). However, the use of immunosuppressants is limited by side effects, especially nephrotoxicity, and probably should be considered as a last resort to treat asthma or dermatitis if oral steroids are inactive.

N. Nitric Oxide

The gas, NO, is formed from L-arginine by a family of enzymes collectively known as NO synthases and mediates its widespread biological effects primarily through the stimulation of soluble guanylyl cyclase and the subsequent activation of the cyclic GMP/cyclic GMP-dependent protein kinase cascade. Three NO synthases are currently defined. Two of these, eNOS and nNOS, are constitutively expressed and are so denoted to reflect the tissue (endothelial and neuronal, respectively) where they were first identified. The third isoenzyme is called iNOS as it can be *induced* by LPS and certain cytokines. eNOS, nNOS, and iNOS are the products of different genes and have deduced molecular masses of 133, 160, and 131 kDa, respectively. See Moncada et al. (1997) for further details.

Many years elapsed after NO was identified and its significance appreciated before del Pozo et al. (1997) identified mRNA transcripts and protein for iNOS in human peripheral blood eosinophils and the human eosinophilic cell line,Eol-3. Subcloning of a 259-bp fragment from three different human eosinophil iNOS cDNAs revealed 97% sequence identity with macrophage/monocyte iNOS (del Pozo et al., 1997). The iNOS in eosinophils and Eos-3 cells is functional as evinced from the finding that nitrite is released into the supernatant of cultured cells by a mechanism that is blocked by the NO synthase inhibitor, L-NMA (del Pozo et al., 1997). Using a panel of antibodies, Kobzik et al. (1997) also has identified the endothelial form of NO synthase in normal human eosinophils using an immunoperoxidase-based staining technique and extended those findings to eosinophils present in the airways of asthmatic lung. Prominent labeling of nitrotyrosine was detected within eosinophils in the same lung sections demonstrating that NO is synthesized in vivo and may be contributing to the ongoing inflammatory response (Kobzik et al., 1997). In laboratory animals, iNOS and eNOS protein have been localized to the cytoplasmic granules of rat peritoneal eosinophils where they are expressed by approximately 30% and 25% of cells, respectively (Zanardo et al., 1997). Moreover, these cells generate nitrite in response to LPS or a combination of LPS with IL-8 or IFNγ and this is blocked with L-NIO (Oliveira et al., 1998).

1. Apoptosis. Beauvais and colleagues (1995b) have reported that the NO donors azide and hydroxylamine suppress programmed cell death of cytokine-deprived eosinophils purified from human peripheral blood. That effect is presumably a cyclic GMP-driven process for it was mimicked by dibutyryl cyclic GMP and attenuated by an inhibitor of soluble guanylyl cyclase, LY 83583. Those original observations have, to some degree, been confirmed. Using an eosinophil/U937 coculture system, Hebestreit and coworkers (1998) reported that anti-CD95 promoted eosinophil apoptosis by a mechanism that was prevented when LPS and IFNγ were introduced into the culture medium. This pharmacological intervention up-regulates iNOS gene expression in the U937 cell population, with a consequent increase in the liberation of NO that is proposed to then act on the eosinophils. Compelling evidence that NO was the U937-derived, antiapoptotic mediator was the finding that L-NMMA and LY 83583 abolished the ability of LPS/IFNγ to rescue eosinophils from CD95-mediated death. In addition, SNAP, azide, and hydroxylamine prevented apoptosis of a pure population of freshly isolated human eosinophils which was mimicked by IBMX (Hebestreit et al., 1998). On face value, the ability of IBMX to suppress apoptosis is not unexpected; however, eosinophils lack PDE isoenzymes that hydrolyze cyclic GMP (Dent et al., 1991, 1994; Souness et al., 1991; Hatzelmann et al., 1995) which raises the possibility that the antiapoptotic activity of IBMX is due to the inhibition of PDE4, the predominant PDE in these cells. Indeed, this contention is consistent with the greater ability of dibutyryl cAMP to rescue eosinophils from CD95-mediated apoptosis when compared to its sister analog, cyclic GMP (Hebestreit et al., 1998). However, neither rolipram nor denbufylline promote human eosinophil apoptosis (Hallsworth et al., 1996), raising the possibility that IBMX acts via a PDE-independent process.

Studies in a number of cells including human eosinophils (Hebestreit et al., 1998) have established that apoptosis resulting from activation of CD95 is associated with the stimulation an acidic sphingomyelinase (Cifone et al., 1993; Tepper et al., 1995), resulting in ceramide formation, an increase in the activity of the MAP kinase family, JNK (Cahill et al., 1996; Wilson et al., 1996), and the activation of a number of proteinases that are essential components of the biochemical machinery that execute cell death (Martin and Green, 1995). Those enzymes degrade nuclear proteins, such as lamins [which

turnover very slowly in nonapoptotic cells (Oberhammer et al., 1994)], whose fragments are required for the packaging of condensed chromatin into apoptotic bodies (Lazebnik et al., 1995).

In human eosinophils, the site(s) at which NO could act to prevent CD95-mediated apoptosis has been investigated. In a detailed study, Hebestreit and colleagues (1998) noted that NO did not reduce CD95 expression or the CD95-dependent liberation of ceramide. However, SNAP and dibutyryl cyclic GMP effectively blocked C_2-ceramide-induced eosinophil apoptosis and the activation of JNK, which was assessed by measuring the phosphorylation of c-*jun*, a component of the transcription factor AP-1. Furthermore, SNAP and/or dibutyryl cyclic GMP prevented the activation of various proteinases that are activated in eosinophils following ligation of CD95 (Hebestreit et al., 1998) and the degradation of a 74-kDa nuclear protein, lamin B_1. Thus, these data suggest that NO blocks CD95-mediated apoptosis by acting at the level of, or proximal to, JNK but distal to the generation of ceramide by sphingomyelinase (Hebestreit et al., 1998).

2. Chemotaxis. The only other index of eosinophil activation where NO has been shown to play a role is in chemotaxis. Zanardo and colleagues (1997) demonstrated that incubation of rat eosinophils with the NO synthase inhibitors, L-NAME (nonselective), AMT (iNOS-selective), and TRIM (nNOS/iNOS-selective) inhibited fMLP- and LTB_4-induced migration under conditions where D-NAME was inactive. The finding that the L-NAME-induced effect was completely restored by SNP and dibutyryl cyclic GMP and mimicked by the putative inhibitor of soluble guanylyl cyclase, ODQ, strongly supports the idea that NO promotes chemotaxis of rat eosinophils by a cyclic GMP-dependent mechanism (Zanardo et al., 1997). In another study eosinophils, isolated from rats treated chronically with L-NAME in the drinking water, were significantly less sensitive to the chemotaxins, fMLP, PAF, and SOZ when studied ex vivo. The mechanism of that effect was attributed to the inhibition of NO synthesis, because D-NAME was inactive and L-arginine, but not D-arginine, reversed the effect of L-NAME (Ferreira et al., 1996). The regulation of chemotaxis by NO also is seen in human peripheral blood eosinophils (Robbins et al., 1995). Thus, chemotaxis effected by fMLP, PAF, IL-3, and IL-5 is attenuated by L-NMMA, L-NAME, and aminoguanidine, and unaffected by the inactive enantiomer, D-NMMA; consistent with the rat data described above, L-arginine and SNP partially restored the effect of L-NAME (Robbins et al., 1995). Collectively, these data suggest that many agents that promote chemotaxis do so by stimulating the production of NO which enhances the locomotor activity of eosinophils.

3. In Vivo Effects. The effect of chronic treatment of rats with L-NAME on bradykinin-, PAF-, LPS-, and carageenan-induced eosinophil migration in model of pleurisy has been reported (Ferreira et al., 1996). Intrapleural injection of the aforementioned chemotaxins resulted in a significant increase in the number of eosinophils found in the pleural cavity at 24 h that was attenuated in rats given L-NAME but not the inactive enantiomer D-NAME. Comparable findings have been reported in a sensitized animal model of pulmonary eosinophilia (Feder et al., 1997; Ferreira et al., 1998). In one representative study, the effect of four NOS inhibitors (L-NAME, L-NMMA, L-NIL, aminoguanidine) administered to sensitized B6D2F1/J mice by the i.p. route were evaluated for their ability to modify the number of eosinophils that appeared in BAL fluid and lung tissue 24 h after antigen challenge. Consistent with all the currently available data, NAME attenuated pulmonary eosinophil recruitment in an enantio-selective manner by a mechanism that was largely prevented by L-arginine. The same result was obtained with aminoguanidine and L-NMMA, but not with the iNOS inhibitor L-NIL. The latter observation is significant, as allergen challenge had no effect on steady-state level of iNOS mRNA transcripts or protein in the lungs, but increased the level of nitrite in the BAL fluid. Thus, it is unlikely that inducible forms of NO synthase are involved in the regulation of eosinophilia. In the same study, L-NAME had no effect on the reduction of eosinophils in the bone marrow that followed allergen challenge, indicating that NO-dependent pulmonary eosinophil recruitment is not due to an enhancement of eosinophil efflux from the marrow. The site of NO production is unclear but Feder et al. (1997) have speculated that pulmonary vascular endothelial cells could be involved in the extravasation of eosinophils from the circulation into the lung since these cells are under the control of eNOS and may thus provide a source of NO. Alternatively, the ability of NO to inhibit the activity of IFNγ-secreting cells could increase the proliferation of Th2 lymphocytes and thereby the elaboration of IL-5.

O. Cetirizine and Other Second-Generation Histamine H_1 Receptor Antagonists

Cetirizine, a carboxylated metabolite of hydroxyzine, is a potent, long-acting, second-generation histamine H_1 receptor antagonist used in the treatment of atopic dermatitis, urticaria, and allergic rhinitis (Spencer et al., 1993). In addition, cetirizine exerts pharmacological effects distinct from those which arise from an interaction at H_1 receptors (Walsh, 1993, 1997b). With respect to the eosinophil, cetirizine inhibits several indices of activation that might contribute to its therapeutic activity in the treatment of allergic disorders. In vitro studies have demonstrated that PAF-induced hyperadherence of human eosinophils to HUVECs is suppressed by cetirizine in the nanomolar range (Sehmi et al., 1993). Higher concentrations of cetirizine (100 μg/ml) also attenuate the adherence of fMLP-stimulated eosinophils to resting cultured endothelial cells and the adherence of

unstimulated eosinophils to endothelial cells exposed to IL-1 (Kyan Aung et al., 1992), although the clinical significance of those effects is unclear. Other in vitro functional responses where cetirizine, at concentrations achieved therapeutically, is inhibitory include adhesion-dependent processes such as PAF-induced, eosinophil-enhanced complement, and IgG-dependent rosette formation and complement-dependent cytotoxicity (Walsh et al., 1991b), superoxide anion generation from eosinophils purified from the blood of allergic subjects (Okada et al., 1994) and eosinophil migration in response to fMLP, IL-8, C5a, and LTB_4 (Leprevost et al., 1988; De Vos et al., 1989; Sehmi et al., 1993). Significantly this latter effect is not mimicked by the antihistamine dexchlorpheniramine (Leprevost et al., 1988), suggesting that the histamine H_1 receptor is not involved. Integrin-dependent activation of the NADPH oxidase in human normodense eosinophils also is blocked by cetirizine but high concentrations are required (Piacentini et al., 1996).

In vivo studies have established that cetirizine attenuates cutaneous, pleural, and pulmonary eosinophil infiltration in response to several stimuli which is consistent with its ability to block adhesion and chemotaxis in vitro. In sensitized rats cetirizine, given by the i.p. route, prevents allergen-induced eosinophil accumulation into the pleural cavity and can resolve existing eosinophilia when injected directly into the thorax (Pasquale et al., 1992). Similar data have been obtained using PAF and compound 48/80 to induce eosinophil emigration in the same model (Martins et al., 1992). Neither of those effects seem to be related to histamine H_1 receptor blockade. In another study, topical exposure of the airways of *ascaris*-sensitized beagle dogs to cetirizine blocks pulmonary eosinophilia following allergen provocation (Johnson et al., 1992). However, a proportion of that effect is certainly attributable to the antagonism of mast cell-derived histamine at H_1 receptors since mepyramine and terfenadine were equally active (Johnson et al., 1992). Comparable data have been obtained in a number of human studies (Fadel et al., 1987, 1990, 1991; Michel et al., 1988; Charlesworth et al., 1989). In a double-blind, placebo-controlled crossover study in pollen-sensitive subjects, oral cetirizine inhibited the infiltration of eosinophils into the superficial dermis after antigen challenge (Michel et al., 1988). Those data were largely supported in a subsequent investigation where cetirizine decreased eosinophil infiltration into the skin of ragweed-sensitive subjects by almost 80% (Charlesworth et al., 1989). However, unlike the results obtained in beagle dogs (Johnson et al., 1992), cetirizine was not acting as a histamine H_1 receptor antagonist because it failed to block the effect of exogenous histamine, and another antihistamine, astemizole, was inactive. Using a "window" technique to measure the number of resident and invading leukocytes in the skin, oral cetirizine significantly attenuates eosinophil migration in response to pollen, PAF, and fMLP in allergic subjects under conditions where intradermal injection of histamine is unaffected (Fadel et al., 1987, 1990,). A preliminary report also documents the ability of cetirizine to attenuate skin reactions in normal and atopic subjects in response to bradykinin given intradermally and by the skin-prick technique (see Walsh, 1997b). It is noteworthy that two studies, which evaluated the effect of cetirizine on allergen-induced LPRs in atopic subjects, failed to detect any suppression of the cellular infiltrate in response to allergen [although in some subjects eosinophil numbers were decreased (Varney et al., 1992; Taborda Barata et al., 1996)]. However, this apparent discrepancy could be methodological (Walsh, 1997b) since punch biopsies, which measure the gross cell content at the reaction site, were used to enumerate cell numbers in those investigations, whereas the skin window technique, which permits the enumeration of cells that have left the postcapillary venules in the dermis, was used in the earlier assessments.

The biochemical basis of the histamine H_1-independent effects of cetirizine is unknown although an interaction at other cell surface receptors is unlikely (Snyder and Snowman, 1987). Low concentrations (0.7–1 μg/ml) of cetirizine increase the lipid order in the exterior part of eosinophil membranes, decrease membrane heterogeneity, and block PAF-induced changes in membrane fluidity (Kantar et al., 1994, 1996). However, the extent to which those physical effects contribute to the aforementioned pharmacological actions of cetirizine remain to be established.

Several other second generation antihistamines have been synthesized that exhibit inhibitory/anti-inflammatory properties unrelated to H_1 receptor blockade. These drugs and and the functional effects they produce in eosinophils are detailed in Table 21.

XV. Concluding Remarks

The last decade has witnessed significant advances in our understanding of the basic pharmacology and immunopharmacology of eosinophils to a level that now is challenging discoveries made by classical parasitologists and immunologists. Almost certainly, this is due to significant refinements in eosinophil purification, the development of new biochemical assays and the application of molecular biological techniques with which to study the regulation of cell surface receptors, G proteins, ion channels, and second and tertiary messenger molecules. Despite this new knowledge and over 100 years of research experience that has encompassed many disciplines, the role of the eosinophil in health and disease still is equivocal. It is true that support has shifted away from the original concept that eosinophils participate in tissue preservation and protection of the host against invading parasites. However, although the trend in the 1990s has been to consider the eosinophil as a proinflammatory cell (see Table 1), proof-of-concept clinical stud-

TABLE 21
Inhibitory effects on eosinophils of second generation histamine H_1 receptor antagonists

Drug	Species	Effect	Reference
Azelastine	Human	Inhibits fMLP-, PMA, and A 23187-superoxide anion generation	Busse et al. (1989)
Azelastine	Eo1 cells	Inhibits PAF- and fMLP-induced Ca^{2+} influx and actin polymerization	Morita et al. (1993)
Azelastine	Eo1 cells	Inhibits A 23187-induced ECP release	Morita et al. (1993)
Azelastine	Human	Suppresses allergen-induced eosinophilia in the nose of allergic subjects	Ciprandi et al. (1996)
Azelastine	Guinea pig	Suppresses allergen-induced eosinophilia in the BAL fluid	Chand et al. (1992b)
Azelastine	Human	Inhibits generation of PAF-like material evoked by fMLP	Shindo and Fukumura (1996b)
Loratadine	Human	Inhibits PAF-induced chemotaxis and superoxide anion generation	Eda et al. (1993b)
Terfenadine	Human	Inhibits PAF-induced chemotaxis and superoxide anion generation	Eda et al. (1994)
Emedastine	Human	Inhibits PAF-induced chemotaxis	Elshazly et al. (1996b)
Epinastine	Human	Inhibits IL-8 release	Kohyama et al. (1997)

ies still are not possible. It is conceivable that other aspects of eosinophil physiology and pathophysiology will dominate research in the immediate future before the role of this cell in allergic diseases is determined. Indeed, the potential involvement of eosinophils in wound healing and, more importantly, cancer immunity (see Table 1) makes this likely. Nevertheless, it is hoped that the advent of new technologies will shed light on the function(s) of one of the most poorly understood leukocytes in the near future.

Acknowledgments. Research performed by us at the time of writing was supported by the Wellcome Trust, Medical Research Council, British Lung Foundation, and National Asthma Campaign (UK). We are indebted to Dr. Terry Newman (Imperial College School of Medicine at the National Heart & Lung Institute, UK) and Professor Bastien Gomperts (University College London, UK) for the beautiful electron micrographs and freeze-fracture replicas shown in Figs. 1, 3, 8, 9, and 11. We also thank Drs. Duncan Rogers, Anne Burke-Gaffney (Imperial College School of Medicine at the National Heart and Lung Institute, UK), and Jonas Erjefält (University of Lund, Sweden) for permission to present Figs. 7, 6, and 10, respectively. We express special thanks to Dr. Gordon Dent (University of Southampton, UK) and Professor Paul Hellewell (University of Sheffield, UK) for the arduous task of proofreading the manuscript.

REFERENCES

Aalbers R, Kauffman HF, Groen H, Koeter GH and de Monchy JG (1991) The effect of nedocromil sodium on the early and late reaction and allergen-induced hyperresponsiveness. *J Allergy Clin Immunol* **87**:993–1000.
Abdelaziz MM, Devalia JL, Khair OA, Rusznak C, Calderon M, Sapsford RJ, Bayram H and Davies RJ (1997) The effect of nedocromil sodium on human airway epithelial cell-induced eosinophil chemotaxis and adherence to human endothelial cell *in vitro*. *Eur Respir J* **10:**851–857.
Abdelilah SG, Bouchaib L, Morita M, Delphine A, Marika S, Andre C and Monique C (1998) Molecular characterization of the low-affinity IgE receptor FcεRII/CD23 expressed by human eosinophils. *Int Immunol* **10:**395–404.
Abraham WM (1989) Effect of nedocromil sodium on antigen-induced airway responses in allergic sheep. *Drugs* **37:**78–86.
Abramovitz M, Boie Y, Nguyen T, Rushmore TH, Bayne MA, Metters KM, Slipetz DM and Grygorczyk R (1994) Cloning and expression of a cDNA for the human prostanoid FP receptor. *J Biol Chem* **269:**2632–2636.
Abu Ghazaleh RI, Fujisawa T, Mestecky J, Kyle RA and Gleich GJ (1989) IgA-induced eosinophil degranulation. *J Immunol* **142:**2393–2400.
Ackerman SJ, Loegering DA and Gleich GJ (1980) The human eosinophil Charcot-Leyden crystal protein: biochemical characteristics and measurement by radioimmunoassay. *J Immunol* **125**: 2118–2126.
Ackerman SJ, Loegering DA, Venge P, Olsson I, Harley JB, Fauci AS and Gleich GJ (1983) Distinctive cationic proteins of the human eosinophil granule: Major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin. *J Immunol* **131:**2977–2982.
Ackerman SJ, Weil GJ and Gleich GJ (1982) Formation of Charcot-Leyden crystals by human basophils. *J Exp Med* **155:**1597–1609.
Adachi T, Motojima S, Hirata A, Fukuda T, Kihara N, Kosaku A, Ohtake H and Makino S (1996) Eosinophil apoptosis caused by theophylline, glucocorticoids, and macrolides after stimulation with IL-5. *J Allergy Clin Immunol* **98:**S207–S215.
Adelroth E, Rosenhall L, Johansson SA, Linden M and Venge P (1990) Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage: The effect of anti-asthmatic treatment with budesonide and terbutaline. *Am Rev Respir Dis* **142:**91–99.
Agrawal DK, Ali N and Numao T (1992) PAF receptors and G-proteins in human blood eosinophils and neutrophils. *J Lipid Mediat* **5:**101–104.
Agrawal DK, Sarmiento EU, Nabe M, Miyagawa H and Townley RG (1996) Increased hypodense eosinophils after activation with PAF-acether and calcium ionophore in asthmatic subjects. *J Asthma* **33:**213–219.
Ahearn JM and Fearon DT (1989) Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). *Adv Immunol* **46:**183–219.
Aizawa T, Kakuta Y, Yamauchi K, Ohkawara Y, Maruyama N,Nitta Y, Tamura G, Sasaki H and Takishima T (1992) Induction of granule release by intracellular application of calcium and guanosine-5′-O-(3-thiotriphosphate) in human eosinophils. *J Allergy Clin Immunol* **90:**789–795.
Aizawa T, Tamura G, Ohtsu H and Takishima T (1990) Eosinophil and neutrophil production of leukotriene C_4 and B_4: Comparison of cells from asthmatic subjects and healthy donors. *Ann Allergy* **64:**287–292.
Akutagawa H, Ohshima Y, Morita M, Uenoyama Y, Nambu M, Horiguchi Y, Mayumi M and Mikawa H (1994) Effect of dibutyryl cAMP and interferon-γ on Fcγ receptor expression on eosinophils. *Hematol Pathol* **8:**85–97.
Akutsu I, Fukuda T, Numao T and Makino S (1990) Inhibitory effect of FK506 on the development of late asthmatic response and on increased bronchial responsiveness. *Arerugi* **39:**605–609.
Alam R, Forsythe P, Stafford S and Fukuda Y (1994) Transforming growth factor β abrogates the effects of hematopoietins on eosinophils and induces their apoptosis. *J Exp Med* **179:**1041–1045.
Alam R and Grant JA (1995) The chemokines and the histamine-releasing factors: modulation of function of basophils, mast cells and eosinophils. *Chem Immunol* **61:**148–160.
Alam R, Pazdrak K, Stafford S and Forsythe P (1995) The interleukin-5/receptor interaction activates Lyn and Jak2 tyrosine kinase and propagates signals via the Ras-Raf-1-MAP kinase and Jak-STAT pathways in eosinophils. *Int Arch Allergy Immunol* **107:**226–227.
Alam R, Stafford S, Forsythe P, Harrison R, Faubion D, Lett Brown MA and Grant JA (1993) RANTES is a chemotactic and activating factor for human eosinophils. *J Immunol* **150:**3442–3448.
Aldebert D, Lamkhioued B, Desaint C, Gounni AS, Goldman M, Capron A, Prin L and Capron M (1996) Eosinophils express a functional receptor for interferon α: inhibitory role of interferon α on the release of mediators. *Blood* **87:**2354–2360.
Alexander AA, Barnes NC and Kay AB (1992) Trial of cyclosporin in corticosteroid-dependent chronic severe asthma. *Lancet* **339:**324–328.
Ali S, Mustafa SJ and Metzger WJ (1992) Modification of allergen-induced airway obstruction and bronchial hyper-responsivness in the allergic rabbit by theophylline aerosol. *Agents Actions* **37:**165–173.
Aliakbari J, Sreedharan SP, Turck CW and Goetzl EJ (1987) Selective localization of vasoactive intestinal peptide and substance P in human eosinophils. *Biochem Biophys Res Commun* **148:**1440–1445.
Allen JM and Seed B (1989) Isolation and expression of functional high-affinity Fc receptor complementary DNAs. *Science (Wash DC)* **243:**378–381.
Allen JN and Davis WB (1994) Eosinophilic lung diseases. *Am J Respir Crit Care Med* **150:**1423–1438.
Aloui R, Gormand F, Prigent AF, Perrinfayolle M and Pacheco Y (1996) Increased respiratory burst and phosphodiesterase activity in alveolar eosinophils in chronic eosinophilic pneumonia. *Eur Respir J* **9:**377–379.
Altman LC, Hill JS, Hairfield WM and Mullarkey MF (1981) Effects of corticosteroids on eosinophil chemotaxis and adherence. *J Clin Invest* **67:**28–36.
Aloe L, Bracci-Laudiero L, Bonini S and Manni L (1997) The expanding role of nerve growth factor: From neurotrophic activity to immunologic diseases. *Allergy* **52**: 883–894.
Alvarez de Toledo G and Fernandez JM (1990) Patch-clamp measurements reveal multimodal distribution of granule sizes in rat mast cells. *J Cell Biol* **110:**1033–1039.
Alves AC, Pires ALA, Cruz HN, Serra MF, Diaz BL, Cordeiro RSB, Lagente V, Martins MA and Silva PMRE (1996) Selective inhibition of phosphodiesterase type IV suppresses the chemotactic responsiveness of rat eosinophils *in vitro*. *Eur J Pharmacol* **312:**89–96.
Ames RS, Li Y, Sarau HM, Nuthulaganti P, Foley JJ, Ellis C, Zeng Z, Su K, Jurewicz AJ, Hertzberg RP, Bergsma DJ and Kumar C (1996) Molecular cloning and characterization of the human anaphylatoxin C3a receptor. *J Biol Chem* **271:** 20231–20234.
Ames RS, Tornetta MA, Foley JJ, Hugli TE and Sarau HM (1997) Evidence that the receptor for C4a is distinct from the C3a receptor. *Immunpharmacology* **38:**87–92.
Anderson DC, Miller LJ, Schmalsteig FC, Rothlein R and Springer TA (1986) Contributions of the Mac-1 glycoprotein family to adherence-dependent granulo-

cyte functions: Structure-function assessment employing subunit-specific monoclonocal antibodies. *J Immunol* **137:**15–27.
Andersson M, Andersson P, Venge P and Pipkorn U (1989) Eosinophils and eosinophil cationic protein in nasal lavages in allergen-induced hyperresponsiveness: Effects of topical glucocorticosteroid treatment. *Allergy* **44:**342–348.
Anwar AR and Kay AB (1977) The ECF-A tetrapeptides and histamine selectively enhance human eosinophil complement receptors. *Nature (Lond)* **269:**522–524.
Anwar AR and Kay AB (1980) H_1-receptor dependence of histamine-induced enhancement of human eosinophil C3b rosettes. *Clin Exp Immunol* **42:**196–199.
Anwar AR, Moqbel R, Walsh GM, Kay AB and Wardlaw AJ (1993) Adhesion to fibronectin prolongs eosinophil survival. *J Exp Med* **177:**839–843.
Anwar AR, Walsh GM, Cromwell O, Kay AB and Wardlaw AJ (1994) Adhesion to fibronectin primes eosinophils via $\alpha_4\beta_1$ (VLA-4). *Immunology* **82:**222–228.
Aoki I, Shindoh Y, Nishida T, Nakai S, Hong YM, Mio M, Saito T and Tasaka K (1991) Sequencing and cloning of the cDNA of guinea pig eosinophil major basic protein. *FEBS Lett* **279:**330–334.
Aoki S, Boubekeur K, Kristersson A, Morley J and Sanjar S (1988) Is allergic airway hyper-reactivity of the guinea-pig dependent on eosinophil accumulation in the lung (Abstract)? *Br J Pharmacol* **94**:365P.
Araki R, Komada T, Nakatani K, Naka M, Shima T and Tanaka T (1995) Protein kinase C-independent activation of Raf-1 and mitogen- activated protein kinase by leukotriene B_4 in guinea pig eosinophils. *Biochem Biophys Res Commun* **210:**837–843.
Arch JR, Ainsworth AT, Cawthorne MA, Piercy V, Sennitt MV, Thody VE, Wilson C and Wilson S (1984) Atypical β-adrenoceptor on brown adipocytes as target for anti-obesity drugs. *Nature (Lond)* **309:**163–165.
Arch JRS and Kaumann AJ (1993) β_3-agonists and atypical β-adrenoceptors. *Med Res Rev* **13:**663–729.
Archer RK (1963) *The Eosinophil Leukocytes,* Blackwell Scientific, Oxford.
Aridor M, Rajmilevich G, Beaven MA and Sagi Eisenberg R (1993) Activation of exocytosis by the heterotrimeric G protein Gi_3. *Science (Wash DC)* **262:**1569–1572.
Arima M, Yukawa T and Makino S (1995) Effect of YM264 on the airway hyperresponsiveness and the late asthmatic response in a guinea-pig model of asthma. *Chest* **108:**529–534.
Arima M, Yukawa T, Terashi Y and Makino S (1991) Cyclosporin A inhibits allergen-induced late asthmatic response an increase of airway hyperresponsiveness in guinea pigs. *Jpn J Thorac Dis* **29:**1089–1095.
Arnoux B, Denjean A, Page CP, Nolibe D, Morley J and Benveniste J (1988) Accumulation of platelets and eosinophils in baboon lung after paf-acether challenge. Inhibition by ketotifen. *Am Rev Respir Dis* **137:**855–860.
Asano M, Inamura N,Nakakara K, Nagayoshi A, Isono T, Hamada K, Oku T, Notsu Y, Kohsaka M and Ono T (1992) A 5-lipoxygenase inhibitor, FR 110302, suppresses airway hyper-responsiveness and lung eosinophilia induced by Sephadex particles in rats. *Agents Actions* **36:**215–221.
Asmis R and Jorg A (1990) Calcium-ionophore-induced formation of platelet-activating factor and leukotrienes by horse eosinophils: A comparative study. *Eur J Biochem* **187:**475–480.
Atsuta J, Fujisawa T, Iguchi K, Terada A, Kamiya H and Sakurai M (1995) Inhibitory effect of transforming growth factor beta 1 on cytokine-enhanced eosinophil survival and degranulation. *Int Arch Allergy Immunol* **108 (Suppl 1):**31–35.
Atsuta J, Sterbinsky SA, Schweibert LA, Bochner BS and Schleimer RP (1998) Phenotyping and cytokine regulation of the human bronchial epithelial cell line BEAS-2B: demonstration of inducible expression of the adhesion molecules VCAM-1 and ICAM-1. *Am J Respir Cell Mol Biol* **18:**999.
Austen KF (1995) From slow reacting substance of anaphylaxis to leukotriene C_4 synthase. *Int Arch Allergy Immunol* **107:**19–24.
Azuma M, Nakamura Y, Sano T, Okano Y and Sone S (1996) Adhesion molecule expression on eosinophils in idiopathic eosinophilic pneumonia. *Eur Respir J* **9:**2494–2500.
Babior BM, Kipnes RS and Curnutte JT (1973) Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *J Clin Invest* **52:**741–744.
Bach MK, Brashler JR, Sanders ME, Furitsu T and Ishizaka T (1990) How similar are *in vitro* differentiated, cord blood derived eosinophils to peripheral blood eosinophils? A comparison of their peroxidase and eosinophil-derived neurotoxin contents and of their responses to various activators. *Int Arch Allergy Appl Immunol* **93:**323–329.
Bach MK, Brashler JR, Stout BK, Johnson HG and Sanders ME (1991) Platelet-derived growth factor can activate purified primate, phorbol myristate acetate-primed eosinophils. *Int Arch Allergy Appl Immunol* **94:**167–168.
Bach MK, Brashler JR, Stout BK, Johnson HG, Sanders ME, Lin AH, Gorman RR, Bienkowski MJ and Ishizaka T (1992) Activation of human eosinophils by platelet-derived growth factor. *Int Arch Allergy Immunol* **97:**121–129.
Baecker PA, Obernolte R, Bach C, Yee C and Shelton ER (1994) Isolation of a cDNA encoding a human rolipram-sensitive cAMP phosphodiesterase (PDE IVD). *Gene* **138:**253–256.
Baigelman W, Chodosh S, Pizzuto D and Cupples LA (1983) Sputum and blood eosinophils during corticosteroid treatment of acute exacerbations of asthma. *Am J Med* **75:**929–936.
Bainton DF and Farquhar MG (1970) Segregation and packaging of granule enzymes in eosinophilic leukocytes. *J Cell Biol* **45:**54–73.
Balch WE (1990) Small GTP-binding proteins in vesicular transport. *Trends Biochem Sci* **15:**473–477.
Bankers-Fulbright JL, Kephart GM, Loegering DA, Bradford AL, Okada S, Kita H and Gleich GJ (1998) Sulfonylureas inhibit cytokine-induced eosinophil survival and activation. *J Immunol* **160:**5546–5553.
Banner KH, Marchini F, Buschi A, Moriggi E, Semararo C and Page CP (1995) The effect of selective phosphodiesterases inhibitors in comparison with other anti-asthma drugs on allergen-induced eosinophilia in guinea pig airways. *Pulmonary Pharmacol* **8:**37–42.
Banner KH and Page CP (1995a) Theophylline and selective phosphodiesterase inhibitors as anti-inflammatory drugs in the treatment of bronchial asthma. *Eur Respir J* **8:**996–1000.
Banner KH and Page CP (1995b) Acute *versus* chronic administration of phosphodiesterase inhibitors on allergen-induced pulmonary cell influx in sensitized guinea-pigs. *Br J Pharmacol* **114:**93–98.
Banner KH and Page CP (1996) Anti-inflammatory effects of theophylline and selective phosphodiesterase inhibitors. *Clin Exp Allergy* **26** (**Suppl 2):**2–9.
Barber DL, McGuire ME and Ganz MB (1989) Beta-adrenergic and somatostatin receptors regulate Na-H exchange independent of cAMP. *J Biol Chem* **264:**21038–21042.
Barinaga M (1994) Cell suicide: by ICE, not fire. *Science (Wash DC)* **263:**754–756.
Barker RL, Gleich GJ and Pease LR (1988) Acidic precursor revealed in human eosinophil granule major basic protein cDNA. *J Exp Med* **168:**1493–1498.
Barker RL, Loegering DA, Arakawa KC, Pease LR and Gleich GJ (1990) Cloning and sequence analysis of the human gene encoding eosinophil major basic protein. *Gene* **86:**285–289.
Barker RL, Loegering DA, Ten RM, Hamann KJ, Pease LR and Gleich GJ (1989) Eosinophil cationic protein cDNA. Comparison with other toxic cationic proteins and ribonucleases. *J Immunol* **143:**952–955.
Barnes PJ (1990) Effect of corticosteroids on airway hyper-responsiveness. *Am Rev Respir Dis* **141:**S70–S76.
Barnes PJ (1993) Anti-inflammatory therapy in asthma. *Annu Rev Med* **44:**229–249.
Barnes PJ (1995) Inhaled glucocorticoids for asthma. *N Engl J Med* **332:**868–875.
Barnes PJ (1997) Glucocorticosteroids. in *Allergy and Allergic Diseases* (Kay AB ed) pp 619–641, Blackwell Scientific, London.
Barnes PJ, Chung KF and Page CP (1988) Platelet activating factor as a mediator of allergic disease. *J Allergy Clin Immunol* **81:**919–934.
Barnes PJ and Pedersen S (1993) Efficacy and safety of inhaled steroids in asthma. *Am Rev Respir Dis* **148:**S1–S26.
Barnette MS, Bartus JO, Burman M, Christensen SB, Cieslinski LB, Esser KM, Prabhakar US, Rush JA and Torphy TJ (1996) Association of the anti-inflammatory activity of phosphodiesterase 4 (PDE4) inhibitors with either inhibition of PDE4 catalytic activity or competition for [^{3}H]rolipram binding. *Biochem Pharmacol* **51:**949–956.
Barnette MS, Grous M, Cieslinski LB, Burman M, Christensen SB and Torphy TJ (1995a) Inhibitors of phosphodiesterase IV (PDE IV) increase acid secretion in rabbit isolated gastric glands: Correlation between function and interaction with a high-affinity rolipram binding site. *J Pharmacol Exp Ther* **273:**1396–1402.
Barnette MS, Manning CD, Cieslinski LB, Burman M, Christensen SB and Torphy TJ (1995b) The ability of phosphodiesterase IV inhibitors to suppress superoxide production in guinea pig eosinophils is correlated with inhibition of phosphodiesterase IV catalytic activity. *J Pharmacol Exp Ther* **273:**674–679.
Bartolazzi A, Nocks A, Aruffo A, Spring F and Stamenkovic I (1996) Glycosylation of CD44 is implicated in CD44-mediated cell adhesion to hyaluronan. *J Cell Biol* **132:**1199–1208.
Bascom R, Pipkorn U, Proud D, Dunnette S, Gleich GJ, Lichtenstein LM and Naclerio RM (1989) Major basic protein and eosinophil-derived neurotoxin concentrations in nasal-lavage fluid after antigen challenge: Effect of systemic corticosteroids and relationship to eosinophil influx. *J Allergy Clin Immunol* **84:**338–346.
Baskar P, Silberstein DS and Pincus SH (1990) Inhibition of IgG-triggered human eosinophil function by IL-4. *J Immunol* **144:**2321–2326.
Bass DA, Grover WH, Lewis JC, Szejda P, DeChatelet LR and McCall CE (1980) Comparison of human eosinophils from normals and patients with eosinophilia. *J Clin Invest* **66:**1265–1273.
Bass DA, Lewis JC, Szejda P, Cowley L and McCall CE (1981) Activation of lysosomal acid phosphatase of eosinophil leukocytes. *Lab Invest* **44:**403–409.
Bates ME, Bertics PJ and Busse WW (1996) IL-5 activates a 45-kilodalton mitogen-activated protein (MAP) kinase and Jak-2 tyrosine kinase in human eosinophils. *J Immunol* **156:**711–718.
Bates ME, Bertics PJ, Calhoun WJ and Busse WW (1993) Increased protein kinase C activity in low density eosinophils. *J Immunol* **150:**4486–4493.
Bates ME, Busse WW and Bertics PJ (1998) Interleukin-5 signals through Shc and Grb2 in human eosinophils. *Am J Respir Cell Mol Biol* **18:**75–83.
Baumhueter S, Dybdal N, Kyle C and Lasky LA (1994) Global vascular expression of murine CD34, a sialomucin-like endothelial ligand for L-selectin. *Blood* **84:**2554–2565.
Beasley RCW, Featherstone RL, Church MK, Rafferty P, Varley JG, Harris A, Robinson C and Holgate ST (1989) Effect of a thromboxane receptor antagonist on PGD_2- and allergen-induced bronchoconstriction. *J Appl Physiol* **66:**1685–1693.
Beauvais F, Michel L and Dubertret L (1995a) Human eosinophils in culture undergo a striking and rapid shrinkage during apoptosis. Role of K^+ channels. *J Leukoc Biol* **57:**851–855.
Beauvais F, Michel L and Dubertret L (1995b) The nitric oxide donors, azide and hydroxylamine, inhibit the programmed cell death of cytokine-deprived human eosinophils. *FEBS Lett* **361:**229–232.
Beavo JA (1988) Multiple isozymes of cyclic nucleotide phosphodiesterase. *Adv Second Messenger Phosphoprotein Res* **22:**1–38.
Beavo JA, Conti M and Heaslip RJ (1994) Multiple cyclic nucleotide phosphodiesterases. *Mol Pharmacol* **46:**399–405.
Begley CG, Lopez AF, Nicola NA, Warren DJ, Vadas MA, Sanderson CJ and Metcalf D (1986) Purified colony-stimulating factors enhance the survival of human neutrophils and eosinophils *in vitro*: A rapid and sensitive microassay for colony-stimulating factors. *Blood* **68:**162–166.
Beil WJ, Weller PF, Peppercorn MA, Galli SJ and Dvorak AM (1995) Ultrastructural immunogold localization of subcellular sites of TNF-α in colonic Crohn's disease. *J Leukoc Biol* **58:**284–298.
Beil WJ, Weller PF, Tzizik DM, Galli SJ and Dvorak AM (1993) Ultrastructural immunogold localization of tumor necrosis factor-α to the matrix compartment of eosinophil secondary granules in patients with idiopathic hypereosinophilic syndrome. *J Histochem Cytochem* **41:**1611–1615.

Belkner J, Wiesner R, Rathman J, Barnett J, Sigal E and Kuhn H (1993) Oxygenation of lipoproteins by mammalian lipoxygenases. *Eur J Biochem* **213:**251–261.
Beller DI, Springer TA and Schreiber RD (1982) Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor. *J Exp Med* **156:**1000–1009.
Bellibas SE (1996) The effect of human calcitonin gene-related peptide on eosinophil chemotaxis in the rat airway. *Peptides* **17:**563–564.
Ben Baruch A, Xu L, Young PR, Bengali K, Oppenheim JJ and Wang JM (1995) Monocyte chemotactic protein-3 (MCP3) interacts with multiple leukocyte receptors. C-C CKR1, a receptor for macrophage inflammatory protein-1α/RANTES, is also a functional receptor for MCP3. *J Biol Chem* **270:**22123–22128.
Beninati W, Derdak S, Dixon PF, Grider DJ, Strollo DC, Hensley RE and Lucey DR (1993) Pulmonary eosinophils express HLA-DR in chronic eosinophilic pneumonia. *J Allergy Clin Immunol* **92:**442–449.
Berends C, Dijkhuizen B, de Monchy JGR, Dubois AEJ, Gerritsen J and Kauffman HF (1997) Inhibition of PAF-induced expression of CD11b and shedding of L-selectin on human neutrophils and eosinophils by the type IV selective PDE inhibitor, rolipram. *Eur Respir J* **10:**1000–1007.
Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, Weissman IL, Hamann A and Butcher EC (1993) Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell* **74:**185.
Beswick PH and Kay AB (1981) The effects of an ECF-A and formyl methionyl chemotactic peptides on oxidative metabolism of human eosinophils and neutrophils. *Clin Exp Immunol* **43:**399–407.
Bevilacqua MP and Nelson RM (1993) Selectins. *J Clin Invest* **91:**379–387.
Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS and Gimbrone MA (1987) Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc Natl Acad Sci USA* **84:**9238–9242.
Bianco S, Pieroni MG, Refine RM, Rottoli L and Siestini P (1989) Protective effect of inhaled furosemide on allergen-induced early and late asthmatic reactions. *N Engl J Med* **321:**1069–1073.
Bianco S, Vaghi A, Robuschi M and Pasargiklian M (1988) Prevention of exercise-induced bronchoconstriction by inhaled frusemide. *Lancet* **2:**252–255.
Bisgaard H, Gronborg H, Mygind N, Dahl R, Lindqvist N and Venge P (1990) Allergen-induced increase of eosinophil cationic protein in nasal lavage fluid: Effect of the glucocorticoid budesonide. *J Allergy Clin Immunol* **85:**891–895.
Bjerke T, Gaustadnes M, Nielsen S, Nielsen LP, Schiotz PO, Rudiger N, Reimert CM, Dahl R, Christensen I and Poulsen LK (1996) Human blood eosinophils produce and secrete interleukin 4. *Respir Med* **90:**271–277.
Bjornson BH, Harvey JM and Rose L (1985) Differential effect of hydrocortisone on eosinophil and neutrophil proliferation. *J Clin Invest* **76:**924–929.
Blease K, Burke-Gaffney A and Hellewell PG (1998) Modulation of cell adhesion molecule expression and function on human lung microvascular endothelial cells by inhibitors of phosphodiesterases 3 and 4. *Br J Pharmacol* **124:**229–237.
Blom M, Tool AT, Kok PT, Koenderman L, Roos D and Verhoeven AJ (1994) Granulocyte-macrophage colony-stimulating factor, interleukin-3 (IL-3), and IL-5 greatly enhance the interaction of human eosinophils with opsonized particles by changing the affinity of complement receptor type 3. *Blood* **83:**2978–2984.
Blom M, Tool AT, Roos D and Verhoeven AJ (1992) Priming of human eosinophils by platelet-activating factor enhances the number of cells able to bind and respond to opsonized particles. *J Immunol* **149:**3672–3677.
Blom M, Tool AT, Wever PC, Wolbink GJ, Brouwer MC, Calafat J, Egesten A, Knol EF, Hack CE, Roos D and Verhoeven AJ (1998) Human eosinophils express, relative to other circulating leukocytes, large amounts of secretory 14-kDa phopholipase A_2. *Blood* **91:**3037–3043.
Bochner BS, Friedman B, Krishnaswami G, Schleimer RP, Lichtenstein LM and Kroegel C (1991b) Episodic eosinophilia-myalgia-like syndrome in a patient without L-tryptophan use: Association with eosinophil activation and increased serum levels of granulocyte/macrophage colony-stimulating factor. *J Allergy Clin Immunol* **88:**629–636.
Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP (1995) IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. *J Immunol* **154:**799–803.
Bochner BS, Luscinskas FW, Gimbrone MA, Newman W, Sterbinsky SA, Derse Anthony CP, Klunk D and Schleimer RP (1991a) Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: Contributions of endothelial cell adhesion molecules. *J Exp Med* **173:**1553–1557.
Bochner BS, Sterbinsky SA, Bickel CA, Werfel S, Wein M and Newman W (1994) Differences between human eosinophils and neutrophils in the function and expression of sialic acid-containing counterligands for E-selectin. *J Immunol* **152:**774–782.
Boichot E, Richard MP and Paubert Braquet M (1993) Effect of cefadroxil on antigen-induced bronchial hyperresponsiveness and eosinophil accumulation in lung from sensitized guinea pigs. *Int Arch Allergy Immunol* **102:**87–93.
Boie Y, Sawyer N, Slipetz DM, Metters KM and Abramovitz M (1995) Molecular cloning and characterization of the human prostanoid DP receptor. *J Biol Chem* **270:**18910–18916.
Boie Y, Sawyer N, Slipetz DM, Metters KM, Abramovitz M, Rushmore TH, Darmon Goodwin A and Grygorczyk R (1994) Cloning and expression of a cDNA for the human prostanoid IP receptor. *J Biol Chem* **269:**12173–12178.
Boise LH, Gonzalez Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G and Thompson CB (1993) bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* **74:**597–608.
Bokoch GM (1994) Regulation of the human neutrophil NADPH oxidase by the Rac GTP-binding proteins. *Curr Opin Cell Biol* **6:**212–218.
Bolger G, Michaeli T, Martins T, St. John T, Steiner B, Rodgers L, Riggs M, Wigler M and Ferguson K (1993) A family of human phosphodiesterases homologous to the *dunce* learning and memory gene product of *Drosophila melanogaster* are potential targets for antidepressant drugs. *Mol Cell Biol* **13:**6558–6571.
Bolscher BG, Koenderman L, Tool AT, Stokman PM and Roos D (1990) NADPH:O_2 oxidoreductase of human eosinophils in the cell-free system. *FEBS Lett* **268:**269–273.
Bolscher BG, Plat H and Wever R (1984) Some properties of human eosinophil peroxidase, a comparison with other peroxidases. *Biochim Biophys Acta* **784:**177–186.
Bonham CA and Thompson AW (1997) Immunosuppressants (drugs and monoclonal antibodies). in *Allergy and Allergic Diseases* (Kay AB ed) pp 642–663, Blackwell Scientific, London.
Borgeat P, Fruteau de Laclos B, Rabinovitch H, Picard S, Braquet P, Hebert J and Laviolette M (1984) Eosinophil-rich human polymorphonuclear leukocyte preparations characteristically release leukotriene C_4 on ionophore A23187 challenge. *J Allergy Clin Immunol* **74:**310–315.
Borland G, Ross JA and Guy K (1998) Forms and functions of CD44. *Immunology* **93:**139–148.
Bos AJ, Wever R, Hamers MN and Roos D (1981) Some enzymatic characteristics of eosinophil peroxidase from patients with eosinophilia and from healthy donors. *Infect Immun* **32:**427–431.
Bosse M, Audette M, Ferland C, Pelletier G, Chu HW, Dakhama A, Lavigne S, Boulet LP and Laviolette M (1996) Gene expression of interleukin-2 in purified human peripheral blood eosinophils. *Immunology* **87:**149–154.
Bosse M, Lavigne S, Laviolette M and Laval PQ (1998) Detection of B7–2 (CD86) on human blood eosinophils (Abstract). *Am J Respir Crit Care Med* **157:**A520.
Boulay F, Mery L, Tardif M, Brouchon L and Vignais P (1991) Expression cloning of a receptor for C5a anaphylatoxin on differentiated HL-60 cells. *Biochemistry* **30:**2993–2999.
Boulay F, Tardif M, Brouchon L and Vignais P (1990a) Synthesis and use of a novel N-formyl peptide derivative to isolate a human N-formyl peptide receptor cDNA. *Biochem Biophys Res Commun* **168:**1103–1109.
Boulay F, Tardif M, Brouchon L and Vignais P (1990b) The human N-formylpeptide receptor. Characterization of two cDNA isolates and evidence for a new subfamily of G-protein-coupled receptors. *Biochemistry* **29:**11123–11133.
Bouvier M, Collins S, O'Dowd BF, Campbell PT, De Blasi A, Kobilka BK, MacGregor C, Irons GP, Caron MG and Lefkowitz RJ (1989) Two distinct pathways for cAMP-mediated down-regulation of the β_2-adrenergic receptor. Phosphorylation of the receptor and regulation of its mRNA level. *J Biol Chem* **264:**16786–16792.
Bowden JJ, Sulakvelidze I and McDonald DM (1994) Inhibition of neutrophil and eosinophil adhesion to venules of rat trachea by β_2-adrenergic agonist formoterol. *J Appl Physiol* **77:**397–405.
Boyce JA, Friend D, Gurish MF, Austen KF and Owen WF (1996) Constitutive production of granulocyte/macrophage colony-stimulating factor by hypodense mononuclear eosinophils developed *in vitro* from hybrid eosinophil/basophil granulocytes. *Proc Natl Acad Sci USA* **93:**2436–2441.
Bozza PT, Castro Faria Neto HC, Penido C, Larangeira AP, das Gracas M, Henriques MO, Silva PM, Martins MA, dos Santos RR and Cordeiro RS (1994b) Requirement for lymphocytes and resident macrophages in LPS-induced pleural eosinophil accumulation. *J Leukoc Biol* **56:**151–158.
Bozza PT, Castro Faria Neto HC, Penido C, Larangeira AP, Silva PM, Martins MA and Cordeiro RS (1994a) IL-5 accounts for the mouse pleural eosinophil accumulation triggered by antigen but not by LPS. *Immunopharmacology* **27:**131–136.
Bozza PT, Payne JL, Goulet JL and Weller PF (1996a) Mechanisms of platelet-activating factor-induced lipid body formation: Requisite roles for 5-lipoxygenase and *de novo* protein synthesis in the compartmentalization of neutrophil lipids. *J Exp Med* **183:**1515–1525.
Bozza PT, Payne JL, Morham SG, Langenbach R, Smithies O and Weller PF (1996b) Leukocyte lipid body formation and eicosanoid generation: Cyclooxygenase-independent inhibition by aspirin. *Proc Natl Acad Sci USA* **93:**11091–11096.
Bozza PT, Yu W, Cassara J and Weller PF (1998) Pathways for eosinophil lipid body induction: Differing signal transduction in cells from normal and hypereosinophilic subjects. *J Leukoc Biol* **64:**563–569.
Bozza PT, Yu W, Penrose JF, Morgan ES, Dvorak AM and Weller PF (1997a) Eosinophil lipid bodies: specific, inducible intracellular sites for enhanced eicosanoid formation. *J Exp Med* **186:**909–920.
Bozza PT, Yu W and Weller PF (1997b) Mechanisms of formation and function of eosinophil lipid bodies: inducible intracellular sites involved in arachidonic acid metabolism. *Mem Inst Oswaldo Cruz* **92:**135–140.
Brach MA, Sott C, Kiehntopf M and Herrmann F (1994) Expression of the transforming growth factor-α gene by human eosinophils is regulated by interleukin-3, interleukin-5, and granulocyte-macrophage colony-stimulating factor. *Eur J Immunol* **24:**646–650.
Bradding P, Redington AE, Djukanovic R, Conrad DJ and Holgate ST (1995) 15-Lipoxygenase immunoreactivity in normal and in asthmatic airways. *Am Rev Respir Dis* **151:**1201–1204.
Brandtzaeg P (1981) Transport models for secretory IgA and secretory IgM. *Clin Exp Immunol* **44:**221–232.
Brash AR, Murray JJ and Oates JA (1985) The 5-lipoxygenase and 15-lipoxygenase of neutrophils and eosinophils. *Prog Clin Biol Res* **199:**143–152.
Braughler JM, Chase RL, Neff GL, Yonkers PA, Day JS, Hall ED, Sethy VH and Lahti RA (1988) A new 21-aminosteroid antioxidant lacking glucocorticoid activity stimulates adrenocorticotropin secretion and blocks arachidonic acid release from mouse pituitary tumor (AtT-20) cells. *J Pharmacol Exp Ther* **244:**423–427.
Braun RK, Franchini M, Erard F, Rihs S, De Vries IJ, Blaser K, Hansel TT and Walker C (1993) Human peripheral blood eosinophils produce and release interleukin-8 on stimulation with calcium ionophore. *Eur J Immunol* **23:**956–960.
Brinckmann R, Schurr K, Heydeck D, Rosenbach T, Kolde G and Kuhn H (1998) Membrane translocation of 15-lipoxygenase in hematopoietic cells is calcium-dependent and activates the oxygenase activity of the enzyme. *Blood* **91:**64–74.
Briskin MJ, McEvoy LM and Butcher EC (1993) MAdCAM-1 has homology to immunoglobulin and mucin-like adhesion receptors and to IgA_1. *Nature (Lond)* **363:**461–464.
Brogden RN and Sorkin EM (1993) Nedocromil sodium: An updated review of its pharmacological propreties and therapeutic efficacy in asthma. *Drugs* **45:**693–715.

Broide DH, Humber D and Sriramarao P (1998a) Inhibition of eosinophil rolling and recruitment in P-selectin- and intracellular adhesion molecule-1-deficient mice. *Blood* **91:**2847–2856.
Broide DH, Paine MM and Firestein GS (1992) Eosinophils express interleukin 5 and granulocyte macrophage-colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics. *J Clin Invest* **90:**1414–1424.
Broide DH, Sullivan S, Gifford T and Sriramarao P (1998b) Inhibition of pulmonary eosinophilia in P-selectin- and ICAM-1-deficient mice. *Am J Respir Cell Mol Biol* **18:**218–225.
Brooks DG, Qiu WQ, Luster AD and Ravetch JV (1989) Structure and expression of human IgG FcRII (CD32). Functional heterogeneity is encoded by the alternatively spliced products of multiple genes. *J Exp Med* **170:**1369–1385.
Brown JR, Kleimberg J, Marini M, Sun G, Bellini A and Mattoli S (1998) Kinetics of eotaxin expression and its relationship to eosinophil accumulation and activation in bronchial biopsies and bronchoalveolar lavage (BAL) of asthmatic patients after allergen inhalation. *Clin Exp Immunol* **114:**137–146.
Brown TR (1898) Studies on trichinosis with especial reference to the increase of the eosinophilic cells in the blood and muscle, the origin of these cells and their diagnostic importance. *J Exp Med* **3:**315–347.
Bruijnzeel PL, Hamelink ML, Kok PT and Kreukniet J (1989) Nedocromil sodium inhibits the A23187- and opsonized zymosan-induced leukotriene formation by human eosinophils but not by human neutrophils. *Br J Pharmacol* **96:**631–636.
Bruijnzeel PL, Kok PT, Hamelink ML, Kijne AM and Verhagen J (1987) Platelet-activating factor induces leukotriene C_4 synthesis by purified human eosinophils. *Prostaglandins* **34:**205–214.
Bruijnzeel PL, Kuijper PH, Rihs S, Betz S, Warringa RA and Koenderman L (1993a) Eosinophil migration in atopic dermatitis. I: Increased migratory responses to N-formyl-methionyl-leucyl-phenylalanine, neutrophil-activating factor, platelet-activating factor, and platelet factor 4. *J Invest Dermatol* **100:**137–142.
Bruijnzeel P, Storz E, van der Donk E and Bruijnzeel Koomen C (1993b) Skin eosinophilia in patients with allergic asthma, patients with nonallergic asthma, and healthy controls. II: 20-Hydroxy-leukotriene B_4 is a potent *in vivo* and *in vitro* eosinophil chemotactic factor in nonallergic asthma. *J Allergy Clin Immunol* **91:**634–642.
Bruijnzeel PL, Warringa RA, Kok PT and Kreukniet J (1990) Inhibition of neutrophil and eosinophil induced chemotaxis by nedocromil sodium and sodium cromoglycate. *Br J Pharmacol* **99:**798–802.
Bruijnzeel PLB and Verhagen J (1989) Lipid metabolism by eosinophils, in *Eosinophils and Asthma* (Morley J and Colditz I eds) pp 69–85, Academic Press, San Diego.
Bruns C, Weckbecker G, Raulf F, Lubbert H and Hoyer D (1995) Characterization of somatostatin receptor subtypes. *CIBA Found Symp* **190:**89–110.
Bruynzeel PL, Koenderman L, Kok PT, Hameling ML and Verhagen J (1986) Platelet-activating factor (PAF-acether) induced leukotriene C_4 formation and luminol-dependent chemiluminescence by human eosinophils. *Pharmacol Res Commun* **18 (Suppl):**61–69.
Bruynzeel PL, Kok PT, Hamelink ML, Kijne AM and Verhagen J (1985a) Exclusive leukotriene C_4 synthesis by purified human eosinophils induced by opsonized zymosan. *FEBS Lett* **189:**350–354.
Bruynzeel PL, Kok PT, Vietor RJ and Verhagen J (1985b) On the optimal conditions of LTC_4 formation by human eosinophils *in vitro*. *Prostaglandins Leukot Med* **20:**11–22.
Bryant DH and Kay AB (1977) Cutaneous eosinophil accumulation in atopic and non-atopic individuals: The effect of an ECF-A tetrapeptide and histamine. *Clin Allergy* **7:**211–217.
Bryant DH, Turnbull LW and Kay AB (1977) Eosinophil chemotaxis to an ECF-A tetrapeptide and histamine: the response in various disease states. *Clin Allergy* **7:**219–226.
Buijs J, Egbers MW, Lokhorst WH, Savelkoul HF and Nijkamp FP (1995) Toxocara-induced eosinophilic inflammation. Airway function and effect of anti-IL-5. *Am J Respir Crit Care Med* **151:**873–878.
Bujak JS and Root RK (1974) The role of peroxidase in the bactericidal activity of human blood eosinophils. *Blood* **43:**727–736.
Buma P, Roubos EW and Buijs RM (1984) Ultrastructural demonstration of exocytosis of neural, neuroendocrine and endocrine secretions with an *in vitro* tannic acid (TARI-) method. *Histochemistry* **80:**247–256.
Burke LA, Crea AE, Wilkinson JR, Arm JP, Spur BW and Lee TH (1990) Comparison of the generation of platelet-activating factor and leukotriene C_4 in human eosinophils stimulated by unopsonized zymosan and by the calcium ionophore A23187: The effects of nedocromil sodium. *J Allergy Clin Immunol* **85:**26–35.
Burke-Gaffney A and Hellewell PG (1996) Eotaxin stimulates eosinophil adhesion to human lung microvascular endothelial cells. *Biochem Biophys Res Commun* **227:** 35–40.
Burke-Gaffney A and Hellewell PG (1998) A CD18/ICAM-1-dependent pathway mediates eosinophil adhesion to human bronchial epithelial cells. *Am J Respir Cell Mol Biol* **19:**408–418.
Busse W, Randlev B and Sedgwick J (1989) The effect of azelastine on neutrophil and eosinophil generation of superoxide. *J Allergy Clin Immunol* **83:**400–405.
Butchers PR and Vardey CJ (1990) The effect of prostanoids on the function of human eosinophils. *Agents Actions Suppl* **31:**103–112.
Butterfield JH, Ackerman SJ, Weiler D, Eisenbrey AB and Gleich GJ (1986) Effects of glucocorticoids on eosinophil colony growth. *J Allergy Clin Immunol* **78:**450–457.
Butterfield JH and Leiferman KM (1993) Eosinophil-associated diseases, in *Immunopharmacology of Eosinophils* (Smith H and Cook RM eds) pp151–192, Academic Press, London.
Butterfield JH, Leiferman KM, Abrams J, Silver JE, Bower J, Gonchoroff N and Gleich GJ (1992) Elevated serum levels of interleukin-5 in patients with the syndrome of episodic angioedema and eosinophilia. *Blood* **79:**688–692.
Butterfield JH, Leiferman KM and Gleich GJ (1993) Nodules, eosinophilia, rheumatism, dermatitis and swelling (NERDS): A novel eosinophilic disorder. *Clin Exp Allergy* **23:**571–580.
Butterworth AE, Remold HG, Houba V, David JR, Franks D, David PH and Sturrock RF (1977) Antibody-dependent eosinophil-mediated damage to ^{51}Cr-labeled schistosomula of *Schistosoma mansoni*: Mediation by IgG, and inhibition by antigen-antibody complexes. *J Immunol* **118:**2230–2236.
Butterworth AE and Thorne KJI (1993) Eosinophils and parasitic diseases, in *Immunopharmacology of Eosinophils* (Smith H and Cook RM eds) pp 119–150, Academic Press, London.
Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Ruffolo RR and Trendelenburg U (1994) International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol Rev* **46:**121–136.
Cahill MA, Peter ME, Kischkel FC, Chinnaiyan AM, Dixit VM, Krammer PH and Nordheim A (1996) CD95 (APO-1/Fas) induces activation of SAP kinases downstream of ICE-like proteases. *Oncogene* **13:**2087–2096.
Calafat J, Janssen H, Tool A, Dentener MA, Knol EF, Rosenberg HF and Egesten A (1998) The bactericidal/permeability increasing protein (BPI) is present in specific granules of human eosinophils. *Blood* **91:**4770–4775.
Calhoun WJ, Lavins BJ, Minkwitz MC, Evans R, Gleich GJ and Cohn J (1998) Effect of zafirlukast (Accolate) on cellular mediators of inflammation: Bronchoalveolar lavage fluid findings after segmental antigen challenge. *Am J Respir Crit Care Med* **157:**1381–1389.
Calhoun W, Hinton KL, Brick JJ and Vuchinien T (1995) Effects of salmeterol on eosinophil recruitment to the airway folowing segmental antigen challenge in atopic asthmatics, in *Acute and Chronic Inflammation in the Respiratory Tract* (Johnson M ed) p 62, Colwood House, London.
Calhoun W, Sedgwick JB, Jariour N, Swenson CA and Busse WW (1993) The effect of mast cell activation on eosinophil recruitment to the airways after antigen challenge. *Am Rev Respir Dis* **147:**A241.
Camp RDR, Coutts AA, Greaves MW, Kay AB and Walport MJ (1983) Responses of human skin to intradermal injection of leukotrienes C_4, D_4 and B_4. *Br J Pharmacol* **80:**497–502.
Capron A, Dessaint JP, Haque A and Capron M (1982) Antibody-dependent cell-mediated cytotoxicity against parasites. *Prog Allergy* **31:**234–267.
Capron M (1991) Eosinophils and parasites. *Ann Parasitol Hum Comp* **66** (**Suppl 1):**41–45.
Capron M (1992) Dual function of eosinophils in pathogenesis and protective immunity against parasites. *Mem Inst Oswaldo Cruz* **87** (**Suppl 5):**83–89.
Capron M, Benveniste J, Braquet P and Capron A (1988b) Role of PAF-acether in IgE-dependent activation of eosinophils, in *New Trends in Lipid Mediators Research: The Role of Platelet-Activating factor in Immune Disease* (Braquet P ed) pp 10–17, Karger, Basel.
Capron M and Capron A (1987) The IgE receptor of human eosinophils, in *Allergy and Inflammation* (Kay AB ed) pp 151–159, Academic Press, London.
Capron M and Capron A (1994) Immunoglobulin E and effector cells in schistosomiasis. *Science (Lond)* **264:**1876–1877.
Capron M, Capron A, Dessaint JP, Torpier G, Johansson SG and Prin L (1981) Fc receptors for IgE on human and rat eosinophils. *J Immunol* **126:**2087–2092.
Capron M, Grangette C, Torpier G and Capron A (1989) The second receptor for IgE in eosinophil effector function. *Chem Immunol* **47:**128–178.
Capron M and Joseph M (1991) The low affinity receptor for IgE on eosinophils and platelets. *Monogr Allergy* **29:**63–75.
Capron M, Jouault T, Prin L, Joseph M, Ameisen JC, Butterworth AE, Papin JP, Kusnierz JP and Capron A (1986) Functional study of a monoclonal antibody to IgE Fc receptor (FcεR2) of eosinophils, platelets, and macrophages. *J Exp Med* **164:**72–89.
Capron M, Kusnierz JP, Prin L, Spiegelberg HL, Khalife J, Tonnel AB and Capron A (1985) Cytophilic IgE on human blood and tissue eosinophils. *Int Arch Allergy Appl Immunol* **77:**246–248.
Capron M, Morita M, Woerly G, Lengrand F, Gounni AS, Delaporte E and Capron A (1997) Differentiation of eosinophils from cord blood cell precursors—kinetics of FcεRI and FcεRII expression. *Int Arch Allergy Immunol* **113:**48–50.
Capron M, Spiegelberg HL, Prin L, Bennich H, Butterworth AE, Pierce RJ, Ouaissi MA and Capron A (1984) Role of IgE receptors in effector function of human eosinophils. *J Immunol* **132:**462–468.
Capron M, Tomassini M, Ven der Vorst E, Kusnierz JP, Pepin KG and Capron A (1988a) Existence et fonctions d'un recepteur pour l'immunoglobuline A sur les eosinophiles humaines. *CRC Acad Sci Immunol* **307:**397–405.
Capron M, Truong MJ, Aldebert D, Gruart V, Suemura M, Delespesse G, Tourvieille B and Capron A (1991) Heterogeneous expression of CD23 epitopes by eosinophils from patients. Relationships with IgE-mediated functions. *Eur J Immunol* **21:** 2423–2429.
Caproni M, Dagata A, Cappelli G and Fabbri P (1996) Modulation of serum eosinophil cationic protein levels by cyclosporin in severe atopic dermatitis. *Br J Dermatol* **135:**336–337.
Capsoni F, Mininzio F, Ongari AM and Zanussi C (1989) A new simplified filter assay for "in vitro" evaluation of chemotaxis of ^{51}Cr-labeled polymorphonuclear leukocytes. *J Immunol Methods* **120:**125–131.
Carlson M, Hakansson L, Kampe M, Stalenheim G, Peterson C and Venge P (1992) Degranulation of eosinophils from pollen-atopic patients with asthma is increased during pollen season. *J Allergy Clin Immunol* **89:**131–139.
Carlson M, Hakansson L, Peterson C, Stalenheim G and Venge P (1991) Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. *J Allergy Clin Immunol* **87:**27–33.
Carlson M, Peterson C and Venge P (1993) The influence of IL-3, IL-5, and GM-CSF on normal human eosinophil and neutrophil C3b-induced degranulation. *Allergy* **48:**437–442.
Carlson MG, Peterson CG and Venge P (1985) Human eosinophil peroxidase: Purification and characterization. *J Immunol* **134:**1875–1879.
Carpen O, Pallai P, Staunton DE and Springer TA (1992) Association of intercellular

adhesion molecule-1 (ICAM-1) with actin-containing cytoskeleton and alpha-actinin. *J Cell Biol* **118:**1223–1234.
Caulfield JP, Hein A, Rothenberg ME, Owen WF, Soberman RJ, Stevens RL and Austen KF (1990) A morphometric study of normodense and hypodense human eosinophils that are derived *in vivo* and *in vitro*. *Am J Pathol* **137:**27–41.
Chabot-Fletcher MC, Underwood DC, Breton JJ, Adams JL, Kagey Sobotka A, Griswold DE, Marshall LA, Sarau HM, Winkler JD and Hay DW (1995) Pharmacological characterization of SB 202235, a potent and selective 5-lipoxygenase inhibitor: Effects in models of allergic asthma. *J Pharmacol Exp Ther* **273:**1147–1155.
Chakir J, Minshall EM, Toda M, Olivenstein R, Zhu Z, Laviolette M, Elias JA and Hamid Q (1999) Interleukin-11 messenger RNA expression is increased in severe asthma: Localization to eosinophils (Abstract). *Am J Respir Crit Care Med* **159:**A450.
Chan BM, Elices MJ, Murphy E and Hemler ME (1992) Adhesion to vascular cell adhesion molecule 1 and fibronectin. Comparison of $\alpha_4\beta_1$ (VLA-4) and $\alpha_4\beta_7$ on the human B cell line JY. *J Biol Chem* **267:**8366–8370.
Chan CC, McKee K, Tagari P, Chee P and Ford Hutchinson A (1990) Eosinophil-eicosanoid interactions: inhibition of eosinophil chemotaxis *in vivo* by a LTD_4-receptor antagonist. *Eur J Pharmacol* **191:**273–280.
Chand N, Harrison JE, Rooney S, Pillar J, Jakubicki R, Nolan K, Diamantis W and Sofia RD (1992a) Anti-IL-5 monoclonal antibody inhibits allergic late phase bronchial eosinophilia in guinea pigs: A therapeutic approach. *Eur J Pharmacol* **211:**121–123.
Chand N,Nolan K, Diamantis W and Sofia RD (1992b) Inhibition of aeroallergen-induced bronchial eosinophilia by azelastine in guinea-pigs. *Int Arch Allergy Immunol* **97:**229–232.
Chanez P, Dent G, Yukawa T, Barnes PJ and Chung KF (1990) Generation of oxygen free radicals from blood eosinophils from asthma patients after stimulation with PAF or phorbol ester. *Eur Respir J* **3:**1002–1007.
Charcot JM and Robin C (1853) Observation de leucocythemie. *CR Mem Soc Biol* **5:**454–450.
Charlesworth EN, Kagey Sobotka A, Norman PS and Lichtenstein LM (1989) Effect of cetirizine on mast cell mediator release and cellular traffic during the cutaneous late phase reaction. *J Allergy Clin Immunol* **83:**905–912.
Charlesworth EN, Kagey Sobotka A, Schleimer RP, Norman PS and Lichtenstein LM (1991) Prednisone inhibits the appearance of inflammatory mediators and the influx of eosinophils and basophils associated with the cutaneous late-phase response to allergen. *J Immunol* **146:**671–676.
Cherry JA and Davis RL (1995) A mouse homolog of *dunce*, a gene important for learning and memory in *Drosophila*, is preferentially expressed in olfactory receptor neurones. *J Neurobiol* **28:**102–113.
Chihara J, Hayashi N, Kakazu T, Yamamoto T, Kurachi D and Nakajima S (1994) RANTES augments radical oxygen products from eosinophils. *Int Arch Allergy Immunol* **104** (**Suppl 1):**52–53.
Chihara J, Kakazu T, Higashimoto I, Yamamoto T, Kurachi D and Nakajima S (1995a) Increased eosinophil oxidative metabolism by treatment with soluble intercellular adhesion molecule-1. *Int Arch Allergy Immunol* **108** (**Suppl 1):**45–47.
Chihara J and Nakajima S (1989) Induction of hypodense eosinophils and nuclear hypersegmentation of eosinophils by various chemotactic factors and lymphokines *in vitro*. *Allergy Proc* **10:**27–32.
Chihara J, Plumas J, Gruart V, Tavernier J, Prin L, Capron A and Capron M (1990) Characterization of a receptor for interleukin 5 on human eosinophils: Variable expression and induction by granulocyte/macrophage colony-stimulating factor. *J Exp Med* **172:**1347–1351.
Chihara J, Yamamoto T, Kurachi D, Kakazu T, Higashimoto I and Nakajima S (1995b) Possible release of eosinophil granule proteins in response to signaling from intercellular adhesion molecule-1 and its ligands. *Int Arch Allergy Immunol* **108** (**Suppl 1):**52–54.
Chin JE, Winterrowd GE, Hatfield CA, Brashler JR, Griffin RL, Vonderfecht SL, Kolbasa KP, Fidler SF, Shull KL, Krzesicki RF, Ready KA, Dunn CJ, Sly LM, Staite ND and Richards IM (1998) Involvement of intercellular adhesion molecule-1 in the antigen-induced infiltration of eosinophils and lymphocytes into the airways in a murine model of pulmonary inflammation. *Am J Respir Cell Mol Biol* **18:**158–167.
Chuang TT, Iacovelli L, Sallese M and De Blasi A (1996) G protein-coupled receptors: heterologous regulation of homologous desensitization and its implications. *Trends Pharmacol Sci* **17:**416–421.
Chuang TT, Sallese M, Ambrosini G, Parruti G and De Blasi A (1992) High expression of β-adrenergic receptor kinase in human peripheral blood leukocytes. Isoproterenol and platelet activating factor can induce kinase translocation. *J Biol Chem* **267:**6886–6892.
Chung KF (1996) Theophylline in chronic asthma–evidence for disease-modifying properties. *Clin Exp Allergy* **26** (**Suppl 2):**22–27.
Chung KF and Barnes PJ (1991) Platelet-activating factor and asthma, in *Asthma: Its Pathology and Treatment* (Kaliner MA, Barnes PJ, and Persson CGA eds) pp 267–300, Marcel Dekker, New York.
Chung KF and Barnes PJ (1992) Loop diuretics and asthma. *Pulm Pharmacol* **5:**1–7.
Church MK, Hutson PA and Holgate ST (1989) Effect of nedocromil sodium on early and late responses to allergen challenge in guinea-pigs. *Drugs* **37:**101–108.
Cifone MG, De Maria R, Roncaioli P, Rippo MR, Azuma M, Lanier LL, Santoni A and Testi R (1993) Apoptotic signaling through CD95 (Fas/Apo-1) activates an acidic sphingomyelinase. *J Exp Med* **177:**1547–1552.
Ciprandi G, Pronzato C, Passalacqua G, Ricca V, Bagnasco M, Grogen J, Mela GS and Canonica GW (1996) Topical azelastine reduces eosinophil activation and intercellular adhesion molecule-1 expression on nasal epithelial cells—an antiallergic activity. *J Allergy Clin Immunol* **98:**1088–1096.
Clark EA and Brugge JS (1995) Integrins and signal transduction pathways: The road taken. *Science (Wash DC)* **268:**233–239.
Clark RA, Gallin JI and Fauci AS (1979) Effects of *in vivo* prednisone on *in vitro* eosinophil and neutrophil adherence and chemotaxis. *Blood* **53:**633–641.
Clark RA, Gallin JI and Kaplan AP (1975) The selective eosinophil chemotactic activity of histamine. *J Exp Med* **142:**1462–1476.
Clark RAF, Sandler JA, Gallin JI and Kaplan AP (1977) Histamine modulation of eosinophil migration. *J Immunol* **118:**137–145.
Clipstone NA and Crabtree GR (1993) Calcineurin is a key signaling enzyme in T lymphocyte activation and the target of the immunosuppressive drugs cyclosporin A and FK506. *Ann NY Acad Sci* **696:**20–30.
Clutterbuck EJ, Hirst EM and Sanderson CJ (1989) Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: Comparison and interaction with IL-1, IL-3, IL-6, and GMCSF. *Blood* **73:**1504–1512.
Clutterbuck EJ and Sanderson CJ (1990) Regulation of human eosinophil precursor production by cytokines: A comparison of recombinant human interleukin-1 (rhIL-1), rhIL-3, rhIL-5, rhIL-6, and rh granulocyte-macrophage colony-stimulating factor. *Blood* **75:**1774–1779.
Cockcroft DW and Murdock KY (1987) Protective effect of inhaled albuterol, cromolyn, beclomethasone and placebo on allergen-induced early asthmatic responses, late asthmatic responses and allergen-induced increases in bronchial responsiveness to inhaled histamine. *J Allergy Clin Immunol* **79:**734–740.
Coeffier E, Joseph D and Vargaftig BB (1991a) LTB_4, a potent chemotactic factor for purified guinea-pig eosinophils: Interference of PAF-acether antagonists. *Int J Immunopharmacol* **13:**273–280.
Coeffier E, Joseph D and Vargaftig BB (1991b) Activation of guinea pig eosinophils by human recombinant IL-5. Selective priming to platelet-activating factor-acether and interference of its antagonists. *J Immunol* **147:**2595–2602.
Coffer PJ, Schweizer RC, Dubois GR, Maikoe T, Lammers JW and Koenderman L (1998) Analysis of signal transduction pathways in human eosinophils activated by chemoattractants and the T-helper 2-derived cytokines interleukin-4 and interleukin-5. *Blood* **91:**2547–2557.
Cohan VL, Johnson KL, Breslow R, Cheng JB and Showell HJ (1992) PDE IV is the predominant PDE isozyme regulating chemotactic factor-mediated guinea-pig functions *in vitro* (Abstract). *J Allergy Clin Immunol* **89:**663.
Cohan VL, Showell HJ, Fisher DA, Pazoles CJ, Watson JW, Turner CR and Cheng JB (1996) *In vitro* pharmacology of the novel phosphodiesterase type 4 inhibitor, CP-80633. *J Pharmacol Exp Ther* **278:**1356–1361.
Cohen RA, Gebhardt BM and Bazan NG (1994) A platelet activating factor antagonist reduces corneal allograft inflammation and neovascularization. *Curr Eye Res* **13:**139–144.
Cohen SG and Ottesen EA (1983) The eosinophil, eosinophilia, and eosinophil-related disorders, in *Allergy: Principles and Practice* (Middleton E, Reed CE, and Ellis EF eds) pp 293–312, Mosby, St. Louis.
Coleman RA, Eglen RM, Jones RL, Narumiya S, Shimizu T, Smith WL, Dahlen SE, Drazen JM, Gardiner PJ and Jackson WT (1995) Prostanoid and leukotriene receptors: a progress report from the IUPHAR working parties on classification and nomenclature. *Adv Prostaglandin Thromboxane Leukotriene Res* **23:**283–285.
Coleman RA, Smith WL and Narumiya S (1994) International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol Rev* **46:**205–229.
Colicelli J, Birchmeier C, Michaeli T, O'Neill K, Riggs M and Wigler M (1989) Isolation and characterization of a mammalian gene encoding a high-affinity cAMP phosphodiesterase. *Proc Natl Acad Sci USA* **86:**3599–3603.
Collins PD, Marleau S, Griffiths Johnson DA, Jose PJ and Williams TJ (1995) Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation *in vivo*. *J Exp Med* **182:**1169–1174.
Collins PD, Weg VB, Faccioli LH, Watson ML, Moqbel R and Williams TJ (1993) Eosinophil accumulation induced by human interleukin-8 in the guinea-pig *in vivo*. *Immunology* **79:**312–318.
Combadiere C, Ahuja SK and Murphy PM (1995) Cloning and functional expression of a human eosinophil CC chemokine receptor. *J Biol Chem* **270:**16491–16494.
Connell JT (1968) Morphological changes in eosinophils in allergic disease. *J Allergy Clin Immunol* **41:**1–9.
Corbi AL, Miller LJ, O'Connor K, Larson RS and Springer TA (1987) cDNA cloning and complete primary structure of the α subunit of leukocyte adhesion glycoprotein, p150,95. *EMBO J* **6:**4023–4028.
Cornelis S, Fache I, Van der Heyden J, Guisez Y, Tavernier J, Devos R, Fiers W and Plaetinck G (1995) Characterization of critical residues in the cytoplasmic domain of the human interleukin-5 receptor alpha chain required for growth signal transduction. *Eur J Immunol* **25:**1857–1864.
Corrigan CJ and Kay AB (1996) T-cell/eosinophil interactions in the induction of asthma. *Eur Respir J* **9:**S72–S78.
Costa JJ, Matossian K, Resnick MB, Beil WJ, Wong DT, Gordon JR, Dvorak AM, Weller PF and Galli SJ (1993) Human eosinophils can express the cytokines tumor necrosis factor-α and macrophage inflammatory protein-1α. *J Clin Invest* **91:**2673–2684.
Cowburn AS, Sladek K, Soja J, Adamek L, Nizankowska E, Szczeklik A, Lam BK, Penrose JF, Austen FK, Holgate ST and Sampson AP (1998) Overexpression of leukotriene C_4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest* **101:**834–846.
Cox G (1995) Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. *J Immunol* **154:**4719–4725.
Cox G, Ohtoshi T, Vancheri C, Denburg JA, Dolovich J, Gauldie J and Jordana M (1991) Promotion of eosinophil survival by human bronchial epithelial cells and its modulation by steroids. *Am J Respir Cell Mol Biol* **4:**525–531.
Coyle AJ, Urwin SC, Page CP, Touvay C, Villain B and Braquet P (1988) The effect of the selective PAF antagonist BN 52021 on PAF- and antigen-induced bronchial hyper-reactivity and eosinophil accumulation. *Eur J Pharmacol* **148:**51–58.
Crimi E, Brusasco V and Crimi P (1989) Effect of nedocromil sodium on the late asthmatic reaction to bronchial antigen challenge. *J Allergy Clin Immunol* **83:**985–990.
Cromwell O, Bennett JP, Hide I, Kay AB and Gomperts BD (1991) Mechanisms of granule enzyme secretion from permeabilized guinea pig eosinophils. Dependence on Ca^{2+} and guanine nucleotides. *J Immunol* **147:**1905–1911.

Cromwell O, Moqbel R, Fitzharris P, Kurlak L, Harvey C, Walsh GM, Shaw RJ and Kay AB (1988) Leukotriene C_4 generation from human eosinophils stimulated with IgG-*Aspergillus fumigatus* antigen immune complexes. *J Allergy Clin Immunol* **82:**535–543.
Cromwell O, Wardlaw AJ, Champion A, Moqbel R, Osei D and Kay AB (1990) IgG-dependent generation of platelet-activating factor by normal and low density human eosinophils. *J Immunol* **145:**3862–3868.
Cypcar D, Sorkness R, Sedgwick J, Nagata M, Clough J, Kaplan M and Lemanske RF (1996) Rat eosinophils - isolation and characterization of superoxide production. *J Leukoc Biol* **60:**101–105.
Czarnetzki BM and Csato M (1989) Comparative studies of human eosinophil migration towards platelet-activating factor and leukotriene B_4. *Int Arch Allergy Appl Immunol* **88:**191–193.
Czarnetzki BM and Mertensmeir R (1985) *In vitro* and *in vivo* chemotaxis of guinea-pig leukocytes towards leukotriene B_4 and its ω-oxidation product. *Prostaglandins* **30:**5–12.
Czarnetzki BM and Rosenbach T (1986) Chemotaxis of human neutrophils and eosinophils towards leukotriene B_4 and its 20-ω-oxidation products *in vitro*. *Prostaglandins* **31:**851–858.
Czech W, Barbisch M, Tenscher K, Schopf E, Schroder JM and Norgauer J (1997) Chemotactic 5-oxo-eicosatetraenoic acids induce oxygen radical production, Ca^{2+}-mobilization, and actin reorganization in human eosinophils via a pertussis toxin-sensitive G-protein. *J Invest Dermatol* **108:**108–112.
Czech W, Krutmann J, Budnik A, Schopf E and Kapp A (1993) Induction of intercellular adhesion molecule 1 (ICAM-1) expression in normal human eosinophils by inflammatory cytokines. *J Invest Dermatol* **100:**417–423.
Daaka Y, Luttrell LM and Lefkowitz RJ (1997) Switching of the coupling of the β_2-adrenergic receptor to different G proteins by protein kinase A. *Nature (Lond)* **390:**88–91.
Daffern PJ, Pfeifer PH, Ember JA and Hugli TE (1995) C3a is a chemotaxin for human eosinophils but not for neutrophils. I. C3a stimulation of neutrophils is secondary to eosinophil activation. *J Exp Med* **181:**2119–2127.
Dahinden CA, Geiser T, Brunner T, Von Tscharner V, Caput D, Ferrara P, Minty A and Baggiolini M (1994) Monocyte chemotactic protein 3 is a most effective basophil- and eosinophil-activating chemokine. *J Exp Med* **179:**751–756.
Dahl R, Pedersen B and Larsen BB (1995) The influence of salmeterol on reactions after allergen challenge and the variations in bronchial hyper-reactivity, blood eosinophils and serum ECP and the influence of salmeterol on bronchial mucosa and BAL, in *Acute and Chronic Inflammation in the Respiratory Tract* (Johnson M ed) pp 70–72, Colwood House, London.
Dahl R and Venge P (1978) Blood eosinophil leucocyte and eosinophil cationic protein. *In vivo* study of the influence of $beta_2$-adrenergic drugs and steroid medication. *Scand J Respir Dis* **59:**319–322.
Danahay H and Broadley KJ (1997) Effects of inhibitors of phosphodiesterase, on antigen-induced bronchial hyperreactivity in conscious sensitized guinea-pigs and airway leukocyte infiltration. *Br J Pharmacol* **120:**289–297.
Danahay H and Broadley KJ (1998) PDE4 inhibition and a corticosteroid in chronically antigen exposed conscious guinea-pigs. *Clin Exp Allergy* **28:**513–522.
Daugherty BL, Siciliano SJ, DeMartino JA, Malkowitz L, Sirotina A and Springer MS (1996) Cloning, expression, and characterization of the human eosinophil eotaxin receptor. *J Exp Med* **183:**2349–2354.
David JR, Butterworth AE, Remold HG, David PH, Houba V and Sturrock RF (1977) Antibody-dependent, eosinophil-mediated damage to ^{51}Cr-labeled schistosomula of *Schistosoma mansoni*: effect of metabolic inhibitors and other agents which alter cell function. *J Immunol* **118:**2221–2229.
David JR, Vadas MA, Butterworth AE, de Brito PA, Carvalho EM, David RA, Bina JC and Andrade ZA (1980) Enhanced helminthotoxic capacity of eosinophils from patients with eosinophilia. *N Engl J Med* **303:**1147–1152.
Davies D, Medeiros MS, Keen J, Turner AJ and Haynes LW (1992) Eosinophil chemotactic peptide sequences in rat alpha-CGRP. Activation of a novel trophic action by neutral endopeptidase 24.11. *Ann NY Acad Sci* **657:**405–411.
Davies RJ, Trigg CJ, Wang JH, Manolitsas ND, Macaulay A, Jhalli N and Hamilton S (1993) Regular inhaled salbutamol may exacerbate bronchial inflammation in patients with mild asthma (Abstract). *Thorax* **48:**1060.
Davis RL, Takayasu H, Eberwine M and Myres J (1989) Cloning and characterisation of mammalian homologs of the *Drosophila* dunce$^+$ gene. *Proc Natl Acad Sci USA* **86:**3604–3608.
Davis WB, Fells GA, Sun XH, Gadek JE, Venet A and Crystal RG (1984) Eosinophil-mediated injury to lung parenchymal cells and interstitial matrix. A possible role for eosinophils in chronic inflammatory disorders of the lower respiratory tract. *J Clin Invest* **74:**269–278.
de Andres B, Cardaba B, del Pozo V, Martin Orozco E, Gallardo S, Tramon P, Palomino P and Lahoz C (1994) Modulation of the FcγRII and FcγRIII induced by GM-CSF, IFN-γ and IL-4 on murine eosinophils. *Immunology* **83:**155–160.
de Andres B, del Pozo V, Cardaba B, Martin E, Tramon P, Lopez Rivas A, Palomino P and Lahoz C (1991) Phosphoinositide breakdown is associated with Fc-γRII-mediated activation of 5-lipoxygenase in murine eosinophils. *J Immunol* **146:**1566–1570.
de Andres B, del Pozo V, Martin E, Palomino P and Lahoz C (1990) Release of O_2^- and LTC_4 by murine eosinophils: role of intra- and extracellular calcium. *Immunology* **69:**271–276.
de Andres B, Mueller AL, Blum A, Weinstock J, Verbeek S, Sandor M and Lynch RG (1997a) FcγRII (CD32) is linked to apoptotic pathways in murine granulocyte precursors and mature eosinophils. *Blood* **90:**1267–1274.
de Andres B, Rakasz E, Hagen M, McCormik ML, Mueller AL, Elliot D, Metwali A, Sandor M, Britigan BE, Weinstock JV and Lynch RG (1997b) Lack of Fcϵ receptors on murine eosinophils: Implications for the functional significance of elevated IgE and eosinophils in parasitic infections. *Blood* **89:**3826–3836.
de Groot RP, van Dijk TB, Caldenhoven E, Coffer PJ, Raaijmakers JA, Lammers JWJ, Koenderman L (1997) Activation of 12-*O*-tetradecanoylphorbol-13-acetate response element- and dyad symmetry element-dependent transcription by interleukin-5 is mediated by Jun N-terminal kinase/stress-activated protein kinase kinases. *J Biol Chem* **272:**2319–2325.
de Mendez I, Adams AG, Sokolic RA, Malech HL and Leto TL (1996) Multiple SH3 domain interactions regulate NADPH oxidase assembly in whole cells. *EMBO J* **15:**1211–1220.
De Simone C, Donelli G, Meli D, Molinari A, Rosati F and Sorice F (1982a) Human peripheral eosinophils with receptors for IgM: Demonstration and ultrastructural morphology. *Immunobiology* **162:**116–127.
De Simone C, Donneli G, Meli D, Rosati F and Sorice F (1982b) Human eosinophils and parasitic diseases. II. Characterization of two cell fractions isolated at different densities. *Clin Exp Immunol* **48:**249–255.
De Simone C, Ferrari M, Ferrarelli G, Rumi C, Pugnaloni L and Sorice F (1987) The effects of substance P on human eosinophil receptors and functions. *Ann NY Acad Sci* **496:**226–232.
De Simone C, Salvi MC, Ferrarelli G, De Santis G, Mango G and Sorice F (1986) Human eosinophils and parasitic diseases—III. Beta-interferon increases eosinophil IgG-Fc receptor expression and capacity. *Int J Immunopharmacol* **8:**479–485.
De Vos C, Joseph M, Leprevost C, Vorng H, Tomassini M, Capron M and Capron A (1989) Inhibition of human eosinophil chemotaxis and of the IgE-dependent stimulation of human blood platelets by cetirizine. *Int Arch Allergy Appl Immunol* **88:**212–215.
de Wit TP, Morton HC, Capel PJ and van de Winkel JG (1995) Structure of the gene for the human myeloid IgA Fc receptor (CD89). *J Immunol* **155:**1203–1209.
Debbaghi A, Hidi R, Vargaftig BB and Touqui L (1992) Inhibition of phospholipase A_2 activity in guinea pig eosinophils by human recombinant IL-1β. *J Immunol* **149:**1374–1380.
Degrendele HC, Estess P, Picker LJ and Siegelman MH (1996) CD44 and its ligand hyaluronate mediate rolling under physiological flow: A novel lymphocyte-endothelial cell primary adhesion pathway. *J Exp Med* **183:**1119–1130.
del Pozo V, de Andres B, Martin E, Cardaba B, Fernandez JC, Gallardo S, Tramon P, Leyva Cobian F, Palomino P, and Lahoz C (1992) Eosinophil as antigen-presenting cell: Activation of T cell clones and T cell hybridoma by eosinophils after antigen processing. *Eur J Immunol* **22:**1919–1925.
del Pozo V, de Andres B, Martin E, Maruri N, Zubeldia JM, Palomino P, and Lahoz C (1990) Murine eosinophils and IL-1: Alpha IL-1 mRNA detection by *in situ* hybridization. Production and release of IL-1 from peritoneal eosinophils. *J Immunol* **144:**3117–3122.
del Pozo V, Dearrudachaves E, Deandres B, Cardaba B, Lopezfarre A, Gallardo S, Cortegano I, Vidarte L, Jurado A, Sastre J, Palomino P, and Lahoz C (1997) Eosinophils transcribe and translate messenger RNA for inducible nitric oxide synthase. *J Immunol* **158:**859–864.
Del Prete GF, De Carli M, Almerigogna F, Daniel CK, D'Elios MM, Zancuoghi G, Vinante F, Pizzolo G and Romagnani S (1995) Preferential expression of CD30 by human CD4+ T cells producing Th2-type cytokines. *FASEB J* **9:**81–86.
Dembele Duchesne MJ, Badia E, Etienne Julan M and Capony JP (1991) Identification and tissue localization of an eosinophil 17 kDa protein accumulating in rat uterus upon estradiol treatment. *J Steroid Biochem Mol Biol* **38:**321–330.
Denjean A, Arnoux B, Masse R, Lockhart A and Benveniste J (1983) Acute effect of intratracheal administration of platelet activating factor in baboons. *J Appl Physiol* **55:**799–804.
Dennis EA (1997) The growing phospholipase A_2 superfamily of signal transduction enzymes. *Trends Biochem Sci* **22:**1–2.
Dent G (1991) Pharmacological modulation of eosinophil activity. Mechanisms of cellular activation and inhibition. Ph.D. Thesis, University of London.
Dent G and Barnes PJ (1993) Platelet activating factor stimulates a receptor-coupled membrane GTPase in guinea pig eosinophils. *Life Sci* **52:**1633–1640.
Dent G, Giembycz MA, Evans PM, Rabe KF and Barnes PJ (1994) Suppression of human eosinophil respiratory burst and cyclic AMP hydrolysis by inhibitors of type IV phosphodiesterase: Interaction with the beta adrenoceptor agonist albuterol. *J Pharmacol Exp Ther* **271:**1167–1174.
Dent G, Giembycz MA, Rabe KF and Barnes PJ (1991) Inhibition of eosinophil cyclic nucleotide PDE activity and opsonised zymosan-stimulated respiratory burst by "type IV"-selective PDE inhibitors. *Br J Pharmacol* **103:**1339–1346.
Dent G, Munoz NM, Ruhlmann E, Zhu X, Leff AR, Magnussen H and Rabe KF (1998a) Protein kinase C inhibition enhances platelet-activating factor-induced eicosanoid production in human eosinophils. *Am J Respir Cell Mol Biol* **18:**136–144.
Dent G, Poppe H, Egerland J, Marx D, Szelenyi I, Branscheid D, Magnussen H and Rabe KF (1998b) Effects of a selective PDE4 inhibitor, D-22888, on human airways and eosinophils *in vitro* and late phase allergic pulmonary eosinophilia in guinea pigs *Pulm Pharmacol Ther* **11:**13–21.
Dent G, Ukena D and Barnes PJ (1989) Receptors for platelet-activating factor, in *Platelet-Activating Factor and Human Disease* (Barnes PJ, Page CP and Henson PM eds) pp 58–81, Blackwell Scientific, Oxford.
Dent LA, Strath M, Mellor AL and Sanderson CJ (1990) Eosinophilia in transgenic mice expressing interleukin 5. *J Exp Med* **172:**1425–1431.
Denzler KL, Levin WJ, Lee JJ and Lee NA (1997b) The murine eosinophil peroxidase gene (*epx*) maps to chromosome 11. *Mamm Genome* **8:**381–382.
Denzler KL, Levin WJ, Quiram PA, Lee NA and Lee JJ (1997a) The murine eosinophil major basic protein gene (*prg2*) maps to chromosome 2. *Mamm Genome* **8:**382–383.
Desreumaux P, Janin A, Colombel JF, Prin L, Plumas J, Emilie D, Torpier G, Capron A and Capron M (1992) Interleukin 5 messenger RNA expression by eosinophils in the intestinal mucosa of patients with coeliac disease. *J Exp Med* **175:**293–296.
Desreumaux P, Janin A, Dubucquoi S, Copin MC, Torpier G, Capron A, Capron M and Prin L (1993) Synthesis of interleukin-5 by activated eosinophils in patients with eosinophilic heart diseases. *Blood* **82:**1553–1560.
Dessein AJ, Lee TH, Elsas P, Ravalese J, Silberstein D, David JR, Austen KF and Lewis RA (1986) Enhancement by monokines of leukotriene generation by human eosinophils and neutrophils stimulated with calcium ionophore A23187. *J Immunol* **136:**3829–3838.

Devalia JL, Sapsford RJ, Rusznak C and Davies RJ (1992) The effect of human eosinophils on cultured human nasal epithelial cell activity and the influence of nedocromil sodium *in vitro*. *Am J Respir Cell Mol Biol* **7:**270–277.
Devos R, Guisez Y, Cornelis S, Verhee A, Van der Heyden J, Manneberg M, Lahm HW, Fiers W, Tavernier J and Plaetinck G (1993) Recombinant soluble human interleukin-5 (hIL-5) receptor molecules. Cross-linking and stoichiometry of binding to IL-5. *J Biol Chem* **268:**6581–6587.
Di Lorenzo G, Morici G, Norrito F, Mansueto P, Melluso M, Purello D and Barbagallo Sangiorgi G (1995) Comparison of the effects of salmeterol and salbutamol on clinical activity and eosinophil cationic protein serum levels during the pollen season in atopic asthmatics. *Clin Exp Allergy* **25:**951–956.
Diamant Z, Hiltermann JT, van Rensen EL, Callenbach PM, Veselic-Charvat M, van der Veen H, Sont JK and Sterk PJ (1997) The effect of inhaled leukotriene D_4 and methacholine on sputum cell differentials in asthma. *Am J Respir Crit Care Med* **155:**1247–1253.
Diamond MS, Garcia Aguilar J, Bickford JK, Corbi AL and Springer TA (1993) The I domain is a major recognition site of the leukocyte integrin Mac-1 (CD11b/CD18) for four distinct adhesion ligands. *J Cell Biol* **120:**1031–1043.
Dibbert B, Daigle I, Braun D, Schranz C, Weber K, Blaser K, Zangemeister-Wittke U, Akbar AN and Simon H-U (1998) Role of Bcl-x_L in delayed eosinophil apoptosis mediated by granulocyte-macrophage colony-stimulating factor and interleukin-5. *Blood* **92:**778–783.
Dixon RAF, Diehl RE, Opas E, Rands E, Vickers PJ, Evans JF, Gillard JW and Miller DK (1990) Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. *Nature (Lond)* **343:**282–284.
Djukanovic R, Finnerty JP, Lee C, Wilson S, Madden J and Holgate ST (1995) The effect of theophylline on mucosal inflammation in asthmatic airways: Biopsy results. *Eur Respir J* **8:**831–833.
Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls RS, Roche WR, Howarth PH and Holgate ST (1992) Effect of inhaled corticosteroid on airway inflammation and symptoms in asthma. *Am Rev Respir Dis* **145:**669–674.
Dobrina A, Menegazzi R, Carlos TM, Nardon E, Cramer R, Zacchi T, Harlan JM and Patriarca P (1991) Mechanisms of eosinophil adherence to cultured vascular endothelial cells. Eosinophils bind to the cytokine-induced ligand vascular cell adhesion molecule-1 via the very late activation antigen-4 integrin receptor. *J Clin Invest* **88:**20–26.
Dougall JG, Harper D, Jackson DM and Leff P (1991) Estimation of the efficacy and affinity of the β_2-adrenoceptor agonist salmeterol in guinea-pig trachea. *Br J Pharmacol* **104:**1057–1064.
Dri P, Cramer R, Spessotto P, Romano M and Patriarca P (1991) Eosinophil activation on biologic surfaces. Production of O_2^- in response to physiologic soluble stimuli is differentially modulated by extracellular matrix components and endothelial cells. *J Immunol* **147:**613–620.
Druilhe A, Arock M, Le Goff L and Pretolani M (1998) Human eosinophils express Bcl-2 family proteins: modulation of Bcl-1 expression by IFNγ. *Am J Respir Cell Mol Biol* **18:**315–322.
Druilhe A, Cai Z, Haile S, Chouaib S and Pretolani M (1996) Fas-mediated apoptosis in cultured human eosinophils. *Blood* **87:**2822–2830.
Dubois GR, Bruijnzeel Koomen CA and Bruijnzeel PL (1994) IL-4 induces chemotaxis of blood eosinophils from atopic dermatitis patients, but not from normal individuals. *J Invest Dermatol* **102:**843–846.
Dubois GR and Bruijnzeel PL (1994) IL-4-induced migration of eosinophils in allergic inflammation. *Ann NY Acad Sci* **725:**268–273.
Dubucquoi S, Desreumaux P, Janin A, Klein O, Goldman M, Tavernier J, Capron A and Capron M (1994) Interleukin 5 synthesis by eosinophils: Association with granules and immunoglobulin-dependent secretion. *J Exp Med* **179:**703–708.
Duffus WP, Thorne K and Oliver R (1980) Killing of juvenile *Fasciola hepatica* by purified bovine eosinophil proteins. *Clin Exp Immunol* **40:**336–344.
Duncan JR and Prasse KW (1986) *Veterinary Laboratory Medicine Clinical Pathology,* Iowa State University Press, Ames.
Dunzendorfer S, Meierhofer C and Wiedermann CJ (1998a) Signaling in neuropeptide-induced migration of human eosinophils. *J Leukoc Biol* **64:**828–834.
Dunzendorfer S, Schratzberger P, Reinisch N, Kahler CM and Wiedermann CJ (1998b) Secretaneurin, a novel neuropeptide, is a potent chemoattractant for human eosinophils. *Blood* **91:**1527–1532.
Durack DT, Ackerman SJ, Loegering DA and Gleich GJ (1981) Purification of human eosinophil-derived neurotoxin. *Proc Natl Acad Sci USA* **78:**5165–5169.
Durack DT, Sumi SM and Klebanoff SJ (1979) Neurotoxicity of human eosinophils. *Proc Natl Acad Sci USA* **76:**1443–1447.
Dussault P, Guilbert M, Ferland C, Laviolette M, Battistini B and Ste-Foy PQ (1999) Constitutive and stimulated production of endothelins from human blood eosinophils: A comparison betwen normal and mild asthmatic subjects (Abstract). *Am J Respir Crit Care Med* **159:**A96.
Dvorak AM (1991) Subcellular morphology and biochemistry of eosinophils, in *Blood Cell Biochemistry: Megakaryocytes, Platelets, Macrophages and Eosinophils* (Harris JR ed) pp 237–344, Plenum Press, London.
Dvorak AM, Ackerman SJ, Furitsu T, Estrella P, Letourneau L and Ishizaka T (1992a) Mature eosinophils stimulated to develop in human-cord blood mononuclear cell cultures supplemented with recombinant human interleukin-5. II. Vesicular transport of specific granule matrix peroxidase, a mechanism for effecting piecemeal degranulation. *Am J Pathol* **140:**795–807.
Dvorak AM, Furitsu T, Letourneau L, Ishizaka T and Ackerman SJ (1991) Mature eosinophils stimulated to develop in human cord blood mononuclear cell cultures supplemented with recombinant human interleukin-5. Part I. Piecemeal degranulation of specific granules and distribution of Charcot-Leyden crystal protein. *Am J Pathol* **138:**69–82.
Dvorak AM, Klebanoff SJ, Henderson WR, Monahan RA, Pyne K and Galli SJ (1985) Vesicular uptake of eosinophil peroxidase by guinea pig basophils and by cloned mouse mast cells and granule-containing lymphoid cells. *Am J Pathol* **118:**425–438.
Dvorak AM, Letourneau L, Login GR, Weller PF and Ackerman SJ (1988) Ultrastructural localization of the Charcot-Leyden crystal protein (lysophospholipase) to a distinct crystalloid-free granule population in mature human eosinophils. *Blood* **72:**150–158.
Dvorak AM, Mihm Jr MC, Osage JE, Kwan TH, Austen KF and Wintroub BU (1982) Bullous pemphigoid, an ultrastructural study of the inflammatory response: eosinophil, basophil and mast cell granule changes in multiple biopsies from one patient. *J Invest Dermatol* **78:**91–101.
Dvorak AM, Morgan E, Schleimer RP, Ryeom SW, Lichtenstein LM and Weller PF (1992b) Ultrastructural immunogold localization of prostaglandin endoperoxide synthase (cyclooxygenase) to non-membrane bound cytoplasmic lipid bodies in human lung mast cells, alveolar macrophages type II pneumocytes and neutrophils. *J Histochem Cytochem* **40:**759–769.
Dvorak AM, Morgan ES, Tzizik DM and Weller PF (1994) Prostaglandin endoperoxide synthase (cyclooxygenase): Ultrastructural localization to nonmembrane-bound cytoplasmic lipid bodies in human eosinophils and 3T3 fibroblasts. *Int Arch Allergy Immunol* **105:**245–250.
Dvorak AM, Onderdonk AB, McLeod RS, Monahan Earley RA, Antonioli DA, Cullen J, Blair JE, Cisneros R, Letourneau L, Morgan E, Silen W and Cohen Z (1993) Ultrastructural identification of exocytosis of granules from human gut eosinophils *in vivo*. *Int Arch Allergy Immunol* **102:**33–45.
Dvorak AM, Saito H, Estrella P, Kissell S, Arai N and Ishizaka T (1989) Ultrastructure of eosinophils and basophils stimulated to develop in human cord blood mononuclear cell cultures containing recombinant human interleukin-5 or interleukin-3. *Lab Invest* **61:**116–132.
Dvorak AM, Weller PF, Monahan Earley RA, Letourneau L and Ackerman SJ (1990) Ultrastructural localization of Charcot-Leyden crystal protein (lysophospholipase) and peroxidase in macrophages, eosinophils, and extracellular matrix of the skin in the hypereosinophilic syndrome. *Lab Invest* **62:**590–607.
Dykman TR, Cole JL, Iida K and Atkinson JP (1983a) Structural heterogeneity of the C3b/C4b receptor (CR1) on human peripheral blood cells. *J Exp Med* **157:**2160–2165.
Dykman TR, Cole JL, Iida K and Atkinson JP (1983b) Polymorphism of human erythrocyte C3b/C4b receptor. *Proc Natl Acad Sci USA* **80:**1698–1702.
Eady RP and Norris AA (1997) Nedocromil sodium and sodium cromoglycate: pharmacology and putative modes of action, in *Allergy and Allergic Diseases* (Kay AB ed) pp 584–595, Blackwell Scientific, London.
Ebisawa M, Bochner BS, Georas SN and Schleimer RP (1992) Eosinophil transendothelial migration induced by cytokines. I. Role of endothelial and eosinophil adhesion molecules in IL-1β-induced transendothelial migration. *J Immunol* **149:**4021–4028.
Ebisawa M, Yamada T, Bickel C, Klunk D and Schleimer RP (1994) Eosinophil transendothelial migration induced by cytokines. III. Effect of the chemokine RANTES. *J Immunol* **153:**2153–2160.
Eda R, Sugiyama H, Hopp RJ, Bewtra AK and Townley RG (1993b) Effect of loratadine on human eosinophil function *in vitro*. *Ann Allergy* **71:**373–378.
Eda R, Sugiyama H, Hopp RJ, Okada C, Bewtra AK and Townley RG (1993a) Inhibitory effects of formoterol on platelet-activating factor induced eosinophil chemotaxis and degranulation. *Int Arch Allergy Immunol* **102:**391–398.
Eda R, Townley RG and Hopp RJ (1994) Effect of terfenadine on human eosinophil and neutrophil chemotactic response and generation of superoxide. *Ann Allergy* **73:**154–160.
Egesten A, Alumets J, von Mecklenburg C, Palmegren M and Olsson I (1986) Localization of eosinophil cationic protein, major basic protein, and eosinophil peroxidase in human eosinophils by immunoelectron microscopic technique. *J Histochem Cytochem* **34:**1399–1403.
Ehrlich P (1879) Ueber die specifischen granulationen des blut. *Arch Anat Physiol Lpz 3, Physiol Abstr* 571–579.
Elmalek M and Hammel I (1987) Morphometric evidence that the maturation of the eosinophil granules is independent of volume change. *J Submicrosc Cytol* **19:**265–268.
Elovic A, Galli SJ, Weller PF, Chang AL, Chiang T, Chou MY, Donoff RB, Gallagher GT, Matossian K, McBride J, Tsai M, Todd R and Wong DTW (1990) Production of transforming growth factor α by hamster eosinophils. *Am J Pathol* **137:**1425–1434.
Elovic AE, Ohyama H, Sauty A, McBride J, Tsuji T, Nagai M, Weller PF and Wong DT (1998) IL-4-dependent regulation of TGFα and TGFβ_1 expression in human eosinophils. *J Immunol* **160:**6121–6127.
Elovic A, Wong DT, Weller PF, Matossian K and Galli SJ (1994) Expression of transforming growth factors-α and β_1 messenger RNA and product by eosinophils in nasal polyps. *J Allergy Clin Immunol* **93:**864–869.
Elsas P, Lee TH, Lenzi HL and Dessein AJ (1987) Monocytes activate eosinophils for enhanced helminthotoxicity and increased generation of leukotriene C_4. *Ann Inst Pasteur Immunol* **138:**97–116.
Elshazly AE, Masuyama K, Eura M and Ishikawa T (1996a) Immunoregulatory effect of substance P in human eosinophil migratory function. *Immunol Invest* **25:**191–201.
Elshazly AE, Masuyama K, Samejima Y, Eura M and Ishikawa T (1996b) Inhibition of human eosinophil chemotaxis *in vitro* by the anti-allergic agent emedastine difumarate. *Immunopharmacol Immunotoxicol* **18:**587–595.
Elsner J, Dichmann S, Dobos GJ and Kapp A (1996a) Actin polymerization in human eosinophils, unlike human neutrophils, depends on intracellular calcium mobilization. *J Cell Physiol* **167:**548–555.
Elsner J, Dichmann S and Kapp A (1995) Activation of the respiratory burst in human eosinophils by chemotaxins requires intracellular calcium fluxes. *J Invest Dermatol* **105:**231–236.
Elsner J, Hochstetter R, Kimmig D and Kapp A (1996b) Human eotaxin represents a potent activator of the respiratory burst of human eosinophils. *Eur J Immunol* **26:**1919–1925.
Elsner J, Hochstetter R, Spiekermann K and Kapp A (1996c) Surface and mRNA expression of the CD52 antigen by human eosinophils but not by neutrophils. *Blood* **88:**4684–4693.
Elsner J, Oppermann M, Czech W, Dobos G, Schopf E, Norgauer J and Kapp A (1994)

C3a activates reactive oxygen radical species production and intracellular calcium transients in human eosinophils. *Eur J Immunol* **24:**518–522.
Elsner J, Opperman M, Kapp A (1996d) Detection of C5a receptors on human eosinophils and inhibition of eosinophil effector functions by anti-C5a receptor (CD88) antibodies. *Eur J Immunol* **25:**1560–1564.
Elsner J, Petering H, Kluthe C, Kimmig D, Smolarski R, Ponath P and Kapp A (1998) Eotaxin-2 activates chemotaxis-related events and release of reactive oxygen species via pertussis toxin-sensitive G proteins in human eosinophils. *Eur J Immunol* **28:**2152–2158.
Elwood W, Lotvall JO, Barnes PJ and Chung KF (1992) The effect of dexamethasone and cyclosporin A on allergen-induced airway hyperresponsiveness and inflammatory cell responses in sensitized Brown-Norway rats. *Am Rev Respir Dis* **145:** 1289–1294.
Elwood W, Sun J, Barnes PJ, Giembycz MA and Chung KF (1995) Inhibition of allergen-induced lung eosinophilia by type IV and combined type III- and IV-selective phosphodiesterase inhibitors in Brown Norway rats. *Inflamm Res* **44:**83–86.
Ema H, Suda T, Nagayoshi K, Miura Y, Civin CI and Nakauchi H (1990) Target cells for granulocyte colony-stimulating factor, interleukin-3, and interleukin-5 in differentiation pathways of neutrophils and eosinophils. *Blood* **76:**1956–1961.
Ember JA and Hugli TE (1997) Complement factors and their receptors. *Immunopharmacology* **38:**3–15.
Emery DL, Djokic TD, Graf PD and Nadel JA (1989) Prostaglandin D_2 causes accumulation of eosinophils in the lumen of the dog trachea. *J Appl Physiol* **67:**959–962.
Engels F, Kessels GCR, Henricks PAJ and Nijkamp FP (1996) Preferential formation of 13-hydroxylinoleic acid by human peripheral blood eosinophils. *Prostaglandins* **52:**117–124.
Engels P, Fichtel K and Lubbert H (1994) Expression and regulation of human and rat phosphodiesterase type IV isogenes. *FEBS Lett* **350:**291–295.
Engels P, Sullivan M, Muller T and Lubbert H (1995) Molecular cloning and functional expression in yeast of a human cAMP-specific phosphodiesterase subtype (PDE IV-C). *FEBS Lett* **358:**305–310.
Enokihara H, Nagashima S, Noma T, Kajitani H, Hamaguchi H, Saito K, Furusawa S, Shishido H and Honjo T (1988) Effect of human recombinant interleukin 5 and G-CSF on eosinophil colony formation. *Immunol Lett* **18:**73–76.
Enomoto T and Kitani T (1986) Electron microscopic studies on peroxidase and acid phosphatase reaction in human leukocytes in normal and leukemic cells and on phagocytosis. *Acta Hematol Jpn* **29:**554–570.
Erger RA and Casale TB (1995) Interleukin-8 is a potent mediator of eosinophil chemotaxis through endothelium and epithelium. *Am J Physiol* **268:**L117–L122.
Erger RA and Casale TB (1996) Eosinophil migration in response to three molecular species of platelet activating factor. *Inflamm Res* **45:**265–267.
Erjefalt JS, Andersson M, Greiff L, Korsgren M, Gizycki M, Jeffery PK and Persson GA (1998) Cytolysis and piecemeal degranulation as distinct modes of activation of airway mucosal eosinophils. *J Allergy Clin Immunol* **102:**286–294.
Erjefalt JS, Korsgren M, Nilsson MC, Sundler F and Persson CG (1997a) Association between inflammation and epithelial damage-restitution processes in allergic airways *in vivo*. *Clin Exp Allergy* **27:**1344–1355.
Erjefalt JS, Sundler F and Persson CG (1996) Eosinophils, neutrophils, and venular gaps in the airway mucosa at epithelial removal-restitution. *Am J Respir Crit Care Med* **153:**1666–1674.
Erjefalt JS, Sundler F and Persson CG (1997b) Epithelial barrier formation by airway basal cells. *Thorax* **52:**213–217.
Erle DJ, Briskin MJ, Butcher EC, Garcia Pardo A, Lazarovits AI and Tidswell M (1994) Expression and function of the MAdCAM-1 receptor, integrin $\alpha_4\beta_7$, on human leukocytes. *J Immunol* **153:**517–528.
Etienne A, Soulard C, Thonier F and Braquet P (1989a) Modulation by drugs of eosinophil recruitment induced by immune challenge in the rat. Possible roles of interleukin 5 and platelet-activating factor. *Int Arch Allergy Appl Immunol* **88:** 216–221.
Etienne A, Soulard C, Thonier F and Braquet P (1989b) Modulation of eosinophil recruitment in the rat by the platelet-activating factor (PAF) antagonist, BN 52021, the somatostatin analog, BIM 23014, and by cyclosporin A. *Prostaglandins* **37:**345–357.
Eum SY, Zuany Amorim C, Lefort J, Pretolani M and Vargaftig BB (1997) Inhibition by the immunosuppressive agent FK-506 of antigen-induced airways eosinophilia and bronchial hyperreactivity in mice. *Br J Pharmacol* **120:**130–136.
Evans DJ, Barnes PJ, Cluzel M and O'Connor BJ (1997) Effects of a potent platelet-activating factor antagonist, SR27417A, on allergen-induced asthmatic responses. *Am J Respir Crit Care Med* **156:**11–16.
Evans DJ, Barnes PJ, Spaethe SM, van Alstyne EL, Mitchell MI and O'Connor BJ (1996) Effect of a leukotriene B_4 receptor antagonist, LY293111, on allergen induced responses in asthma. *Thorax* **51:**1178–1184.
Evans DJ, Lindsay MA, Webb, BLJ, Kankaanranta H, Giembycz MA, O'Connor BJ and Barnes PJ (1999) Expression and activation of protein kinase Cζ in eosinophils following allergen challenge. *Am J Physiol,* in press.
Evans PM, Barnes PJ and Chung KF (1990) Inhibition of release of superoxide anions from stimulated human eosinophils by corticosteroids (Abstract). *Br J Pharmacol* **100:**446P.
Evans PM, O'Connor BJ, Fuller RW, Barnes PJ and Chung KF (1993) Effect of inhaled corticosteroids on peripheral blood eosinophil counts and density profiles in asthma. *J Allergy Clin Immunol* **91:**643–650.
Ezeamuzie CI and Al-Hage M (1998) Differential effects of salbutamol and salmeterol on human eosinophil responses. *J Pharmacol Exp Ther* **284:**25–31.
Fabian I, Kletter Y, Mor S, Geller Bernstein C, Ben Yaakov M, Volovitz B and Golde DW (1992a) Activation of human eosinophil and neutrophil functions by haematopoietic growth factors: comparisons of IL-1, IL-3, IL-5 and GM-CSF. *Br J Haematol* **80:**137–143.
Fabian I, Lass M, Kletter Y and Golde DW (1992b) Differentiation and functional activity of human eosinophilic cells from an eosinophil HL-60 subline: Response to recombinant hematopoietic growth factors. *Blood* **80:**788–794.
Faccioli LH, Nourshargh S, Moqbel R, Williams FM, Sehmi R, Kay AB and Williams TJ (1991) The accumulation of ^{111}In-eosinophils induced by inflammatory mediators, *in vivo*. *Immunology* **73:**222–227.
Fadel R, David B, Herpin Richard N, Borgnon A, Rassemont R and Rihoux JP (1990) *In vivo* effects of cetirizine on cutaneous reactivity and eosinophil migration induced by platelet-activating factor (PAF-acether) in man. *J Allergy Clin Immunol* **86:**314–320.
Fadel R, David B, Rassemont R, Herpin Richard N, Borgnon A and Rihoux JP (1991) Eosinophil infiltration: Effects of H_1 antihistamines. *J Am Acad Dermatol* **24:** 1094–1096.
Fadel R, Herpin Richard N, Rihoux JP and Henocq E (1987) Inhibitory effect of cetirizine 2HCl on eosinophil migration *in vivo*. *Clin Allergy* **17:**373–379.
Fajac I, Braunstein G, Ickovic MR, Lacronique J and Frossard N (1995) Selective recruitment of eosinophils by substance P after repeated allergen exposure in allergic rhinitis. *Allergy* **50:**970–975.
Falini B, Pileri S, Pizzolo G, Durkop H, Flenghi L, Stirpe F, Martelli MF and Stein H (1995) CD30 (Ki-1) molecule: A new cytokine receptor of the tumor necrosis factor receptor superfamily as a tool for diagnosis and immunotherapy. *Blood* **85:**1–14.
Fallman M, Andersson R and Andersson T (1993) Signaling properties of CR3 (CD11b/CD18) and CR1 (CD35) in relation to phagocytosis of complement-opsonized particles. *J Immunol* **151:**330–338.
Farmer SG (1995) Bradykinin, in *Airways Smooth Muscle: Peptide Receptors, Ion Channels and Signal Transduction* (Raeburn D and Giembycz MA eds) pp 51–65, Birkhauser, Basel.
Farmer SG, Wilkins DE, Meeker SA, Seeds EA and Page CP (1992) Effects of bradykinin receptor antagonists on antigen-induced respiratory distress, airway hyper-responsiveness and eosinophilia in guinea-pigs. *Br J Pharmacol* **107:**653–659.
Fattah D, Page KR, Bezbaruah S, Priest RC, Horgan CM and Solari R (1996) A rapid activation assay for human eosinophils based on adhesion to immobilized ICAM-1, VCAM-1 and IgG. *Cytokine* **8:**248–259.
Fearon DT (1979) Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. *Proc Natl Acad Sci USA* **76:**5867–5871.
Fearon DT (1985) Human complement receptors for C3b (CR1) and C3d (CR2). *J Invest Dermatol* **85:**53s–57s.
Fechter M, Egger D, Auer H and H (1986) Experimental eosinophilia and inflammation—The effect of various inflammatory mediators and chemoattractants. *Exp Pathol* **29:**153–158.
Feder LS, Stelts D, Chapman RW, Manfra D, Crawley Y, Jones H, Minnicozzi M, Fernandez X, Paster T, Egan RW, Kreutner W and Kung TT (1997) Role of nitric oxide on eosinophilic lung inflammation in allergic mice. *Am J Respir Cell Mol Biol* **17:**436–442.
Fernandes B and Crankshaw D (1995) Functional characterization of the prostanoid DP receptor in human myometrium. *Eur J Pharmacol* **283:**73–81.
Fernvik E, Gronneberg R, Lundahl J, Hed J, Andersson O, Johansson SGO and Hallden G (1996) The degree of natural allergen exposure modifies eosinophil activity markers in the circulation of patients with mild asthma. *Allergy* **51:**697–705.
Fernvik E, Hallden G, Hed J and Lundahl J (1995) Intracellular and surface distribution of CD9 in human eosinophils. *APMIS* **103:**699–706.
Ferreira HH, Bevilacqua E, Gagioti SM, De Luca IM, Zanardo RC, Teixeira CE, Sannomiya P, Antunes E and De Nucci G (1998) Nitric oxide modulates eosinophil infiltration in antigen-induced airway inflammation in rats. *Eur J Pharmacol* **358:**253–259.
Ferreira HHA, Medeiros MV, Lima CSP, Flores CA, Sannomiya P, Antunes E and De Nucci G (1996) Inhibition of eosinophil chemotaxis by chronic blockade of nitric oxide biosynthesis. *Eur J Pharmacol* **310:**201–207.
Filley WV, Holley KE, Kephart, GM and Gleich GJ (1982) Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet* **2:**11–16.
Finnerty JP, Lee C, Wilson S, Madden J, Djukanovic R and Holgate ST (1996) Effects of theophylline on inflammatory cells and cytokines in asthmatic subjects: a placebo-controlled parallel group study. *Eur Respir J* **9:**1672–1677.
Finney PA, Belvisi MG, Adcock IM, Donnelly LE, Barnes PJ. and Giembycz MA (1998) Chronic treatment with salbutamol causes *in vivo* heterologous desensitization of β_2-adrenoceptors and down-regulation of Gsα in Sprague-Dawley rats (Abstract). *Naunyn-Schmiedeberg's Arch Pharmacol* **358:**R648, P 21.12.
Finotto S, Ohno I, Marshall JS, Gauldie J, Denburg JA, Dolovich J, Clark DA and Jordana M (1994) TNF-α production by eosinophils in upper airways inflammation (nasal polyposis). *J Immunol* **153:**2278–2289.
Fiore S, Maddox JF, Perez HD and Serhan CN (1994) Identification of a human cDNA encoding a functional high affinity lipoxin A_4 receptor. *J Exp Med* **180:**253–260.
Fischer E, Capron M, Prin L, Kusnierz JP and Kazatchkine MD (1986) Human eosinophils express CR1 and CR3 complement receptors for cleavage fragments of C3. *Cell Immunol* **97:**297–306.
Fischer N and Czarnetzki BM (1982) Comparative studies on eosinophil chemotactic factors during leukocyte migration under agarose. *J Invest Dermatol* **79:**222–226.
Fisher DA, Smith JF, Pillar JS, St. Denis SH and Cheng JB (1998a) Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. *Biochem Biophys Res Commun* **246:**570–577.
Fisher DA, Smith JF, Pillar JS, St. Denis SH and Cheng JB (1998b) Isolation and characterization of PDE9A, a novel human cGMP-specific phosphodiesterase. *J Biol Chem* **273:**15559–15564.
Fitzharris P, Moqbel R, Thorne KJ, Richardson BA, Hartnell A, Cromwell O, Butterworth AE and Kay AB (1986) The effects of eosinophil activating factor on

IgG-dependent sulphidopeptide leukotriene generation by human eosinophils. *Clin Exp Immunol* **66:**673–680.
Flanagan WM, Corthesy B, Bram RJ and Crabtree GR (1991) Nuclear association of a T-cell transcription factor blocked by FK506 and cyclosporin A. *Nature (Lond)* **352:**803–807.
Foegh ML, Maddox YT and Ramwell PW (1986) Human peritoneal eosinophils and formation of arachidonate cyclooxygenase products. *Scand J Immunol* **23:**599–603.
Folkard SG, Hogarth PJ, Taylor MJ and Bianco AE (1996) Eosinophils are the major effector cells of immunity to microfilariae in a mouse model of onchocerciasis. *Parasitology* **112:**323–329.
Ford-Hutchinson AW (1991) Arachidonate 15-lipoxygenase: Charactersitics and potential biological significance. *Eicosanoids* **4:**65–74.
Forssmann U, Uguccioni M, Loetscher P, Dahinden CA, Langen H, Thelen M and Baggiolini M (1997) Eotaxin-2, a novel CC chemokine that is selective for the chemokine receptor CCR3, and acts like eotaxin on human eosinophil and basophil leukocytes. *J Exp Med* **185:**2171–2176.
Foster A and Chan CC (1991) Peptide leukotriene involvement in pulmonary eosinophil migration upon antigen challenge in the actively sensitized guinea pig. *Int Arch Allergy Appl Immunol* **96:**279–284.
Foster AP and Cunningham FM (1998) Histamine-induced adherence and migration of equine eosinophils. *Am J Vet Res* **59:**1153–1159.
Foster AP, Lees P and Cunningham FM (1992) Platelet activating factor is a mediator of equine neutrophil and eosinophil migration *in vitro*. *Res Vet Sci* **53:**223–229.
Foster AP, Lees P and Cunningham FM (1995) Actions of PAF receptor antagonists in horses with the allergic skin disease sweet itch. *Inflamm Res* **44:**412–417.
Foster AP, McCabe PJ, Sanjar S and Cunningham FM (1997) Agonist-induced adherence of equine eosinophils to fibronectin. *Vet Immunol Immunopathol* **56:**205–220.
Francischi JN, Conry DM, Cloutier S and Sirois P (1993a) Reduction of Sephadex-induced lung inflammation and bronchial hyper-reactivity by rapamycin. *Braz J Med Biol Res* **26:**1105–1110.
Francischi JN, Conroy DM, Maghni K and Sirois P (1993b) Inhibition by rapamycin of leukocyte migration and bronchial hyperreactivity induced by injection of Sephadex beads to guinea-pigs. *Br J Pharmacol* **110:**1381–1386.
Francischi JN, Conry DM, Maghni K and Sirois P (1993c) Rapamycin inhibits airway leukocyte infiltration and hyperreactivity in guinea-pigs. *Agents Actions* **39:**C139–C141.
Fredens K, Dahl R and Venge P (1982) The Gordon phenomenon induced by the eosinophil cationic protein and eosinophil protein X. *J Allergy Clin Immunol* **70:**361–366.
Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden TK, Jacobson KA, Leff P and Williams M (1994) Nomenclature and classification of purinoceptors. *Pharmacol Rev* **46:**143–156.
Freiburghaus J and Jorg A (1990) Isolation of bovine eosinophils and characterization of their leukotriene formation. *Agents Actions* **31:**16–22.
Freiburghaus J, Jorg A and Muller T (1991) Luminol-dependent chemiluminescence in bovine eosinophils and neutrophils: Differential increase of intracellular and extracellular chemiluminescence induced by soluble stimulants. *J Biolumin Chemilumin* **6:**115–121.
Freitag A, Watson RM, Matsos G, Eastwood C and O'Byrne PM (1993) Effect of platelet activating factor antagonist, WEB 2086, on allergen-induced asthmatic responses. *Thorax* **48:**589–594.
Frick WE, Sedgwick JB and Busse WW (1988) Hypodense eosinophils in allergic rhinitis. *J Allergy Clin Immunol* **82:**119–125.
Fruman DA, Burakoff SJ and Bierer BE (1994) Molecular actions of cyclosporin A, FK 506 and rapamycin, in *Immunosuppressive Drugs: Developments in Anti-Rejection Therapy* (Thompson AW and Starzl TE eds) pp 15–35, Edward Arnold, London.
Fugner A (1989) Formation of oedema and accumulation of eosinophils in the guinea pig lung. Inhibition by inhaled β-stimulants. *Int Arch Allergy Appl Immunol* **88:**225–227.
Fujisawa T, Abu Ghazaleh R, Kita H, Sanderson CJ and Gleich GJ (1990) Regulatory effect of cytokines on eosinophil degranulation. *J Immunol* **144:**642–646.
Fujisawa T, Fukuda S, Atsuta J, Ichimi R, Kamiya H and Sakurai M (1994) Interferon-γ induces interleukin-3 release from peripheral blood eosinophils. *Int Arch Allergy Immunol* **104** (**Suppl 1):**41–43.
Fujita Y, Rubinstein E, Greco DB, Reisman RE and Arbesman CE (1975) Antigen-antibody complexes in or on eosinophils in nasal secretions. *Int Arch Allergy Appl Immunol* **48:**577–583.
Fujitani Y, Trifilieff A, Tsuyuki S, Coyle AJ and Bertrand C (1997) Endothelin receptor antagonists inhibit antigen-induced lung inflammation in mice. *Am J Respir Crit Care Med* **155:**1890–1894.
Fukuda T (1994) Immunosuppressive agents and asthma. *Clin Rev Allergy* **12:**95–108.
Fukuda T, Ackerman SJ, Reed CE, Peters MS, Dunnette SL and Gleich GJ (1985a) Calcium ionophore A23187 calcium-dependent cytolytic degranulation in human eosinophils. *J Immunol* **135:**1349–1356.
Fukuda T, Dunnette SL, Reed CE, Ackerman SJ, Peters MS and Gleich GJ (1985b) Increased numbers of hypodense eosinophils in the blood of patients with bronchial asthma. *Am Rev Respir Dis* **132:**981–985.
Fukuda T and Gleich GJ (1989) Heterogeneity of human eosinophils. *J Allergy Clin Immunol* **83:**369–373.
Fukuda T, Numao T, Akutsu I and Makino S (1992) Platelet-activating factor (PAF)-induced chemotaxis and PAF binding to human eosinophils. *J Lipid Mediat* **5:**145–149.
Gabig TG, Crean CD, Mantel PL and Rosli R (1995) Function of wild-type or mutant Rac2 and Rap1a GTPases in differentiated HL60 cell NADPH oxidase activation. *Blood* **85:**804–811.
Galli SJ, Gordon JR and Wershil BK (1991) Cytokine production by mast cells and basophils. *Curr Opin Immunol* **3:**865–872.
Gao JL and Murphy PM (1995) Cloning and differential tissue-specific expression of three mouse β chemokine receptor-like genes, including the gene for a functional macrophage inflammatory protein-1α receptor. *J Biol Chem* **270:**17494–17501.
Gao JL, Sen AI, Kitaura M, Yoshie O, Rothenberg ME, Murphy PM and Luster AD (1996) Identification of a mouse eosinophil receptor for the CC chemokine eotaxin. *Biochem Biophys Res Commun* **223:**679–684.
Garcia Zepeda EA, Combadiere C, Rothenberg ME, Sarafi MN, Lavigne F, Hamid Q, Murphy PM and Luster AD (1996b) Human monocyte chemoattractant protein (MCP)-4 is a novel CC chemokine with activities on monocytes, eosinophils, and basophils induced in allergic and non allergic inflammation that signals through the CC chemokine receptors (CCR)-2 and -3. *J Immunol* **157:**5613–5626.
Garcia Zepeda EA, Rothenberg ME, Ownbey RT, Celestin J, Leder P and Luster AD (1996a) Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. *Nat Med* **2:**449–456.
Gauchat JF, Henchoz S, Fattah D, Mazzei G, Aubry JP, Jomotte T, Dash L, Page K, Solari R, Aldebert D, Capron M, Dahinden C and Bonnefoy J-Y (1995) CD40 ligand is functionally expressed on human eosinophils. *Eur J Immunol* **25:**863–865.
Gauderer CA and Gleich GJ (1978) Inhibition of eosinophil chemotaxis by chloroquine and corticosteroids. *Proc Soc Exp Biol Med* **157:**129–133.
Gauvreau GM, Jordana M, Watson RM, Cockcroft DW and O'Byrne PM (1997) Effect of regular inhaled albuterol on allergen-induced late responses and sputum eosinophils in asthmatic subjects. *Am J Respir Crit Care Med* **156:**1738–1745.
Gavett SH, O'Hearn DJ, Li X, Huang SK, Kinkelman FD and Wills-Karp M (1995) Interleukin-12 inhibits antigen-induced airway hyper-responsiveness, inflammation and Th2 cytokine expression in mice. *J Exp Med* **182:**1527–1536.
Geng JG, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim JM, Bliss GA, Zimmerman GA and McEver RP (1990) Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature (Lond)* **343:**757–760.
Georas SN, McIntyre BW, Ebisawa M, Bednarczyk JL, Sterbinsky SA, Schleimer RP and Bochner BS (1993) Expression of a functional laminin receptor ($\alpha_6\beta_1$, very late activation antigen-6) on human eosinophils. *Blood* **82:**2872–2879.
Gerard C and Gerard NP (1994) The pro-inflammatory seven-transmembrane segment receptors of the leukocyte. *Curr Opin Immunol* **6:**140–145.
Gerard NP and Gerard C (1991) The chemotactic receptor for human C5a anaphylatoxin. *Nature (Lond)* **349:**614–617.
Gerard NP, Hodges MK, Drazen JM, Weller PF and Gerard C (1989) Characterization of a receptor for C5a anaphylatoxin on human eosinophils. *J Biol Chem* **264:**1760–1766.
Gerdes J, Naiem M, Mason DY and Stein H (1982) Human complement (C3b) receptors defined by a mouse monoclonal antibody. *Immunology* **45:**645–653.
Ghiabi M, Gallagher GT and Wong DT (1992) Eosinophils, tissue eosinophilia, and eosinophil-derived transforming growth factor α in hamster oral carcinogenesis. *Cancer Res* **52:**389–393.
Ghidoni JJ and Goldberg AF (1966) Light and electron microscopic localization of acid phosphatase activity in human eosinophils. *Am J Clin Pathol* **45:**402–405.
Giembycz MA (1996) Phosphodiesterase 4 and tolerance to β_2-adrenoceptor agonists in asthma. *Trends Pharmacol Sci* **17:**331–336.
Giembycz MA, Kroegel C and Barnes PJ (1990) Platelet activating factor stimulates cyclo-oxygenase activity in guinea pig eosinophils. Concerted biosynthesis of thromboxane A_2 and E-series prostaglandins. *J Immunol* **144:**3489–3497.
Giembycz MA and Souness JE (1994) Characteristics and properties of the cyclic AMP-specific phosphodiesterase in eosinophil leukocytes: a potential target for asthma therapy?, in *Bronchitis V* (Postma DS and Gerritsen J eds) pp 318–332, Van Gorcum, Assen.
Ginsel LA, Onderwater JJM, Fransen JAM, Verhoeven AJ and Roos D (1990) Localization of the low-Mr subunit of cytochrome b_{558} in human blood phagocytes by immunoelectron microscopy. *Blood* **76:**2105–2116.
Gladue RP, Carroll LA, Milici AJ, Scampoli DN, Stukenbrok HA, Pettipher ER, Salter ED, Contillo L and Showell HJ (1996) Inhibition of leukotriene B_4-receptor interaction suppresses eosinophil infiltration and disease pathology in a murine model of experimental allergic encephalomyelitis. *J Exp Med* **183:**1893–1898.
Gleich GJ, Hunt LW, Bochner BS and Schleimer RP (1996) Glucocorticoid effects on human eosinophils, in *Inhaled Glucocortocoids in Asthma. Lung Biology in Health and Disease* (Schleimer RP, Busse WW, O'Byrne PM eds) vol 97, pp 279–308, Marcel Dekker, New York.
Gleich GJ and Loegering DA (1984) Immunobiology of eosinophils. *Annu Rev Immunol* **2:**429–459.
Gleich GJ, Loegering DA, Bell MP, Checkel JL, Ackerman SJ and McKean DJ (1986) Biochemical and functional similarities between human eosinophil-derived neurotoxin and eosinophil cationic protein: Homology with ribonuclease. *Proc Natl Acad Sci USA* **83:**3146–3150.
Gleich GJ, Loegering DA, Kueppers F, Bajaj SP and Mann KG (1974) Physiochemical and biological properties of the major basic protein from guinea pig eosinophil granules. *J Exp Med* **140:**313–332.
Gleich GJ, Loegering DA and Maldonado JE (1973) Identification of a major basic protein in guinea pig eosinophil granules. *J Exp Med* **137:**1459–1471.
Gleich GJ, Schroeter AL, Marcoux JP, Sachs MI, O'Connell EJ and Kohler PF (1984) Episodic angioedema associated with eosinophilia. *N Engl J Med* **310:**1612–1626.
Glovsky MM, Hugli TE, Ishizaka T, Lichtenstein LM and Erickson BW (1979) Anaphylatoxin-induced histamine release with human leukocytes: studies with C3a leukocyte binding and histamine release. *J Clin Invest* **64:**804–811.
Goers JW, Glovsky MM, Hunkapiller MW, Farnsworth V and Richards JH (1984) Studies on C3a binding to human eosinophils: Characterization of binding. *Int Arch Allergy Appl Immunol* **74:**147–151.
Goetzl EJ (1982) The conversion of leukotriene C_4 to isomers of leukotriene B_4 by human eosinophil peroxidase. *Biochem Biophys Res Commun* **106:**270–275.
Goetzl EJ and Austen KF (1975) Purification and synthesis of eosinophilotactic tetrapeptides of human lung tissue: identification as eosinophil chemotactic factor of anaphylaxis. *Proc Natl Acad Sci USA* **72:**4123–4127.

Goetzl EJ and Sreedharan SP (1992) Mediators of communication and adaptation in the neuroendocrine and immune system. *FASEB J* **6:**2646–2652.
Goetzl EJ, Wasserman SI, Gigli I and Austen KF (1975) Modulation of eosinophil function, in *New Directions in Asthma* (Stein M ed) pp 173–186, American College of Physicians, Park Ridge, IL.
Goetzl EJ, Weller PF and Valone FH (1979) Biochemical and functional basis of the regulatory and protective roles of the human eosinophil, in *Advances in Inflammation Research* (Weissman G ed) pp 157–165, Raven Press, New York.
Goetzl EJ, Woods JM and Gorman RR (1977) Stimulation of human eosinophil and neutrophil polymorphonuclear leukocyte chemotaxis and random migration by 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid. *J Clin Invest* **59:**179–183.
Goldman DW and Goetzl EJ (1984) Heterogeneity of human polymorphonuclear leukocyte receptors for leukotriene B_4. Identification of a subset of high affinity receptors that transduce the chemotactic response. *J Exp Med* **159:**1027–1041.
Gomperts BD (1990) G_E: A GTP-binding protein mediating exocytosis. *Annu Rev Physiol* **52:**591–606.
Gomperts BD and Cromwell O (1991) Secretory properties of eosinophils. *Clin Exp Allergy* **21** (**Suppl 1):**24–28.
Gonzalo JA, Lloyd CM, Wen D, Albar JP, Wells TN, Proudfoot A, Martinez A-C, Dorf M, Bjerke T, Coyle AJ and Gutierrez-Ramos JC (1998) The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. *J Exp Med* **188:**157–167.
Gordon DL, Johnson GM and Hostetter MK (1987) Characteristics of iC3b binding to human polymorphonuclear leucocytes. *Immunology* **60:**553–558.
Gorski P, Palczynski C and Grzegorczyk J (1988) Deuterium-oxide-induced degranulation of human eosinophils in allergic asthmatic patients *in vitro. Allergol Immunopathol Madr* **16:**249–251.
Gounni AS, Lamkhioued B, Delaporte E, Dubost A, Kinet JP, Capron A and Capron M (1994b) The high-affinity IgE receptor on eosinophils: from allergy to parasites or from parasites to allergy? *J Allergy Clin Immunol* **94:**1214–1216.
Gounni AS, Lamkhioued B, Ochiai K, Tanaka Y, Delaporte E, Capron A, Kinet JP and Capron M (1994a) High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature (Lond)* **367:**183–186.
Gozzard N, el Hashim A, Herd CM, Blake SM, Holbrook M, Hughes B, Higgs GA and Page CP (1996a) Effect of the glucocorticosteroid budesonide and a novel phosphodiesterase type 4 inhibitor CDP840 on antigen-induced airway responses in neonatally immunised rabbits. *Br J Pharmacol* **118:**1201–1208.
Gozzard N, Herd CM, Blake AM, Holbrooke M, Hughes B, Higgs GA and Page CP (1996b) Effect of theophylline and rolipram on antigen-induced airway responses in neonatally immunised rabbits. *Br J Pharmacol* **117:**1405–1412.
Grant SM, Goa KL, Fitton A and Sorkin EM (1990) Ketotifen. A review of its pharmacodynamic properties, and therapeutic use in asthma and allergic disorders. *Drugs* **40:**412–428.
Grayson MH, Van der Vieren M, Gallatin WM, Hoffman PA and Bochner BS (1997) Expression of a novel β_2 intergrin ($\alpha_d\beta_2$) on human leukocytes and mast cells (Abstract). *J Allergy Clin Immunol* **99:**S386.
Grayson MH, Van der Vieren M, Sterbinsky SA, Michael Gallatin W, Hoffman PA, Staunton DE and Bochner BS (1998) $\alpha_d\beta_2$ integrin is expressed on human eosinophils and functions as an alternative ligand for vascular cell adhesion molecule 1 (VCAM-1). *J Exp Med* **188:**2187–2191.
Greiff L, Erjefalt JS, Andersson M, Svensson C and Persson CG (1998) Generation of clusters of free eosinophil granules (Cfegs) in seasonal allergic rhinitis. *Allergy* **53:**200–203.
Grewe M, Czech W, Morita A, Werfel T, Klammer M, Kapp A, Ruzicka T, Schöpf E and Krutmann J (1998) Human eosinophils produce biologically active IL-12: Implications for control of T-cell responses. *J Immunol* **161:**415–420.
Gristwood RW, Llupia J, Fernandez AG and Berga P (1991) Effect of theophylline compared with prednisolone on late phase airway leukocyte infiltration in guineapigs. *Int Arch Allergy Appl Immunol* **94:**293–294.
Griswold DE, Webb EF, Breton J, White JR, Marshall PJ and Torphy TJ (1993) Effect of selective phosphodiesterase type IV inhibitor, rolipram, on fluid and cellular phases of inflammatory responses. *Inflammation* **17:**333–344.
Grootendorst DC, Diamant Z, Veselic M, Hiemstra PS, DeSmet M, Leff JA, Friedman BS, Peszek I and Sterk PJ (1997) Effect of oral montelukast, a $CysLT_1$-receptor antagonist, on eosinophil counts and ECP in induced sputum before and after allergen challenge in asthmatics (Abstract). *Am J Respir Crit Care Med* **155:**A976.
Gruart V, Truong MJ, Plumas J, Zandecki M, Kusnierz JP, Prin L, Vinatier D, Capron A and Capron M (1992) Decreased expression of eosinophil peroxidase and major basic protein messenger RNAs during eosinophil maturation. *Blood* **79:** 2592–2597.
Guan EN, Burgess WH, Robinson SL, Goodman EB, McTigue KJ and Tenner AJ (1991) Phagocytic cell molecules that bind the collagen-like region of C1q: Involvement in the C1q-mediated enhancement of phagocytosis. *J Biol Chem* **266:**20345–20355.
Guan EN, Robinson SL, Goodman EB and Tenner AJ (1994) Cell surface protein identified on phagocytic cells modulates the C1q-mediated enhancement of phagocytosis. *J Immunol* **152:**4005–4016.
Guida L, O'Hehir RE and Hawrylowicz CM (1994) Synergy between dexamethasone and interleukin-5 for the induction of major histocompatibility complex class II expression by human peripheral blood eosinophils. *Blood* **84:**2733–2740.
Gulbenkian AR, Egan RW, Fernandez X, Jones H, Kreutner W, Kung T, Payvandi F, Sullivan L, Zurcher JA and Watnick AS (1992) Interleukin-5 modulates eosinophil accumulation in allergic guinea pig lung. *Am Rev Respir Dis* **146:**263–266.
Gulbenkian AR, Fernandez X, Kreutner W, Minnicozzi M, Watnick AS, Kung T and Egan RW (1990) Anaphylactic challenge causes eosinophil accumulation in bronchoalveolar lavage fluid of guinea pigs. Modulation by betamethasone, phenidone, indomethacin, WEB 2086, and a novel antiallergy agent, SCH 37224. *Am Rev Respir Dis* **142:**680–685.
Gullberg U, Widegren B, Arnason U, Egesten A and Olsson I (1986) The cytotoxic eosinophil cationic protein (ECP) has ribonuclease activity. *Biochem Biophys Res Commun* **139:**1239–1242.
Gundel RH, Letts LG and Wegner CD (1991) The role of platelet activating factor in airway inflammation, airway hyperresponsiveness and pulmonary function in monkeys. *Ann NY Acad Sci USA* **629:**205–216.
Gundel RH, Wegner CD and Letts LG (1992) Antigen-induced acute and late phase responses in primates. *Am Rev Respir Dis* **146:**369–373.
Hadjokas NE, Crowley JJ, Bayer CR and Nielson CP (1995) β-Adrenergic regulation of the eosinophil respiratory burst as detected by lucigenin-dependent luminescence. *J Allergy Clin Immunol* **95:**735–741.
Hagan JB, Kita H and Gleich GJ (1998) Inhibition of interleukin-5 mediated eosinophil viability by fluticasone 17-propionate: Comparison with other glucocorticoids. *Clin Exp Allergy* **28:**999–1006.
Hagan P, Blumenthal UJ, Dunn D, Simpson AJ and Wilkins HA (1991) Human IgE, IgG_4 and resistance to reinfection with *Schistosoma haematobium. Nature (Lond)* **349:**243–245.
Hakansson L, Nielsen LS and Teder P (1994) Measurement of neutrophil and eosinophil adhesion to E-selectin, VCAM-1, and ICAM-1 by the use of transfected fibroblast cell lines. *J Immunol Methods* **176:**53–66.
Hakansson L, Venge P (1994) Priming of eosinophil and neutrophil migratory responses by interleukin 3 and interleukin 5. *APMIS* **102:**308–316.
Hall JM (1997) Bradykinin receptors. *Gen Pharmacol* **28:**1–6.
Hall FC and Walport MJ (1993) Hypereosinophilic syndromes: Association with vasculitis, fibrosis and autoimmunity. *Clin Exp Allergy* **23:**542–547.
Hall LR, Mehlotra RK, Higgins AW, Haxhiu MA and Pearlman E (1998) An essential role for interleukin-5 and eosinophils in helminth-induced airway hyperresponsiveness. *Infect Immun* **66:**4425–4430.
Hallam C, Pritchard DI, Trigg S and Eady RP (1982) Rat eosinophil-mediated antibody-dependent cellular cytotoxicity: investigations of the mechanisms of target cell lysis and inhibition by glucocorticoids. *Clin Exp Immunol* **48:**641–648.
Hallgren R, Venge P, Cullhed I and Olsson I (1979) Blood eosinophils and eosinophil cationic protein after acute myocardial infarction or corticosteroid administration. *Br J Haematol* **42:**147–154.
Hallsworth MP, Giembycz MA, Barnes PJ and Lee TH (1996) Cyclic AMP-elevating agents prolong or inhibit eosinophil survival depending on prior exposure to GM-CSF. *Br J Pharmacol* **117:**79–86.
Hallsworth MP, Litchfield TM and Lee TH (1992) Glucocorticoids inhibit granulocyte/macrophage colony-stimulating factor and interleukin-5 enhanced *in vitro* survival of human eosinophils. *Immunology* **75:**382–385.
Hamada A and Greene BM (1987) Clq enhancement of IgG-dependent eosinophil-mediated killing of schistosomula *in vitro. J Immunol* **138:**1240–1245.
Hamada A, Watanabe N, Ohtomo H and Matsuda H (1996) Nerve growth factor enhances survival and cytotoxic activity of human eosinophils. *Br J Haematol* **93:**299–302.
Hamada F, Aoki M, Akiyama T and Toyoshima K (1993) Association of immunoglobulin G Fc receptor II with *Src*-like protein-tyrosine kinase *Fgr* in neutrophils. *Proc Natl Acad Sci USA* **90:**6305–6309.
Hamann KJ, Barker RL, Loegering DA, Pease LR and Gleich GJ (1989) Sequence of human eosinophil-derived neurotoxin cDNA: Identity of deduced amino acid sequence with human nonsecretory ribonucleases. *Gene* **83:**161–167.
Hamann KJ, Barker RL, Ten RM and Gleich GJ (1991) The molecular biology of eosinophil granule proteins. *Int Arch Allergy Appl Immunol* **94:**202–209.
Hamann KJ, Ten RM, Loegering DA, Jenkins RB, Heise MT, Schad CR, Pease LR, Gleich GJ and Barker RL (1990) Structure and chromosome localization of the human eosinophil-derived neurotoxin and eosinophil cationic protein genes: evidence for intronless coding sequences in the ribonuclease gene superfamily. *Genomics* **7:**535–546.
Hamid Q, Barkans J, Meng Q, Ying S, Abrams JS, Kay AB and Moqbel R (1992) Human eosinophils synthesize and secrete interleukin-6, *in vitro. Blood* **80:**1496–1501.
Hammel I, Dvorak AM, Peters SP, Schulman ES, Dvorak HF, Lichtenstein LM and Galli SJ (1985) Differences in the volume distributions of human lung mast cell granules and lipid bodies: Evidence that the size of these organelles is regulated by distinct mechanisms. *J Cell Biol* **100:**1488–1492.
Hammel I, Lagunoff D, Bauza M and Chi E (1983) Periodic, multimodal distribution of granule volumes in mast cells. *Cell Tissue Res* **228:**51–59.
Hammel I, Lagunoff D and Kruger PG (1988) Studies on the growth of mast cells in rats. Changes in granule size between 1 and 6 months. *Lab Invest* **59:**549–554.
Handen JS and Rosenberg HF (1997) Intronic enhancer activity of the eosinophil-derived neurotoxin (*RNS2*) and eosinophil cationic protein (*RNS3*) genes is mediated by an NFAT-1 consensus binding sequence. *J Biol Chem* **272:**1665–1669.
Handzel ZT, Busse WW, Sedgwick JB, Vrtis R, Lee WM, Kelly EAB and Gern JE (1998) Eosinophils bind rhinoviris and activate virus-specific T cells. *J Immunol* **160:**1279–1284.
Hannigan GE, Leunghagesteijn C, Fitzgibbon L, Coppolino MG, Radeva G, Filmus J, Bell JC and Dedhar S (1996) Regulation of cell adhesion and anchorage-dependent growth by a new β_1-integrin-linked protein kinase. *Nature (Lond)* **379:**91–96.
Hanrahan JW, Alles WP and Lewis SA (1985) Single anion-selective channels in basolateral membranes of a mammalian tight membrane. *Proc Natl Acad Sci USA* **82:**7791–7795.
Hansel TT, Braunstein JB, Walker C, Blaser K, Bruijnzeel PL, Virchow JC and Virchow C (1991a) Sputum eosinophils from asthmatics express ICAM-1 and HLA-DR. *Clin Exp Immunol* **86:**271–277.
Hansel TT, De Vries IJ, Carballido JM, Braun RK, Carballido Perrig N, Rihs S, Blaser K and Walker C (1992) Induction and function of eosinophil intercellular adhesion molecule-1 and HLA-DR. *J Immunol* **149:**2130–2136.
Hansel TT, De Vries IJ, Iff T, Rihs S, Wandzilak M, Betz S, Blaser K and Walker C (1991b) An improved immunomagnetic procedure for the isolation of highly purified human blood eosinophils. *J Immunol Methods* **145:**105–110.
Hansel TT, Pound JD, Pilling D, Kitas GD, Salmon M, Gentle TA, Lee SS and

Thompson RA (1989) Purification of human blood eosinophils by negative selection using immunomagnetic beads. *J Immunol Methods* **122:**97–103.
Hansel TT, Pound JD and Thompson RA (1990) Isolation of eosinophils from human blood. *J Immunol Methods* **127:**153–164.
Hansson A, Serhan CN, Haeggstrom J, Ingelman Sundberg M and Samuelsson B (1986) Activation of protein kinase C by lipoxin A and other eicosanoids. Intracellular action of oxygenation products of arachidonic acid. *Biochem Biophys Res Commun* **134:**1215–1222.
Harbinson PL, MacLeod D, Hawksworth R, O'Toole S, Sullivan PJ, Heath P, Kilfeather S, Page CP, Costello JF, Holgate ST and Lee TH (1997) The effect of a novel orally active selective PDE4 isoenzyme inhibitor (CDP840) on allergen-induced responses in asthmatic subjects. *Eur Respir J* **10:**1008–1014.
Hardin JH and Spicer SS (1970) An ultrastructural study of human eosinophil granules: Maturational stages and pyroantimonate reactive cation. *Am J Anat* **128:**283–310.
Harmar T, Arimura A, Gozes I, Journot L, Laburthe M, Pisegna JR, Rawlings SR, Robberechte P, Said SI, Sreedharan SP, Wank SA and Waschek JA (1998) International Union of Pharmacology. XVIII. Nomenclature of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. *Pharmacol Rev* **50:**265–270.
Harris RR, Namovic MT, Hulkower KI, Kolasa TP, Brooks CDW, Bell RL and Carter GW (1997) The effects of the leukotriene inhibitor, ABT-080, in a model of lung eosinophilia in Brown Norway rats (Abstract). *Am J Respir Crit Care Med* **155:** A624.
Hartmann J, Scepek S and Lindau M (1995) Regulation of granule size in human and horse eosinophils by number of fusion events among unit granules. *J Physiol (Lond)* **483:**201–209.
Hartnell A, Kay AB and Wardlaw AJ (1992a) Interleukin-3-induced up-regulation of CR3 expression on human eosinophils is inhibited by dexamethasone. *Immunology* **77:**488–493.
Hartnell A, Kay AB and Wardlaw AJ (1992b) IFN-γ induces expression of FcγRIII (CD16) on human eosinophils. *J Immunol* **148:**1471–1478.
Hartnell A, Moqbel R, Walsh GM, Bradley B and Kay AB (1990) Fcγ and CD11/CD18 receptor expression on normal density and low density human eosinophils. *Immunology* **69:**264–270.
Hartnell A, Robinson DS, Kay AB and Wardlaw AJ (1993) CD69 is expressed by human eosinophils activated *in vivo* in asthma and *in vitro* by cytokines. *Immunology* **80:**281–286.
Hartnell A, Walsh GM, Moqbel R and Kay AB (1989) Phenotypic markers on human eosinophils (Abstract). *J Allergy Clin Immunol* **83:**192.
Hatakeyama M, Mori H, Doi T and Taniguchi T (1989) A restricted cytoplasmic region of IL-2 receptor beta chain is essential for growth signal transduction but not for ligand binding and internalization. *Cell* **59:**837–845.
Hatzelmann A, Tenor H and Schudt C (1995) Differential effects of non-selective and selective phosphodiesterase inhibitors on human eosinophil functions. *Br J Pharmacol* **114:**821–831.
Havill AM, van-Valen RG and Handley DA (1990) Prevention of non-specific airway hyperreactivity after allergen challenge in guinea-pigs by the PAF receptor antagonist SDZ 64–412. *Br J Pharmacol* **99:**396–400.
Hayashi N, Chihara J, Kobayashi Y, Kakazu T, Kurachi D, Yamamoto T and Nakajima S (1994) Effect of platelet-activating factor and platelet factor 4 on eosinophil adhesion. *Int Arch Allergy Immunol* **104** (**Suppl 1**)**:**57–59.
Heath H, Qin SX, Rao P, Wu LJ, Larosa G, Kassam N, Ponath PD and Mackay CR (1997) Chemokine receptor usage by human eosinophils—The importance of CCR3 demonstrated using an antagonistic monoclonal antibody. *J Clin Invest* **99:**178–184.
Hebestreit H, Dibbert B, Balatti I, Braun D, Schapowal A, Blaser K and Simon H (1998) Disruption of fas receptor signaling by nitric oxide in eosinophils. *J Exp Med* **187:**415–425.
Heldin CH (1992) Structural and functional studies on platelet-derived growth factor. *EMBO J* **11:**4251–4259.
Henderson LM, Moule SK and Chappell JB (1993) The immediate activator of the NADPH oxidase is arachidonate not phosphorylation. *Eur J Biochem* **211:**157–162.
Henderson WR and Chi EY (1985) Ultrastructural characterization and morphometric analysis of human eosinophil degranulation. *J Cell Sci* **73:**33–48.
Henderson WR, Chi EY, Jorg A and Klebanoff SJ (1983) Horse eosinophil degranulation induced by the ionophore A23187. Ultrastructure and role of phospholipase A_2. *Am J Pathol* **111:**341–349.
Henderson WR, Harley JB and Fauci AS (1984) Arachidonic acid metabolism in normal and hypereosinophilic syndrome human eosinophils: Generation of leukotrienes B_4, C_4, D_4 and 15-lipoxygenase products. *Immunology* **51:**679–686.
Henderson WR, Harley JB, Fauci AS and Chi EY (1988) Hypereosinophilic syndrome human eosinophil degranulation induced by soluble and particulate stimuli. *Br J Haematol* **69:**13–21.
Henderson WR, Jorg A and Klebanoff SJ (1982) Eosinophil peroxidase-mediated inactivation of leukotrienes B_4, C_4, and D_4. *J Immunol* **128:**2609–2613.
Henderson WR, Lewis DB, Albert RK, Zhang Y, Lamm WJ, Chiang GK, Jones F, Eriksen P, Tien YT, Jonas M and Chi EY (1996) The importance of leukotrienes in airway inflammation in a mouse model of asthma. *J Exp Med* **184:**1483–1494.
Henocq E and Vargaftig BB (1988) Skin eosinophils in atopic patients. *J Allergy Clin Immunol* **81:**691–695.
Henriques GM, Miotla JM, Cordeiro SB, Wolitzky BA, Woolley ST and Hellewell PG (1996) Selectins mediate eosinophil recruitment *in vivo*: A comparison with their role in neutrophil influx. *Blood* **87:**5297–5304.
Henson PM (1969) The adherence of leucocytes and platelets induced by fixed IgG antibody or complement. *Immunology* **16:**107–121.
Her E, Frazer J, Austen KF and Owen WF, Jr (1991) Eosinophil hematopoietins antagonize the programmed cell death of eosinophils. Cytokine and glucocorticoid effects on eosinophils maintained by endothelial cell-conditioned medium. *J Clin Invest* **88:**1982–1987.
Herd CM, Donigi-Gale D, Shoupe TS and Page CP (1992) Effect of a 5-lipoxygenase inhibitor and leukotriene antagonist PF 5901 on PAF-induced airway responses in neonatally immunized rabbits. *Br J Pharmacol* **236:**548–560.
Hessel EM, Cruikshank WW, Van Ark I, De Bie JJ, Van Esch B, Hofman G, Nijkamps FP, Center DM and Van Oosterhout AJ (1998) Involvement of IL-16 in the induction of airway hyper-responsiveness and up-regulation of IgE in a murine model of allergic asthma. *J Immunol* **160:**2998–3005.
Heyneman R (1975) Alkaline phosphatase activity in neutrophil and eosinophil leucocytes. *Arch Int Physiol Biochim* **83:**189–190.
Hibbs ML, Selvaraj P, Carpen O, Springer TA, Kuster H, Jouvin ME and Kinet J (1989) Mechanisms for regulating expression of membrane isoforms of FcγRIII (CD16). *Science (Wash DC)* **246:**1608–1611.
Hibbs MS, Mainardi CL and Kang AH (1982) Type-specific collagen degradation by eosinophils. *Biochem J* **207:**621–624.
Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, Schunack W and Levi-Haas HL (1997) International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol Rev* **49:**253–278.
Hiraguri M, Miike S, Sano H, Kurasawa K, Saito Y, Iwamoto I (1997) Granulocyte/macrophage colony-stimulating factor and IL-5 activate mitogen-activated protein kinase through Jak2 kinase and phosphatidylinositol 3-kinase in human eosinophils. *J Allergy Clin Immunol* **100:**S45–S51.
Hirata K, Maghni K, Borgeat P and Sirois P (1990) Guinea pig alveolar eosinophils and macrophages produce leukotriene B_4 but not peptido-leukotrienes. *J Immunol* **144:**1880–1885.
Hirata K, Pele JP, Robidoux C and Sirois P (1989) Guinea pig lung eosinophil: purification and prostaglandin production. *J Leukoc Biol* **45:**523–528.
Hirata M, Hayashi Y, Ushikubi F, Yokota Y, Kageyama R, Nakanishi S and Narumiya S (1991) Cloning and expression of cDNA for a human thromboxane A_2 receptor. *Nature (Lond)* **349:**617–620.
Hodges MK, Weller PF, Gerard NP, Ackerman SJ and Drazen JM (1988) Heterogeneity of leukotriene C_4 production by eosinophils from asthmatic and from normal subjects. *Am Rev Respir Dis* **138:**799–804.
Hogan SP, Koskinen A, Matthaei KI, Young IG and Foster PS (1998) Interelukin-5-producing $CD4^+$ T-cells play a pivotal role in aeroallergen-induced eosinophilia, bronchial hyperreactivity, and lung damage in mice. *Am J Respir Crit Care Med* **157:**210–218.
Hogg N, Ross GD, Jones DB, Slusarenko M, Walport MJ and Lachmann PJ (1984) Identification of an anti-monocyte monoclonal antibody that is specific for membrane complement receptor type one (CR1). *Eur J Immunol* **14:**236–243.
Holgate ST, Bradding P and Sampson AP (1996) Leukotriene antagonists and synthesis inhibitors: New directions in asthma therapy. *J Allergy Clin Immunol* **98:**1–13.
Holtzman MJ (1991) Arachidonic acid metabolism. Implications of biological chemistry for lung function and disease. *Am Rev Respir Dis* **143:**188–203.
Holtzman MJ, Pentland A, Baenziger NL and Hansbrough JR (1989) Heterogeneity of cellular expression of arachidonate 15-lipoxygenase: Implications for biological activity. *Biochim Biophys Acta* **1003:**204–208.
Hom JT and Estridge T (1993) FK506 and rapamycin modulate the functional activities of human peripheral blood eosinophils. *Clin Immunol Immunopathol* **68:**293–300.
Honda Z, Nakamura M, Miki I, Minami M, Watanabe T, Seyama Y, Okado H, Toh H, Ito K, Miyamoto T and Shimizu T (1991) Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. *Nature (Lond)* **349:**342–346.
Hoogewerf A, Black D, Proudfoot AE, Wells TN and Power CA (1996) Molecular cloning of murine CC CKR-4 and high affinity binding of chemokines to murine and human CC CKR-4. *Biochem Biophys Res Commun* **218:**337–343.
Horie S, Gleich GJ and Kita H (1996) Cytokines directly induce degranulation and superoxide production from human eosinophils. *J Allergy Clin Immunol* **98:**371–381.
Horie S and Kita H (1994) CD11b/CD18 (Mac-1) is required for degranulation of human eosinophils induced by human recombinant granulocyte/macrophage colony-stimulating factor and platelet-activating factor. *J Immunol* **152:**5457–5467.
Horie S, Okubo Y, Hossain M, Momose T, Suzuki J, Isobe M and Sekiguchi M (1997a) Intercellular adhesion molecule-1 on eosinophils is involved in eosinophil protein X release induced by cytokines. *Immunology* **90:**301–307.
Horie S, Okuda Y, Hossain M, Sato E, Nomura H, Koyama S, Suzuki J, Isobe M and Sekiguchi M (1997b) Interleukin-13 but not interleukin-4 prolongs eosinophil survival and induces eosinophil chemotaxis. *Intern Med* **36:**179–185.
Horiuchi T and Weller PF (1997) Expression of vascular endothelial growth factor by human eosinophils: upregulation by granulocyte/macrophage colony-stimulating factor and interleukin-5. *Am J Respir Cell Mol Biol* **17:**70–77.
Horn BR, Robin ED, Theodore J and Van Kessel A (1975) Total eosinophil counts in the management of bronchial asthma. *N Engl J Med* **292:**1152–1155.
Horton MA, Larson KA, Lee JJ and Lee NA (1996) Cloning of the murine eosinophil peroxidase gene (*mEPO*)—Characterization of a conserved subgroup of mammalian hematopoietic peroxidases. *J Leukoc Biol* **60:**285–294.
Horuk R (1994) Molecular properties of the chemokine receptor family. *Trends Pharmacol Sci* **15:**159–165.
Hoshino M (1994) The effect of ketotifen on the inflammation of the bronchial mucosa in atopic asthmatics. *Arerugi* **43:**689–695.
Hoshino M, Nakamura Y, Shin Z and Fukushima Y (1997) Effects of ketotifen on symptoms and on bronchial mucosa in patients with atopic asthma. *Allergy* **52:** 814–820.
Hossain M, Okubo Y, Horie S and Sekiguchi M (1996) Analysis of recombinant human tumour necrosis factor-α-induced CD4 expression on human eosinophils. *Immunology* **88:**301–307.
Hossain M, Okubo Y and Sekiguchi M (1994a) Effects of various drugs (staurosporine, herbimycin A, ketotifen, theophylline, FK506 and cyclosporin A) on eosinophil viability. *Arerugi* **43:**711–717.

Hossain M, Okubo Y and Sekiguchi M (1994b) Eosinophil viability-enhancing activity in mite-sensitive bronchial asthma. *Intern Med* **33:**529–535.
Houslay MD (1996) The N-terminally alternately spliced regions of PDE4A cAMP-specific phosphodiesterases determine intracellular targetting and regulation of catalytic activity. *Biochem Soc Trans* **24:**980–986.
Houslay MD, Scotland G, Pooley L, Spence S, Wilkinson I, McCallum F, Julien P, Rena NG, Michie AM, Erdogan S, Zeng L, O'Donnell JC, Tobias ES and McPhee I (1995) Alternative splicing of the type-IVA cyclic AMP phosphodiesterase gene provides isoform variants with distinct N-terminal domains fused to a common, soluble catalytic unit: "Designer" changes in V_{max}, stability and membrane association. *Biochem Soc Trans* **23:**393–398.
Howarth PH, Roberts JA, Bradding P, Walls AE and Holgate ST (1992) The influence of β-agonists on airway inflammation in asthma, in *Beta-Agonists in the Treatment of Asthma* (Costello JA and Mann RD eds) pp 69–77, Parthenon, Carnforth.
Howell CJ, Pujol JL, Crea AE, Davidson R, Gearing AJ, Godard P and Lee TH (1989) Identification of an alveolar macrophage-derived activity in bronchial asthma that enhances leukotriene C_4 generation by human eosinophils stimulated by ionophore A23187 as a granulocyte/macrophage colony-stimulating factor. *Am Rev Respir Dis* **140:**1340–1347.
Howell RE, Jenkins LP, Fielding LE and Grimes D (1995) Inhibition of antigen-induced pulmonary eosinophilia and neutrophilia by selective inhibitors of phosphodiesterases types 3 or 4 in Brown Norway rats. *Pulm Pharmacol* **8:**83–89.
Howell RE, Sickles BD and Woeppel SL (1993) Pulmonary anti-allergic and bronchodilator effects of isozyme-selective phosphodiesterase inhibitors in guinea-pigs. *J Pharmacol Exp Ther* **264:**609–615.
Hsieh K-H (1991) Effects of a PAF antagonist, BN52021, on the PAF-, methacholine- and allergen-induced bronchoconstriction in asthmatic children. *Chest* **99:**877–882.
Huang WW, Garcia-Zepeda EA, Sauty A, Oettgen HC, Rothenberg ME and Luster AD (1998) Molecular and biological characterization of the murine leukotriene B_4 receptor expressed on eosinophils. *J Exp Med* **188:**1063–1074.
Hubscher T (1975a) Role of the eosinophil in the allergic reactions. I. Edi-an eosinophil-derived inhibitor of histamine release. *J Immunol* **114:**1379–1388.
Hubscher T (1975b) Role of the eosinophil in the allergic reactions. II. Release of prostaglandins from human eosinophilic leukocytes. *J Immunol* **114:**1389–1393.
Hughes B, Howat D, Lisle H, Holbrook M, James T, Gozzard N, Blease K, Hughes P, Kingaby R, Warrelow G, Alexandre R, Head J, Boyd E, Eaton M, Perry M, Wales M, Smith B, Owens R, Catterall C, Lumb S, Russel A, Allen R, Merriman M, Bloxham D and Higgs GA (1996) The inhibition of antigen-induced eosinophia and bronchoconstriction by CDP 840, a novel stereo-selective inhibitor of phosphodiresterase type 4. *Br J Pharmacol* **118:**1183–1191.
Hugli TE (1984) Structure and function of the anaphylatoxins. *Springer Semin Immunopathol* **7:**193–219.
Huizinga TW, de Haas M, Kleijer M, Nuijens JH, Roos D and von dem Borne AE (1990) Soluble Fcγ receptor III in human plasma originates from release by neutrophils. *J Clin Invest* **86:**416–423.
Huizinga TW, van der Schoot CE, Jost C, Klaassen R, Kleijer M, von dem Borne AE, Roos D and Tetteroo PA (1988) The PI-linked receptor FcRIII is released on stimulation of neutrophils. *Nature (Lond)* **333:**667–669.
Huizinga TW, van Kemenade F, Koenderman L, Dolman KM, von dem Borne AE, Tetteroo PA and Roos D (1989) The 40-kDa Fcγ receptor (FcRII) on human neutrophils is essential for the IgG-induced respiratory burst and IgG-induced phagocytosis. *J Immunol* **142:**2365–2369.
Humbles AA, Conroy DM, Marleau S, Rankin SM, Palframan RT, Proudfoot AE, Wells TN, Li D, Jeffery PK, Griffiths-Johnson DA, Williams TJ and Jose PJ (1997) Kinetics of eotaxin generation and its relationship to eosinophil accumulation in allergic airways disease: Analysis in a guinea pig model *in vivo. J Exp Med* **186:**601–612.
Hunt LW, Swedland HA and Gleich GJ (1996) Effect of nebulized lidocaine on severe glucocorticoid-dependent asthma. *Mayo Clin Proc* **71:**361–368.
Hutson PA, Holgate ST and Church MK (1988) Effect of cromolyn sodium and albuterol on early and late phase bronchoconstriction and airway leukocyte infiltration after allergen challenge in non-anesthetized guinea-pigs. *Am Rev Respir Dis* **138:**1157–1163.
Hwang S-B (1988) Identification of a second putative receptor of platelet activating factor from human polymorphonuclear leukocytes. *J Biol Chem* **263:**3225–3233.
Hwang SB, Lam L and Hsu HM (1989) Characterization of platelet activating factor (PAF) receptor by specific binding of [^{3}H]L-659,989, a PAF-receptor antagonist, to rabbit platelet membranes: posible multiple conformations of a single PAF receptor. *J Pharmacol Exp Ther* **254:**48–58.
Ichihara M, Hotta T, Asano H, Inoue C, Murate T, Kobayashi M and Saito H (1994) Effects of stem cell factor (SCF) on human marrow neutrophil, neutrophil/macrophage mixed, macrophage and eosinophil progenitor cell growth. *Int J Hematol* **59:**81–89.
Ikari H and Matsunaga T (1989) Clinical evaluation of five-year continuous prophylaxis of children's bronchial asthma using ketotifen capsule. Improvements in quality of live, serum IgE concentration and peripheral eosinophils. *Pediatr Clin (Jpn)* **39:**3275–3281.
Ingley E and Young IG (1991) Characterization of a receptor for interleukin-5 on human eosinophils and the myeloid leukemia line HL-60. *Blood* **78:**339–344.
Inoue H, Aratani H, Takishima T, Kagoshima M, Iwahisa Y and Terasawa M (1992) Effect of a potent PAF antagonist, Y-24180, on an allergen-induced late asthmatic response in guinea-pigs. *J Lipid Med* **5:**173–176.
Inoue M, Kanbe N, Kurosawa M and Kido H (1998) Cloning and tissue distribution of a novel serine protease esp-1 from human eosinophils. *Biochem Biophys Res Commun* **252:**307–312.
in 't Veen JC, Grootendorst DC, Bel EH, Smits HH, Van Der Keur M, Sterk PJ and Hiemstra PS (1998) CD11b and L-selectin expression on eosinophils and neutrophils in blood and induced sputum of patients with asthma compared with normal subjects. *Clin Exp Allergy* **28:**606–615.
Iozzo RV, MacDonald GH and Wight TN (1982) Immunoelectron microscopic localization of catalase in human eosinophil leukocytes. *J Histochem Cytochem* **30:**697–701.
Ishi Y, Shirato M, Nomura A, Sakamoto T, Uchida Y, Ohtsuka M, Sagai M, Hasegawa S (1998) Cloning of rat eotaxin: Ozone inhalation increases mRNA and protein expression in lungs of brown Norway rats. *Am J Physiol* **274:**L171–L176.
Ishida K, Thompson RJ, Beattie LL, Wiggs B and Schellenberg RR (1990) Inhibition of antigen-induced hyperresponsiveness, but not acute hypoxia nor airway eosinophilia, by an antagonist of platelet activating factor. *J Immunol* **144:**3907–3911.
Ishihara C, Ochiai K, Kagami M, Takashahi H, Matsuyama G, Yoshida S, Tomioka H and Koya N (1997) Human peripheral eosinophils express functional interferon-γ receptors (IFN-γR). *Clin Exp Immunol* **110:**524–529.
Ishikawa T, Wicher K and Arbesman CE (1974) *In vitro* and *in vivo* studies on uptake of antigen-antibody complexes by eosinophils. *Int Arch Allergy Appl Immunol* **46:**230–248.
Israel E, Fischer AR, Rosenberg MA, Lilly CM, Callery JC, Shapiro J, Cohn J, Rubin P and Drazen JM (1993) The pivotal role of 5-lipoxygenase products in the reaction of aspirin-sensitive asthmatics to aspirin. *Am Rev Respir Dis* **148:**1447–1451.
Itoh K, Takahashi E, Mukaiyama O, Satoh Y and Yamaguchi T (1996) Effects of a thromboxane synthase inhibitor (CS-518) on the eosinophil-dependent late asthmatic response and airway hyperresponsiveness in guinea pigs. *Int Arch Allergy Immunol* **109:**79–85.
Itoh N and Nagata S (1993) A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen. *J Biol Chem* **268:**10932–10937.
Iwama T, Nagai H, Suda H, Tsuruoka N and Koda A (1992) Effect of murine recombinant interleukin-5 on the cell population in guinea-pig airways. *Br J Pharmacol* **105:**19–22.
Iwamoto I, Kumano K, Kasai M, Kurasawa K and Nakao A (1996) Interleukin-12 prevents antigen-induced eosinophil recruitment into mouse airways. *Am J Respir Crit Care Med* **154:**1257–1260.
Iwamoto I, Nakagawa N, Yamazaki H, Kimura A, Tomioka H and Yoshida S (1993a) Mechanism for substance P-induced activation of human neutrophils and eosinophils. *Regul Pept* **46:**228–230.
Iwamoto I, Tomoe S, Tomioka H and Yoshida S (1993b) Leukotriene B_4 mediates substance P-induced granulocyte infiltration into mouse skin. Comparison with antigen-induced granulocyte infiltration. *J Immunol* **151:**2116–2123.
Iyengar R (1993a) Multiple families of Gs-regulated adenylyl cyclases. *Adv Second Messenger Phosphoprotein Res* **28:**27–36.
Iyengar R (1993b) Molecular and functional diversity of mammalian Gs-stimulated adenylyl cyclases. *FASEB J* **7:**768–775.
Izumi S, Hirai K, Miyamasu M, Takahashi Y, Misaki Y, Takaishi T, Morita Y, Matsushima K, Ida N,Nakamura H, Kasahara T and Ito K (1997) Expression and regulation of monocyte chemoattractant protein-1 by human eosinophils. *Eur J Immunol* **27:**816–824.
Izumi T, Radmark O and Samuelsson B (1991) Purification of 15-lipoxygenase from human leukocytes, evidence for the presence of isozymes. *Adv Prostaglandin Thromboxane Leukotriene Res* **21A:**101–104.
Jabara HH, Ackerman SJ, Vercelli D, Yokota T, Arai K, Abrams J, Dvorak AM, Lavigne MC, Banchereau J, De Vries J, Leung DYM and Geha RS (1988) Induction of interleukin-4-dependent IgE synthesis and interleukin-5-dependent eosinophil differentiation by supernatants of a human helper T-cell clone. *J Clin Immunol* **8:**437–446.
Jaffar Z, Sullivan P, Page CP and Costello JF (1994) Modulation of T-lymphocyte activity in atopic asthmatics by low dose theophylline therapy (Abstract). *Eur Respir J* **6:**160S.
Jain NC (1986) The eosinophils, *Schalm's Veterinary Hematology,* Lea and Febiger, Philadelphia.
Janecharut T, Hata H, Takahashi H, Yoshida S, Saito H and Kojima S (1992) Effects of recombinant tumour necrosis factor on antibody-dependent eosinophil-mediated damage to *Schistosoma japonicum* larvae. *Parasite Immunol (Oxford)* **14:**605–616.
Janson Bjerklie S, Fahy J, Geaghan S and Golden J (1993) Disappearance of eosinophils from bronchoalveolar lavage fluid after patient education and high-dose inhaled corticosteroids: A case report. *Heart Lung* **22:**235–238.
Jeffery PK, Godfrey RW, Adelroth E, Nelson F, Roger A and Johansson SA (1992) Effects of treatment of airway inflammation and thickening of basement membrane reticular collagen in asthma. *Am Rev Respir Dis* **145:**890–899.
Johnson EH, Muirhead DE, al-Busaidy R and Musa BE (1999) The ultrastructural morphology of the camel eosinophil. *Vet J* **157:**79–84.
Johnson HG, McNee ML and Nugent RA (1992) Canine *in vivo* tracheal chemotaxis of eosinophils to antigen in sensitized dogs: Inhibition by a steroid, a systemic lazaroid U-78517F, and several topical H_1 antihistamines. *Am Rev Respir Dis* **146:**621–625.
Johnson HG and Stout BK (1993) Late phase bronchoconstriction and eosinophilia as well as methacholine hyperresponsiveness in *Ascaris*-sensitive rhesus monkeys were reversed by oral administration of U-83836E. *Int Arch Allergy Appl Immunol* **10:**362–366.
Johnston GI, Cook RG and McEver RP (1989) Cloning of GMP-140, a granule membrane protein of platelets and endothelium: Sequence similarity to proteins involved in cell adhesion and inflammation. *Cell* **56:**1033–1044.
Johnston SL, Bardin PG, Harrison J, Ritter W, Joubert JR and Holgate ST (1992) The effects of an oral thromboxane TP antagonist BAY u3405, on prostaglandin D_2- and histamine-induced bronchoconstriction in asthma, and relationship to plasma drug concentrations. *Br J Clin Pharmacol* **34:**402–408.
Jones DG and Kay AB (1977) Chemotactic activity of guinea pig eosinophils for the ECF-A acidic tetrapeptides, histamine, histamine metabolites, and the effect of H_1- and H_2-receptor antagonists. *Int Arch Allergy Appl Immunol* **55:**277–282.
Jones RE, Finkelman FD, Hester RB and Kayes SG (1994) *Toxocara canis*: Failure to find IgE receptors (FcεR) on eosinophils from infected mice suggests that murine eosinophils do not kill helminth larvae by an IgE-dependent mechanism. *Exp Parasitol* **78:**64–75.
Jones TW (1846) The blood-corpuscle considered in its different phases of develop-

ment in the animal series. Memoir I Vertebrata. *Phil Trans R Soc Lond* **136:**63–87.
Jong EC, Henderson WR and Klebanoff SJ (1980) Bactericidal activity of eosinophil peroxidase. *J Immunol* **124:**1378–1382.
Jorg A, Henderson WR, Murphy RC and Klebanoff SJ (1982a) Leukotriene generation by eosinophils. *J Exp Med* **155:**390–402.
Jorg A, Pasquier JM and Klebanoff SJ (1982b) Purification of horse eosinophil peroxidase. *Biochim Biophys Acta* **701:**185–191.
Jose PJ, Adcock IM, Griffiths Johnson DA, Berkman N, Wells TN, Williams TJ and Power CA (1994a) Eotaxin: cloning of an eosinophil chemoattractant cytokine and increased mRNA expression in allergen-challenged guinea-pig lungs. *Biochem Biophys Res Commun* **205:**788–794.
Jose PJ, Griffiths Johnson DA, Collins PD, Walsh DT, Moqbel R, Totty NF, Truong O, Hsuan JJ and Williams TJ (1994b) Eotaxin: A potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J Exp Med* **179:**881–887.
Joseph SK (1996) The inositol trisphosphate receptor family. *Cell Signal* **8:**1–7.
Jouault T, Capron M, Balloul JM, Ameisen JC and Capron A (1988) Quantitative and qualitative analysis of the Fc receptor for IgE (FcεRII) on human eosinophils. *Eur J Immunol* **18:**237–241.
Jung G, Yang DC and Nakao A (1985) Oxygenation of phosphatidylcholine by human polymorphonuclear leukocyte 15-lipoxygenase. *Biochem Biophys Res Commun* **130:**559–566.
Kadin M, Butmarc J, Elovic A and Wong D (1993) Eosinophils are the major source of transforming growth factor-β_1 in nodular sclerosing Hodgkin's disease. *Am J Pathol* **142:**11–16.
Kagoshima M, Tomomatsu N, Iwahisa Y, Yamaguchi S, Matsuura M, Kawakami Y and Terasawa M (1997) Suppressive effect of Y-24180, a receptor antagonist to platelet activating factor (PAF), on antigen-induced asthmatic responses in guinea-pigs. *Inflamm Res* **46:**147–153.
Kaiser J, Bickel CA, Bochner BS and Schleimer RP (1993) The effects of the potent glucocorticoid budesonide on adhesion of eosinophils to human vascular endothelial cells and on endothelial expression of adhesion molecules. *J Pharmacol Exp Ther* **267:**245–249.
Kajita T, Yui Y, Mita H, Taniguchi N, Saito H, Mishima T and Shida T (1985) Release of leukotriene C_4 from human eosinophils and its relation to the cell density. *Int Arch Allergy Appl Immunol* **78:**406–410.
Kakazu T, Chihara J, Saito A and Nakajima S (1995) Effect of RANTES on eosinophil adhesion to plates coated with recombinant soluble intercellular adhesion molecule-1 and expression of β_2-integrin adhesion molecules on eosinophils. *Int Arch Allergy Immunol* **108** (**Suppl 1):**9–11.
Kameyoshi Y, Dorschner A, Mallet AI, Christophers E and Schroder JM (1992) Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J Exp Med* **176:**587–592.
Kameyoshi Y, Schroder JM, Christophers E and Yamamoto S (1994) Identification of the cytokine RANTES released from platelets as an eosinophil chemotactic factor. *Int Arch Allergy Immunol* **104** (**Suppl 1):**49–51.
Kaneko M, Horie S, Kato M, Gleich GJ and Kita H (1995b) A crucial role for β_2 integrin in the activation of eosinophils stimulated by IgG. *J Immunol* **155:**2631–2641.
Kaneko M, Swanson MC, Gleich GJ and Kita H (1995a) Allergen-specific IgG_1 and IgG_3 through FcγRII induce eosinophil degranulation. *J Clin Invest* **95:**2813–2821.
Kaneko T, Alvarez R, Ueki IF and Nadel JA (1995c) Elevated intracellular cyclic AMP inhibits chemotaxis in human eosinophils. *Cell Signalling* **7:**527–534.
Kankaanranta H, de Souza P, Barnes PJ, Giembycz MA and Lindsay MA (1998) SB 203580, an inhibitor of p38 mitogen-activated protein kinase, enhances constitutive apoptosis of human eosinophils. *J Pharmacol Exp Ther,* in press.
Kanofsky JR, Hoogland H, Wever R and Weiss SJ (1988) Singlet oxygen production by human eosinophils. *J Biol Chem* **263:**9692–9696.
Kantar A, Oggiano N, Giorgi PL and Rihoux JP (1994) A study of the interaction between cetirizine and plasma membrane of eosinophils, neutrophils, platelets and lymphocytes using a fluorescence technique. *Mediat Inflamm* **3:**229–234.
Kantar A, Rihoux JP and Fiorini R (1996) Effect of cetirizine on plasma membrane of human eosinophils, neutrophils and platelets—A dose-response study. *Eur J Pharm Sci* **4:**101–107.
Kapp A, Zeck Kapp G, Czech W and Schopf E (1994) The chemokine RANTES is more than a chemoattractant: characterization of its effect on human eosinophil oxidative metabolism and morphology in comparison with IL-5 and GM-CSF. *J Invest Dermatol* **102:**906–914.
Katayama ML, Federico MH, Brentani RR and Brentani MM (1998) Eosinophil accumulation in rat uterus following estradiol administration is modulated by laminin and its integrin receptors. *Cell Adhes Commun* **5:**409–424.
Kato M, Abraham RT and Kita H (1995) Tyrosine phosphorylation is required for eosinophil degranulation induced by immobilized immunoglobulins. *J Immunol* **155:**357–366.
Kato M, Abraham RT, Okada S and Kita H (1998a) Ligation of the β_2 integrin triggers activation and degranulation of human eosinophils. *Am J Respir Cell Mol Biol* **18:**675–686.
Kato M, Hattori T, Takahashi M, Yanagita N and Nakashima I (1994) Eosinophil cationic protein and prophylactic treatment in pollinosis in natural allergen provocation. *Br J Clin Pract* **48:**299–301.
Kato M, Kephart GM, Talley NJ, Wagner JM, Sarr MG, Bonno M, McGovern TW and Gleich GJ (1998b) Eosinophil infiltration and degranulation in normal human tissue. *Anat Rec* **252:**418–425.
Katoh S, Zheng Z, Oritani K, Shimozato T and Kincade PW (1995) Glycosylation of CD44 negatively regulates its recognition of hyaluronan. *J Exp Med* **182:**419–429.
Katz A, Wu D and Simon MI (1992) Subunits $\beta\gamma$ of heterotrimeric G protein activate β_2 isoform of phospholipase C. *Nature (Lond)* **360:**686–689.
Kauffman HF, van der Belt B, de Monchy JG, Boelens H, Koeter GH and de Vries K (1987) Leukotriene C_4 production by normal-density and low-density eosinophils of atopic individuals and other patients with eosinophilia. *J Allergy Clin Immunol* **79:**611–619.
Kawabe T, Maeda Y, Maekawa N, Tanaka M, Mayumi M, Mikawa H and Yodoi J (1991) Role of PAF and cytokines in the modulation of FcεRII/CD23 expression on human eosinophils. *Adv Prostaglandin Thromboxane Leukotriene Res* **21B:**989–995.
Kawabori S, Soda K, Perdue MH and Bienenstock J (1991) The dynamics of intestinal eosinophil depletion in rats treated with dexamethasone. *Lab Invest* **64:**224–233.
Kawasaki A, Hoshino K, Osaki R, Mizushima Y and Yano S (1992) Effect of ibudilast, a novel anti-asthmatic agent, on airway hyper-reactivity in bronchial asthma. *J Asthma* **29:**245–252.
Kay AB (1985) Eosinophils as effector cells in immunity and hypersensitivity disorders. *Clin Exp Immunol* **62:**1–12.
Kay AB, Glass EJ and Salter DM (1979) Leucoattractants enhance complement receptors on human phagocytic cells. *Clin Exp Immunol* **62:**1–12.
Kay AB, Shin HS and Austen KF (1973) Selective attraction of eosinophils and synergism between eosinophil chemotactic factor of anaphylaxis (ECF-A) and a fragment cleaved from the fifth component of complement (C5a). *Immunology* **24:**969–976.
Kay AB, Walsh GM, Moqbel R, Macdonald AJ, Nagakura T, Carroll MP and Richerson HB (1987) Disodium cromoglycate inhibits activation of human inflammatory cells *in vitro*. *J Allergy Clin Immunol* **80:**1–8.
Keery RJ and Lumley P (1988) AH 6809, a prostaglandin DP-receptor blocking drug on human platelets. *Br J Pharmacol* **94:**745–754.
Kelly JJ, Barnes PJ and Giembycz MA (1996) Phosphodiesterase 4 in macrophages: Relationship between cAMP accumulation, suppression of cAMP hydrolysis and inhibition of [^{3}H]***R***-(−)-rolipram binding by selective inhibitors. *Biochem J* **318:**425–436.
Kernen P, Wymann MP, Von Tscharner V, Deranleau DA, Tai PC, Spry CJ, Dahinden CA and Baggiolini M (1991) Shape changes, exocytosis, and cytosolic free calcium changes in stimulated human eosinophils. *J Clin Invest* **87:**2012–2017.
Khalife J, Capron M, Cesbron JY, Tai PC, Taelman H, Prin L and Capron A (1986) Role of specific IgE antibodies in peroxidase (EPO) release from human eosinophils. *J Immunol* **137:**1659–1664.
Kidney JC, Dominguez M, Taylar P, Rose M, Chung KF and Barnes PJ (1995) Immunomodulation by theophylline: demonstration by withdrawal of therapy. *Am J Respir Crit Care Med* **151:**1907–1914.
Kim CJ, Kane GC, Zangrilli JG, Cho SK, Koh YY and Peters SP (1994) Eosinophils recruited to the lung by segmental antigen challenge show a reduced chemotactic response to leukotriene B_4. *Prostaglandins* **47:**393–403.
Kim JT, Gleich GJ and Kita H (1997) Roles of CD9 molecules in survival and activation of human eosinophils. *J Immunol* **159:**926–933.
Kimani G, Tonnesen MG and Henson PM (1988) Stimulation of eosinophil adherence to human vascular endothelial cells *in vitro* by platelet-activating factor. *J Immunol* **140:**3161–3166.
Kings MA, Chapman I, Kristersson A, Sanjar S and Morley J (1990) Human recombinant lymphokines and cytokines induce pulmonary eosinophilia in guinea-pigs which is inhibited by ketotifen and AH 21–132. *Int Arch Allergy Appl Immunol* **91:**354–361.
Kips JC, Brusselle G, Joos GF, Peleman RA, Devos RR, Tavernier JH and Pauwels RA (1995) Importance of interleukin-4 and interleukin-12 in allergen-induced airway changes in mice. *Int Arch Allergy Immunol* **107:**115–118.
Kirchhausen T, Staunton DE and Springer TA (1993) Location of the domains of ICAM-1 by immunolabeling and single-molecule electron microscopy. *J Leukoc Biol* **53:**342–346.
Kirchmair R, Hogue-Angletti R, Gutierrez J, Fischer-Colbrie R and Winkler H (1993) Secretoneurin—A neuropeptide generated in brain, adrenal medulla and other endocrine tissues by proteolytic processing of secretogranin II (chromogranin C). *Neuroscience* **53:**359–365.
Kirchmair R, Marksteiner J, Troger J, Mahata SK, Mahata M, Donnerer J, Amann R, Fischer-Colbrie R, Winkler H and Saria A (1994) Human and rat primary C-fibre afferents store and release secretoneurin, a novel neuropeptide. *Eur J Neurosci* **6:**861–868.
Kishimoto S, Shimadzu W, Izumi T, Shimizu T, Fukuda T, Makino S, Sugiura T and Waku K (1996a) Regulation by IL-5 of expression of functional platelet-activating factor receptors on human eosinophils. *J Immunol* **157:**4126–4132.
Kishimoto S, Shimazu W, Izumi T, Shimizu T, Fukuda T, Makino S, Sugiura T and Waku K (1996b) Enhanced expression of platelet-activating factor receptor on human eosinophils by interleukin-3, interleukin-5 and granulocyte-macrophage colony-stimulating factor. *Int Arch Allergy Immunol* **111 (Suppl 1):**63–65.
Kishimoto T (1988) IgG receptors on eosinophils using immune scanning electron microscopy. *Scanning Microsc* **2:**2105–2108.
Kishimoto T, Sato T, Ono T, Takahashi K and Kimura I (1990) Effect of ketotifen on the reactivity of eosinophils with the incubation of anti-IgG. *Br J Clin Pract* **44:**226–230.
Kishimoto TK, O'Connor K, Lee A, Roberts TM and Springer TA (1987) Cloning subunit of the β leukocyte adhesion proteins: Homology to an extracellular matrix receptor defines a novel supergene family. *Cell* **48:**681–690.
Kita H, Abu Ghazaleh RI, Gleich GJ and Abraham RT (1991a) Role of pertussis toxin-sensitive G proteins in stimulus-dependent human eosinophil degranulation. *J Immunol* **147:**3466–3473.
Kita H, Abu Ghazaleh RI, Gleich GJ and Abraham RT (1991b) Regulation of Ig-induced eosinophil degranulation by adenosine 3′,5′-cyclic monophosphate. *J Immunol* **146:**2712–2718.
Kita H, Abu Ghazaleh R, Sanderson CJ and Gleich GJ (1991c) Effect of steroids on immunoglobulin-induced eosinophil degranulation. *J Allergy Clin Immunol* **87:**70–77.
Kita H, Abu Ghazaleh RI, Sur S and Gleich GJ (1995) Eosinophil major basic protein induces degranulation and IL-8 production by human eosinophils. *J Immunol* **154:**4749–4758.

Kita H and Gleich GJ (1997) Eosinophils and IgE receptors: a continuing controversy. *Blood* **89:**3497–3501.
Kita H, Kato M, Gleich GJ and Abraham RT (1994) Tyrosine phosphorylation and inositol phosphate production: are early events in human eosinophil activation stimulated by immobilized secretory IgA and IgG? *J Allergy Clin Immunol* **94:** 1272–1281.
Kita H, Ohnishi T, Okubo Y, Weiler D, Abrams JS and Gleich GJ (1991d) Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J Exp Med* **174:**745–748.
Kita H, Weiler DA, Abu Ghazaleh R, Sanderson CJ and Gleich GJ (1992) Release of granule proteins from eosinophils cultured with IL-5. *J Immunol* **149:**629–635.
Kitagaki K, Nagai H, Hayashi S and Totsuka T (1997) Facilitation of apoptosis by cyclosporins A and H, but not FK506 in mouse bronchial eosinophils. *Eur J Pharmacol* **337:**283–289.
Kitagaki K, Niwa S, Hoshiko K, Nagai H, Hayashi S and Totsuka T (1996) Augmentation of apoptosis in bronchial exuded rat eosinophils by cyclosporin A. *Biochem Biophys Res Commun* **222:**71–77.
Kitaura M, Nakajima T, Imai T, Harada S, Combadiere C, Tiffany HL, Murphy PM and Yoshie O (1996) Molecular cloning of human eotaxin, an eosinophil-selective CC chemokine, and identification of a specific eosinophil eotaxin receptor, CC chemokine receptor 3. *J Biol Chem* **271:**7725–7730.
Kitayama J, Fuhlbrigge RC, Puri KD and Springer TA (1997) P-selectin, L-selectin, and α_4 integrin have distinct roles in eosinophil tethering and arrest on vascular endothelial cells under physiological flow conditions. *J Immunol* **159:**3929–3939.
Kitayama J, Mackay CR, Ponath PD and Springer TA (1998) The C-C chemokine receptor CCR3 participates in stimulation of eosinophil arrest on inflammatory endothelium in shear flow. *J Clin Invest* **101:**2017–2024.
Klebanoff SJ, Durack DT, Rosen H and Clark RA (1977) Functional studies on human peritoneal eosinophils. *Infect Immun* **17:**167–173.
Klementsson H and Andersson M (1992) Eosinophil chemotactic activity of topical PAF on the human nasal mucosa. *Eur J Clin Pharmacol* **42:**295–299.
Klickstein LB, Barbashov SF, Liu T, Jack RM and Nicholson Weller A (1997) Complement receptor type 1 (CR1, CD35) is a receptor for C1q. *Immunity* **7:**345–355.
Kloprogge E, de Leeuw AJ, de Monchy GR and Kauffman HF (1989a) Hypodense eosinophilic granulocytes in normal individuals and patients with asthma: generation of a hypodense population *in vitro*. *J Allergy Clin Immunol* **83:**393–400.
Kloprogge E, de Leeuw AJ, de Monchy JG and Kauffman HF (1989b) Cellular communication in leukotriene C_4 production between eosinophils and neutrophils. *Int Arch Allergy Appl Immunol* **90:**20–23.
Klos A, Bank S, Gietz C, Bautsch W, Kohl J, Burg M and Kretzschmar T (1992) C3a receptor on dibutyryl-cAMP-differentiated U937 cells and human neutrophils: the human C3a receptor characterized by functional responses and [^{125}I]-C3a binding. *Biochemistry* **31:**11274–11282.
Knight D, Zheng X, Rocchini C, Jacobson M, Bai T and Walker B (1997) Adenosine A_3 receptor stimulation inhibits migration of human eosinophils. *J Leukoc Biol* **62:**465–468.
Knol EF and Roos D (1996) Mechanisms regulating eosinophil extravasation in asthma. *Eur Respir J* **9:**136S–140S.
Knol EF, Tackey F, Tedder TF, Klunk DA, Bickel CA, Sterbinsky SA and Bochner BS (1994) Comparison of human eosinophil and neutrophil adhesion to endothelial cells under nonstatic conditions. Role of L-selectin. *J Immunol* **153:**2161–2167.
Kobayashi H (1993) Effect of *c-kit* ligand (stem cell factor) in combination with interleukin-5, granulocyte/macrophage colony-stimulating factor, and interleukin-3, on eosinophil lineage. *Int J Hematol* **58:**21–26.
Kobzik L, Imrich A, Massaro A and Drazen JM (1997) Eosinophil expression of nitric oxide synthase-3 and nitrotyrosine antigens (Abstract). *Am J Respir Crit Care Med* **155:**A60.
Koeffler HP, Billing R, Levine AM and Golde DW (1980) Ia antigen is a differentiation marker on human eosinophils. *Blood* **56:**11–14.
Koenderman L and Bruijnzeel PL (1989) Increased sensitivity of the chemoattractant-induced chemiluminescence in eosinophils isolated from atopic individuals. *Immunology* **67:**534–536.
Koenderman L, Hermans SW, Capel PJ and van de Winkel JG (1993) Granulocyte/macrophage colony-stimulating factor induces sequential activation and deactivation of binding via a low-affinity IgG Fc receptor, hFcγRII, on human eosinophils. *Blood* **81:**2413–2419.
Koenderman L, Kuijpers TW, Blom M, Tool AT, Roos D and Verhoeven AJ (1991) Characteristics of CR3-mediated aggregation in human eosinophils: Effect of priming by platelet-activating factor. *J Allergy Clin Immunol* **87:**947–954.
Koenderman L, Maikoe T, Warringa R and Raaijmakers J (1992) Salmeterol is a potent inhibitor of cytokine-primed eosinophil chemotaxis (Abstract). *Am Rev Respir Dis* **145:**A421.
Koenderman L, Tool AT, Roos D and Verhoeven AJ (1990) Priming of the respiratory burst in human eosinophils is accompanied by changes in signal transduction. *J Immunol* **145:**3883–3888.
Koenderman L, Vanderbruggen T, Schweizer RC, Warringa RAJ, Coffer P, Caldenhoven E, Lammers JWJ and Raaijmakers JAM (1996) Eosinophil priming by cytokines - from cellular signal to *in vivo* modulation. *Eur Respir J* **9:**119–125.
Kohi F, Miyagawa H, Agarwal DK, Bewtra AK and Townley RG (1990) Generation of leukotriene B_4 and C_4 from granulocytes of normal controls, allergic rhinitis and healthy donors. *Ann Allergy* **65:**228–232.
Kohno Y, Ji XD, Mawhorter SD, Koshiba M and Jacobson KA (1996) Activation of A_3 adenosine receptors on human eosinophils elevates intracellular calcium. *Blood* **88:**3569–3574.
Kohyama T, Takizawa H, Akiyama N, Sato M, Kawasaki S and Ito K (1997) A novel antiallergic drug epinastine inhibits IL-8 release from human eosinophils. *Biochem Biophys Res Commun* **230:**125–128.
Koike M and Takatsu K (1994) IL-5 and its receptor: which role do they play in the immune response? *Int Arch Allergy Immunol* **104:**1–9.
Kondo M, Takeshita T, Ishii N,Nakamura M, Watanabe S, Arai K and Sugamura K (1993) Sharing of the interleukin-2 (IL-2) receptor γ-chain between receptors for IL-2 and IL-4. *Science (Wash DC)* **262:**1874–1877.
Konig P (1988) Inhaled corticosteroids—Their present and future role in the management of asthma. *J Allergy Clin Immunol* **82:**297–306.
Korth RM (1996) Specific high affinity binding of platelet activating factor to intact human blood neutrophils and eosinophils. *Int Arch Allergy Immunol* **110:**124–131.
Koyanagi H, Wakasugi N, Yoshimatsu K, Takashima Y, Yodoi J, Momoi M, Suda T, Kasahara T and Yamaguchi Y (1995) Role of ADF/TRX and its inhibitor on the release of major basic protein from human eosinophils. *Biochem Biophys Res Commun* **213:**1140–1147.
Kraft M, Bettinger CM, Pak J, Yancey SW, Wisniewski M, Wenzel SE, Ackerman SJ and Martin RJ (1995) Salmeterol decreases nocturnal symptoms and β-agonist use in nocturnal asthma without altering airway inflammation, in *Acute and Chronic Inflammation in the Respiratory Tract* (Johnson M ed) pp 68, Colwood House, London.
Kraft M, Torvik JA, Trudeau JB, Wenzel SE and Martin RJ (1996) Theophylline: Potential antiinflammatory effects in nocturnal asthma. *J Allergy Clin Immunol* **97:**1242–1246.
Kraneveld AD, Van Ark I, Van Der Linde HJ, Fattah D, Nijkamp FP and Van Oosterhout AJ (1997) Antibody to very late activation antigen 4 prevents interleukin-5-induced airway hyperresponsiveness and eosinophil infiltration in the airways of guinea pigs. *J Allergy Clin Immunol* **100:**242–250.
Kremer EJ, Kalatzis V, Baker E, Callen DF, Sutherland GR and Maliszewski CR (1992) The gene for the human IgA Fc receptor maps to 19q13.4. *Hum Genet* **89:**107–108.
Kroegel C, Chilvers ER and Barnes PJ (1990a) Platelet-activating factor (PAF) stimulates phosphoinositide (PI) metabolism in guinea pig eosinophils (Abstract). *Clin Sci (Lond)* **76:**54P.
Kroegel C, Chilvers ER, Giembycz MA, Challiss RA and Barnes PJ (1991) Platelet-activating factor stimulates a rapid accumulation of inositol (1,4,5)trisphosphate in guinea pig eosinophils: Relationship to calcium mobilization and degranulation. *J Allergy Clin Immunol* **88:**114–124.
Kroegel C, Giembycz MA and Barnes PJ (1990b) Characterization of eosinophil cell activation by peptides. Differential effects of substance P, melittin, and fMET-Leu-Phe. *J Immunol* **145:**2581–2587.
Kroegel C, Konig W, Mollay C and Kreil G (1981) Generation of the eosinophil chemotactic factor (ECF) from various cell types by melittin. *Mol Immunol* **18:** 227–236.
Kroegel C, Liu MC, Hubbard WC, Lichtenstein LM and Bochner BS (1994a) Blood and bronchoalveolar eosinophils in allergic subjects after segmental antigen challenge: surface phenotype, density heterogeneity, and prostanoid production. *J Allergy Clin Immunol* **93:**725–734.
Kroegel C and Matthys H (1993) Platelet-activating factor-induced human eosinophil activation. Generation and release of cyclo-oxygenase metabolites in human blood eosinophils from asthmatics. *Immunology* **78:**279–285.
Kroegel C, Pleass R, Yukawa T, Chung KF, Westwick J and Barnes PJ (1989b) Characterization of platelet-activating factor-induced elevation of cytosolic free calcium concentration in eosinophils. *FEBS Lett* **243:**41–46.
Kroegel C, Warner JA, Virchow JC and Matthys H (1994b) Pulmonary immune cells in health and disease: The eosinophil leucocyte (part II). *Eur Respir J* **7:**743–760.
Kroegel C, Yukawa T, Dent G, Chanez P, Chung KF and Barnes PJ (1988) Platelet-activating factor induces eosinophil peroxidase release from purified human eosinophils. *Immunology* **64:**559–561.
Kroegel C, Yukawa T, Dent G, Venge P, Chung KF and Barnes PJ (1989c) Stimulation of degranulation from human eosinophils by platelet-activating factor. *J Immunol* **142:**3518–3526.
Kroegel C, Yukawa T, Westwick J and Barnes PJ (1989a) Evidence for two platelet activating factor receptors on eosinophils: dissociation between PAF-induced intracellular calcium mobilization degranulation and superoxides anion generation in eosinophils. *Biochem Biophys Res Commun* **162:**511–521.
Krych M, Atkinson JP and Holers VM (1992) Complement receptors. *Curr Opin Immunol* **4:**8–13.
Kuhn H, Belkner J, Wiesner R and Brash AR (1990) Oxygenation of biological membranes by the pure reticulocyte lipoxygenase. *J Biol Chem* **265:**18351–18359.
Kuijpers TW, Mul EP, Blom M, Kovach NL, Gaeta FC, Tollefson V, Elices MJ and Harlan JM (1993) Freezing adhesion molecules in a state of high-avidity binding blocks eosinophil migration. *J Exp Med* **178:**279–284.
Kuitert LM, Angus RM, Barnes NC, Barnes PJ, Bone MF, Chung KF, Fairfax AJ, Higgenbotham TW, O'Connor BJ, Piotrowska B, Rozniecki J, Uden S, Hayden E and Willard CJ (1995) Effect of a novel potent platelet activating factot antagonist, modipafant, in clinical asthma. *Am J Respir Crit Care Med* **151:**1331–1335.
Kuitert LM, Hui KP, Uthayarkumar S, Burke W, Newland AC, Uden S and Barnes NC (1993) Effect of platelet-activating factor UK 74,505 on the early and late responses to allergen. *Am Rev Respir Dis* **147:**82–86.
Kuna P, Alam R, Ruta U and Gorski P (1998) RANTES induces nasal mucosal inflammation rich in eosinophils, basophils, and lymphocytes *in vivo*. *Am J Respir Cell Mol Biol* **18:**873–879.
Kuna P, Iyer M, Peerschke EIB, Kaplan AP, Reid KBM and Ghebrehiwet B (1996) Human C1q induces eosinophil migration. *Clin Immunol Immunopathol* **81:**48–54.
Kunapuli SP, Fen Mao G, Bastepe M, Liu Chen LY, Li S, Cheung PP, DeRiel JK and Ashby B (1994) Cloning and expression of a prostaglandin E receptor EP_3 subtype from human erythroleukaemia cells. *Biochem J* **298:**263–267.
Kung TT, Stelts D, Zurcher JA, Watnick AS, Jones H, Mauser PJ, Fernandez X, Umland S, Kruetner W, Chapman RW and Egan RW (1994) Mechanisms of allergic pulmonary eosinophilia in the mouse. *J Allergy Clin Immunol* **94:**1217–1224.
Kuo HP, Yu TR and Yu CT (1994) Hypodense eosinophil number relates to clinical severity, airway hyperresponsiveness and response to inhaled corticosteroids in asthmatic subjects. *Eur Respir J* **7:**1452–1459.
Kuribayashi F, Kumatori A, Suzuki S, Nakamura M, Matsumoto T and Tsuji Y

(1995) Human peripheral eosinophils have a specific mechanism to express gp91^{phox}, the large subunit of cytochrome b_{558}. *Biochem Biophys Res Commun* **209:**146–152.
Kurihara K, Wardlaw AJ, Moqbel R and Kay AB (1989) Inhibition of platelet-activating factor (PAF)-induced chemotaxis and PAF binding to human eosinophils and neutrophils by the specific ginkgolide-derived PAF antagonist, BN 52021. *J Allergy Clin Immunol* **83:**83–90.
Kurosaki T and Ravetch JV (1989) A single amino acid in the glycosyl phosphatidylinositol attachment domain determines the membrane topology of FcγRIII. *Nature (Lond)* **342:**805–807.
Kurup VP, Murali PS, Guo J, Choi H, Banerjee B, Fink JN and Coffman RL (1997) Anti-interleukin (IL)-4 and -IL-5 antibodies downregulate IgE and eosinophilia in mice exposed to *Aspergillus* antigens. *Allergy* **52:**1215–1221.
Kyan Aung U, Hallsworth M, Haskard D, De Vos C and Lee TH (1992) The effects of cetirizine on the adhesion of human eosinophils and neutrophils to cultured human umbilical vein endothelial cells. *J Allergy Clin Immunol* **90:**270–272.
Kyan Aung U, Haskard DO and Lee TH (1991b) Vascular cell adhesion molecule-1 and eosinophil adhesion to cultured human umbilical vein endothelial cells *in vitro*. *Am J Respir Cell Mol Biol* **5:**445–450.
Kyan Aung U, Haskard DO, Poston RN, Thornhill MH and Lee TH (1991a) Endothelial leukocyte adhesion molecule-1 and intercellular adhesion molecule-1 mediate the adhesion of eosinophils to endothelial cells *in vitro* and are expressed by endothelium in allergic cutaneous inflammation *in vivo*. *J Immunol* **146:**521–528.
Lacy P, Levi Schaffer F, Mahmudi-Azer S, Bablitz B, Hagen SC, Velazquez J, Kay AB and Moqbel R (1998) Intracellular localization of interleukin-6 in eosinophils from atopic asthmatics and effects of interferon γ. *Blood* **91:**2508–2516.
Lacy P, Thompson N, Tian M, Solari R, Hide I, Newman TM and Gomperts BD (1995) A survey of GTP-binding proteins and other potential key regulators of exocytotic secretion in eosinophils. Apparent absence of rab3 and vesicle fusion protein homologues. *J Cell Sci* **108:**3547–3556.
Laduron PM (1984) Criteria for receptor sites in binding studies. *Biochem Pharmacol* **33:**833–839.
Lagente V, Carre C, Kyriacopoulos F, Boichot E, Mencia Huerta JM and Braquet P (1994a) Inhibitory effect of cyclosporin A on eosinophil infiltration in the guinea-pig lung induced by antigen, platelet-activating factor and leukotriene B_4. *Eur Respir J* **7:**921–926.
Lagente V, Moodley I, Perrin S, Mottin G and Junien JL (1994b) Effects of isozyme-selective phosphodiesterase inhibitors on eosinophil infiltration in the guinea-pig lung. *Eur J Pharmacol* **255:**253–256.
Lagente V, Pruniaux MP, Junien JL and Moodley I (1995) Modulation of cytokine-induced eosinophil infiltration by phosphodiesterase inhibitors. *Am J Respir Crit Care Med* **151:**1720–1724.
Lahbib-Mansais Y, Yerle M and Gellin J (1995) Localization of IGF1R and EDN genes to pig chromosomes 1 and 7 by *in situ* hybridization. *Cytogenet Cell Genet* **71:**225–227.
Laitinen LA, Laitinen A and Haahtela T (1992) A comparative study of the effects of an inhaled corticosteroid, budesonide, and a β_2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind parallel group control study. *J Allergy Clin Immunol* **90:**32–42.
Laitinen LA, Laitinen A, Haahtela T, Vilkka V, Spur BW and Lee TH (1993) Leukotriene E_4 and granulocytic infiltration into asthmatic airways. *Lancet* **341:**989–990.
Lam BK, Owen WF, Austen KF and Soberman RJ (1989) The identification of a distinct export step following the biosynthesis of leukotriene C_4 by human eosinophils. *J Biol Chem* **264:**12885–12889.
Lamas AM, Leon OG and Schleimer RP (1991) Glucocorticoids inhibit eosinophil responses to granulocyte/macrophage colony-stimulating factor. *J Immunol* **147:**254–259.
Lamas AM, Marcotte GV and Schleimer RP (1989) Human endothelial cells prolong eosinophil survival. Regulation by cytokines and glucocorticoids. *J Immunol* **142:**3978–3984.
Lamas AM, Mulroney CM and Schleimer RP (1988) Studies on the adhesive interaction between purified human eosinophils and cultured vascular endothelial cells. *J Immunol* **140:**1500–1505.
Lambrecht G and Parnham MJ (1986) Kadsurenone distinquishes between different platelet activating factor receptor subtypes on macrophages and polymorphonuclear leukocytes. *Br J Pharmacol* **87:**287–289.
Lamkhioued B, Aldebert D, Gounni AS, Delaporte E, Goldman M, Capron A and Capron M (1995b) Synthesis of cytokines by eosinophils and their regulation. *Int Arch Allergy Immunol* **107:**122–123.
Lamkhioued B, Gounni AS, Gruart V, Pierce, Capron A and Capron M (1995a) Human eosinophils express a receptor for secretory component. Role in secretory IgA-dependent activation. *Eur J Immunol* **25:**117–125.
Lanier LL, Yu G and Phillips JH (1991) Analysis of FcγRIII (CD16) membrane expression and association with CD3ζ and FcεRIγ by site-directed mutation. *J Immunol* **146:**1571–1576.
Larbi KY and Gomperts BD (1997) Complex pattern of inhibition by Mg^{2+} of exocytosis from permeabilised eosinophils. *Cell Calcium* **21:**213–219.
Larson KA, Horton MA, Madden BJ, Gleich GJ, Lee NA and Lee JJ (1995) The identification and cloning of a murine major basic protein gene expressed in eosinophils. *J Immunol* **155:**3002–3012.
Lasky LA (1995) Selectin-carbohydrate interactions and the initiation of the inflammatory response. *Annu Rev Biochem* **64:**113–139.
Lasky LA, Singer MS, Dowbenko D, Imai Y, Henzel W, Fennie C, Watson S and Rosen SD (1992) Glycosylation-dependent cell adhesion molecule 1: A novel mucin-like adhesion ligand for L-selectin. *Cold Spring Harbor Symp Quant Biol* **57:**259–269.
Laudanna C, Melotti P, Bonizzato C, Piacentini G, Boner A, Serra MC and Berton G (1993) Ligation of members of the β_1 or the β_2 subfamilies of integrins by antibodies triggers eosinophil respiratory burst and spreading. *Immunology* **80:**273–280.
Laviolette M, Ferland C, Comtois JF, Champagne K, Bosse M and Boulet LP (1995) Blood eosinophil leukotriene C_4 production in asthma of different severities. *Eur Respir J* **8:**1465–1472.
Laviolette M, Ferland C, Trepanier L, Rocheleau H, Dakhama A and Boulet LP (1994) Effects of inhaled steroids on blood eosinophils in moderate asthma. *Ann N Y Acad Sci* **725:**288–297.
Lawrence TE, Millecchia LL and Fedan JS (1998) Fluticasone propionate and pentamidine isethionate reduce airway hyper-reactivity, pulmonary eosinophilia and pulmonary dendritic cell response in a guinea-pig model of asthma. *J Pharmacol Exp Ther* **284:**222–227.
Lazebnik Y, Takahashi A, Moir RD, Goldman RD, Poirier GG, Kaufmann SH and Earnshaw WC (1995) Studies of the lamin proteinase reveals multiple parallel biochemical pathways during apoptotic execution. *Proc Natl Acad Sci USA* **92:**9042–9046.
Learn DB and Brestel EP (1982) A comparison of superoxide production by human eosinophils and neutrophils. *Agents Actions* **12:**485–488.
Lee AN, Fries FP, Anderson JA and Sporn PHS (1997) Regulation of cyclooxygenase metabolism in human eosinophils (Abstract). *Am J Respir Crit Care Med* **155:**A60.
Lee T, Lenihan DJ, Malone B, Roddy LL and Wasserman SI (1984) Increased biosynthesis of platelet-activating factor in activated human eosinophils. *J Biol Chem* **259:**5526–5530.
Lee TC, Malone B, Wasserman SI, Fitzgerald V and Snyder F (1982) Activities of enzymes that metabolize platelet-activating factor (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine) in neutrophils and eosinophils from humans and the effect of a calcium ionophore. *Biochem Biophys Res Commun* **105:**1303–1308.
Lee TH, Crea AE, Gant V, Spur BW, Marron BE, Nicolaou KC, Reardon E, Brezinski M and Serhan CN (1990) Identification of lipoxin A_4 and its relationship to the sulphidopeptide leukotrienes C_4, D_4 and E_4 in the bronchoalveolar lavage fluids obtained from patients with selected pulmonary diseases. *Am Rev Respir Dis* **141:**1453–1458.
Lee YH, Howe RS, Sha SJ, Teuscher C, Sheehan DM and Lyttle CR (1989) Estrogen regulation of an eosinophil chemotactic factor in the immature rat uterus. *Endocrinology* **125:**3022–3028.
Leff JA, Pizzichini E, Efthimiadis A, Boulet LP, Wei LX, Weinland DE, Hendeles FE and Hargreave FE (1997) Effect of montelukast (MK-0476) on airway eosinophilic inflammation in mildly uncontrolled asthma: A randomized placebo-controlled trial (Abstract). *Am J Respir Crit Care Med* **155:**A977.
Lehman PA and Henderson WR (1990) Retinoid-induced inhibition of eosinophil LTC_4 production. *Prostaglandins* **39:**569–577.
Lehrer RI, Szklarek D, Barton A, Ganz T, Hamann KJ and Gleich GJ (1989) Antibacterial properties of eosinophil major basic protein and eosinophil cationic protein. *J Immunol* **142:**4428–4434.
Lei XF, Ohkawara Y, Stampfli MR, Mastruzzo C, Marr RA, Snider D, Xing Z and Jordana M (1998) Disruption of antigen-induced inflammatory responses in CD40 ligand knockout mice. *J Clin Invest* **101:**1342–1353.
Leiferman KM, Fujisawa T, Gray BH and Gleich GJ (1990) Extracellular deposition of eosinophil and neutrophil granule proteins in the IgE-mediated cutaneous late phase reaction. *Lab Invest* **62:**579–589.
Leiva MC, Xu Q, Galman M and Lyttle CR (1991) Ontogeny of the production of an estrogen-regulated eosinophil chemotactic factor in the rat uterus. *Biol Reprod* **45:**818–823.
Lellouch Tubiana A, Lefort J, Simon MT, Pfister A and Vargaftig BB (1988) Eosinophil recruitment into guinea pig lungs after PAF-acether and allergen administration. Modulation by prostacyclin, platelet depletion, and selective antagonists. *Am Rev Respir Dis* **137:**948–954.
Leonardi A, Borghesan F, Avarello A, Plebani M and Secchi AG (1997) Effect of lodoxamide and disodium cromoglycate on tear eosinophil cationic protein in vernal keratoconjunctivitis. *Br J Ophthalmol* **81:**23–26.
Leonardi A, Borghesan F, Faggian D, Secchi A and Plebani M (1995) Eosinophil cationic protein in tears of normal subjects and patients affected by vernal keratoconjunctivitis. *Allergy* **50:**610–613.
Leprevost C, Capron M, De Vos C, Tomassini M and Capron A (1988) Inhibition of eosinophil chemotaxis by a new antiallergic compound (cetirizine). *Int Arch Allergy Appl Immunol* **87:**9–13.
Levi Schaffer F, Barkans J, Newman TM, Ying S, Wakelin M, Hohenstein R, Barak V, Lacy P, Kay AB and Moqbel R (1996) Identification of interleukin-2 in human peripheral blood eosinophils. *Immunology* **87:**155–161.
Levi Schaffer F, Lacy P, Severs NJ, Newman TM, North J, Gomperts B, Kay AB and Moqbel R (1995) Association of granulocyte/macrophage colony-stimulating factor with the crystalloid granules of human eosinophils. *Blood* **85:**2579–2586.
Levi-Schaffer F, Temkin V, Malamud V, Feld S and Zilberman Y (1998) Mast cells enhance eosinophil survival *in vitro*: role of TNFα and granulocyte/macrophage colony-stimulating factor. *J Immunol* **160:**5554–5562.
Lewis DM, Lewis JC, Loegering DA and Gleich GJ (1978) Localization of the guinea pig eosinophil major basic protein to the core of the granule. *J Cell Biol* **77:**702–713.
Leyden E (1872) Zur Kenntnis des bronchial-asthma. *Arch Pathol Anat* **54:**324–352.
Leyte A, Barr FA, Kehlenbach RH and Huttner WB (1992) Multiple trimeric G-proteins on the trans-Golgi network exert stimulatory and inhibitory effects on secretory vesicle formation. *EMBO J* **11:**4795–4804.
Li MS, Sun L, Satoh T, Fisher LM and Spry CJ (1995) Human eosinophil major basic protein, a mediator of allergic inflammation, is expressed by alternative splicing from two promoters. *Biochem J* **305:**921–927.
Liles WC, Kiener PA, Ledbetter JA, Aruffo A and Klebanoff SJ (1996) Differential expression of Fas (CD95) and Fas ligand on normal human phagocytes: Implications for the regulation of apoptosis in neutrophils. *J Exp Med* **184:**429–440.
Lim KG, Wan HC, Bozza PT, Resnick MB, Wong DT, Cruikshank WW, Kornfeld H, Center DM and Weller PF (1996) Human eosinophils elaborate the lymphocyte chemoattractants IL-16 (lymphocyte chemoattractant factor) and RANTES. *J Immunol* **156:**2566–2570.

Lindau M and Almers W (1995) Structure and function of fusion pores in exocytosis and ectoplasmic membrane fusion. *Curr Opin Cell Biol* **7:**509–517.
Lindau M, Nusse O, Bennett J and Cromwell O (1993) The membrane fusion events in degranulating guinea pig eosinophils. *J Cell Sci* **104:**203–210.
Lindsay MA, Daniels I, Barnes PJ and Giembycz MA (1998a) Activation of *lyn* kinase selectively mediates Ca^{2+}-dependent arachidonic acid release from leukotriene B_4-stimulated eosinophils. *J Biol Chem*, in press.
Lindsay MA, Haddad E, Rousell J, Teixeira MM, Hellewell PG, Barnes PJ and Giembycz MA (1998b) Role of mitogen-activated protein kinases and tyrosine kinases during leukotriene B_4-induced eosinophil activation. *J Leukoc Biol* **64:** 555–562.
Lindsay MA, Perkins RS, Barnes PJ and Giembycz MA (1998c) Leukotriene B_4 activates the NADPH oxidase in eosinophils through a pertussis toxin-sensitive mechanism(s) that is largely independent of arachidonic acid mobilization. *J Immunol* **160:**4526–4534.
Little MM and Casale TB (1991) Comparison of platelet-activating factor-induced chemotaxis of normodense and hypodense eosinophils. *J Allergy Clin Immunol* **88:**187–192.
Liu CM, Bleeker ER, Lichtenstein LM, Kagey Sobotka A, Niv Y, McLemore TL, Permutt S, Proud D and Hubbard WC (1990) Evidence for elevated levels of histamine, prostaglandin D_2 and other bronchoconstricting prostaglandins in the airways of subjects with mild asthma. *Am Rev Respir Dis* **142:**126–132.
Liu J, Farmer JD, Lane WS, Friedman J, Weissman I and Schreiber SL (1991) Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506-complexes. *Cell* **66:**807–815.
Liu S, Miller PD, Holmes SAV, Christmas TJ and Kirby RS (1992) Eosinophilic prostatitis and prostatic specific antigen. *Br J Urol* **69:**61–63.
Livi GP, Kmetz P, McHale MM, Cieslinski LB, Sathe GM, Taylor DP, Davis RL, Torphy TJ and Balcarek JM (1990) Cloning and expression of cDNA for a human low-K_m, rolipram-sensitive cyclic AMP phosphodiesterase. *Mol Cell Biol* **10:**2678–2686.
Lobban M, Shakur Y, Beattie J and Houslay MD (1994) Identification of two splice variant forms of type-IVB cyclic AMP phosphodiesterase, DPD (rPDE-IVB1) and PDE-4 (rPDE-IVB2) in brain: selective localization in membrane and cytosolic compartments and differential expression in various brain regions. *Biochem J* **304:**399–406.
Lohse MJ (1993) Molecular mechanisms of membrane receptor desensitization. *Biochim Biophys Acta* **1179:**171–188.
Lohse MJ, Benovic JL, Codina J, Caron MG and Lefkowitz RJ (1990) β-Arrestin: a protein that regulates β-adrenergic receptor function. *Science (Wash DC)* **248:** 1547–1550.
Lopez AF, Eglinton JM, Gillis D, Park LS, Clark S and Vadas MA (1989) Reciprocal inhibition of binding between interleukin 3 and granulocyte/macrophage colony-stimulating factor to human eosinophils. *Proc Natl Acad Sci USA* **86:**7022–7026.
Lopez AF, Vadas MA, Woodcock JM, Milton SE, Lewis A, Elliott MJ, Gillis D, Ireland R, Olwell E and Park LS (1991) Interleukin-5, interleukin-3, and granulocyte/macrophage colony-stimulating factor cross-compete for binding to cell surface receptors on human eosinophils. *J Biol Chem* **266:**24741–24747.
Lopez AF, Williamson DJ, Gamble JR, Begley CG, Harlan JM, Klebanoff SJ, Waltersdorph A, Wong G, Clark SC and Vadas MA (1986) Recombinant human granulocyte/macrophage colony-stimulating factor stimulates *in vitro* mature human neutrophil and eosinophil function, surface receptor expression, and survival. *J Clin Invest* **78:**1220–1228.
Lowe SW, Schmitt EM, Smith SW, Osborne BA and Jacks T (1993) p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature (Lond)* **362:**847–849.
Lucey DR, Dorsky DI, Nicholson Weller A and Weller PF (1989a) Human eosinophils express CD4 protein and bind human immunodeficiency virus 1 gp120. *J Exp Med* **169:**327–332.
Lucey DR, Nicholson Weller A and Weller PF (1989b) Mature human eosinophils have the capacity to express HLA-DR. *Proc Natl Acad Sci USA* **86:**1348–1351.
Lukacs NW, Standiford TJ, Chensue SW, Kunkel RG, Strieter RM and Kunkel SL (1996a) C-C chemokine-induced eosinophil chemotaxis during allergic airway inflammation. *J Leukoc Biol* **60:**573–578.
Lukacs NW, Strieter RM, Lincoln PM, Brownell E, Pullen DM, Schock HJ, Chensue SW, Taub DD and Kunkel SL (1996b) Stem cell factor (*c-kit* ligand) influences eosinophil recruitment and histamine levels in allergic airway inflammation. *J Immunol* **156:**3945–3951.
Lundahl J, Hallden G and Hed J (1993) Differences in intracellular pool and receptor-dependent mobilization of the adhesion-promoting glycoprotein Mac-1 between eosinophils and neutrophils. *J Leukoc Biol* **53:**336–341.
Lungarella G, Menegazzi R, Gardi C, Spessotto P, de Santi MM, Bertoncin P, Patriarca P, Calzoni P and Zabucchi G (1992) Identification of elastase in human eosinophils: immunolocalization, isolation, and partial characterization. *Arch Biochem Biophys* **292:**128–135.
Luttmann W, Franz P, Matthys H and Virchow JC (1998a) Effects of TGFβ on eosinophil chemotaxis. *Scand J Immunol* **47:**127–130.
Luttmann W, Knoechel B, Foerster M, Matthys H, Virchow JC and Kroegel C (1996) Activation of human eosinophils by IL-13. Induction of CD69 surface antigen, its relationship to messenger RNA expression, and promotion of cellular viability. *J Immunol* **157:**1678–1683.
Luttmann W, Opfer A, Dauer E, Foerster M, Matthys H, Eibel H, Schulze-Osthoff K, Kroegel C and Virchow JC (1998b) Differential regulation of CD95 (Fas/APO-1) expression in human blood eosinophils. *Eur J Immunol* **28:**2057–2065.
Lyttle CR, Medlock KL and Sheehan DM (1984) Eosinophils as the source of uterine nuclear type II estrogen binding sites. *J Biol Chem* **259:**2697–2700.
Lyttle CR, Teuscher C, Medlock KL and Sheehan DM (1989) Estradiol-stimulated increases in uterine eosinophils and nuclear type II estrogen-binding sites are prevented by pertussis toxin. *Endocrinology* **125:**2773–2779.
Maas RL, Brash AR and Oates JA (1981) A second pathway of leukotriene biosynthesis in porcine leukocytes. *Proc Natl Acad Sci USA* **78:**5523–5527.
Mabuchi K, Sugiura T, Ojima Uchiyama A, Masuzawa Y and Waku K (1992) Differential effects of platelet-activating factor on superoxide anion production in human eosinophils and neutrophils. *Biochem Int* **26:**1105–1113.
Macari DM, Teixeira MM and Hellewell PG (1996) Priming of eosinophil recruitment *in vivo* by LPS pretreatment. *J Immunol* **157:**1684–1692.
Macdonald D, Gordon AA, Kajitani H, Enokihara H and Barrett AJ (1990) Interleukin-2 treatment-associated eosinophilia is mediated by interleukin-5 production. *Br J Haematol* **76:**168–173.
MacLeod D and Djukanovic R (1996) Low-dose theophylline: a new anti-inflammatory role in asthma management? *Monaldi Arch Chest Dis* **51:**30–34.
MacMillan DK, Hill E, Sala A, Sigal E, Shuman T, Henson PM and Murphy RC (1994) Eosinophil 15-lipoxygenase is a leukotriene A_4 synthase. *J Biol Chem* **269:**26663–26668.
Macquin-Mavier I, Levame M, Istin N and Harf A (1989) Mechanisms of endothelin-mediated bronchoconstriction in the guinea pig. *J Pharmacol Exp Ther* **250:**740–745.
Maghni K, de Brum Fernandes AJ, Foldes Filep E, Gaudry M, Borgeat P and Sirois P (1991) Leukotriene B_4 receptors on guinea pig alveolar eosinophils. *J Pharmacol Exp Ther* **258:**784–789.
Maguire ME and Erdos JJ (1980) Inhibition of magnesium uptake by β-adrenergic agonists and prostaglandin E_1 is not mediated by cyclic AMP. *J Biol Chem* **255:**1030–1035.
Magyar A, Mihalik R and Olah I (1995) The surface phenotype of swine blood and tissue eosinophil granulocytes. *Vet Immunol Immunopathol* **47:**273–281.
Mahauthaman R, Howell CJ, Spur BW, Youlten LJ, Clark TJ, Lessof MH and Lee TH (1988) The generation and cellular distribution of leukotriene C_4 in human eosinophils stimulated by unopsonized zymosan and glucan particles. *J Allergy Clin Immunol* **81:**696–705.
Makita T and Sanborn EB (1970) The ultrastructural localization of adenosine triphosphatase and alkaline phosphatase activity in eosinophil leukocytes. *Histochemie* **24:**99–105.
Malaviya R and Jakschik BA (1993) Reversible translocation of 5-lipoxygenase in mast cells upon IgE/antigen stimulation. *J Biol Chem* **268:**4939–4944.
Malhotra R, Willis AC, Jensenius JC, Jackson J and Sim RB (1993) Structure and homology of human C1q receptor (collectin receptor). *Immunology* **78:**341–348.
Maliszewski CR, March CJ, Schoenborn MA, Gimpel S and Shen L (1990) Expression cloning of a human Fc receptor for IgA. *J Exp Med* **172:**1665–1672.
Mallett S and Barclay AN (1991) A new superfamily of cell surface proteins related to the nerve growth factor receptor. *Immunol Today* **12:**220–223.
Manetti R, Annunziato F, Biagiotti R, Giudizi M-G, Piccinni M-P, Giannarini L, Sampognaro S, Parronchi P, Vinante F, Pizzolo G, Maggi E and Romagnani S (1994) CD30 expression by $CD8^+$ T cells producing type 2 helper cytokines. Evidence for large numbers of $CD8^+/CD30^+$ T cell clones in human immunodeficiency virus infection. *J Exp Med* **180:**2407–2411.
Manley HC and Haynes LW (1989) Eosinophil chemotactic response to rat CGRP-1 is increased after exposure to trypsin or guinea-pig lung particulate fraction. *Neuropeptides* **13:**29–34.
Manolitsas ND, Wang J, Devalia JL, Trigg CJ, McAulay AE and Davies RJ (1995) Regular albuterol, nedocromil sodium, and bronchial inflammation in asthma. *Am J Respir Crit Care Med* **151:**1925–1930.
Manzini S, Meini S, Giachetti A, Beani L, Borea PA, Antonelli T, Ballati L and Bacciarelli C (1990) Pharmacodynamic profile of isbufylline a new antibronchospastic xanthine devoid of central excitatory actions. *Arzneimittelforschung* **40:** 1205–1213.
Manzini S, Perretti F, Abelli L, Evangelista S, Seeds EAM and Page CP (1993) Isbufylline, a new xanthine derivative, inhibits airway hyper-responsiveness and airways inflammation in guinea-pigs. *Eur J Pharmacol* **249:**251–257.
Markaverich BM, Roberts RR, Alejandro MA and Clark JH (1986) Uterine type II estrogen-binding sites are not of eosinophil origin. *J Biol Chem* **261:**142–146.
Marsden KA, Rao PS, Cavanagh D and Spaziani E (1984) The effect of prostaglandin D_2 (PGD_2) on circulating eosinophils. *Prostaglandins Leukotr Med* **15:**387–397.
Martin SJ and Green DR (1995) Protease activation during apoptosis: Death by a thousand cuts. *Cell* **82:**349–352.
Martin U, Bock D, Arseniev L, Tornetta MA, Ames RS, Bautsch W, Kohl J, Ganser A and Klos A (1997) The human C3a receptor is expressed on neutrophils and monocytes, but not on B or T lymphocytes. *J Exp Med* **186:**199–207.
Martinez FD, Solomon S, Holberg CJ, Graves PE, Baldini M and Erickson RP (1998) Linkage of circulating eosinophils to markers on chromosome 5q. *Am J Respir Crit Care Med* **158:**1739–1744.
Martins MA, Etienne A, Soulard C, Domingo MT and Braquet P (1989) Chemotactic effect of PAF-acether on peritoneal eosinophils from normal rats. *Braz J Med Biol Res* **22:**1151–1154.
Martins MA, Lima MC, Bozza PT, Faria-Neto HC, de Silva PM, Sunkel CE and Cordeiro RS (1993) Interference of the PAF receptor antagonist, PCA 4248, with the rat pleurisy evoked by inflammatory mediators or allergen. *Eur J Pharmacol* **237:**17–22.
Martins MA, Pasquale CP, e Silva PM, Cordeiro RS and Vargaftig BB (1990) Eosinophil accumulation in the rat pleural cavity after mast cell stimulation with compound 48/80 involves protein synthesis and is selectively suppressed by dexamethasone. *Int Arch Allergy Appl Immunol* **92:**416–424.
Martins MA, Pasquale CP, e Silva PM, Pires AL, Ruffie C, Rihoux JP, Cordeiro RS and Vargaftig BB (1992) Interference of cetirizine with the late eosinophil accumulation induced by either PAF or compound 48/80. *Br J Pharmacol* **105:**176–180.
Maruo H, Tanimoto Y, Bewtra AK and Townley RG (1994) Effect of phosphodiesterase IV inhibitor (WAY-PDE-641) on PAF-induced superoxide generation from human eosinophils (Abstract). *J Allergy Clin Immunol* **93:**257.
Masaki T, Vane JR and Vanhoutte PM (1994) International Union of Pharmacology nomenclature of endothelin receptors. *Pharmacol Rev* **46:**137–142.
Mashikian MV, Tarpy RE, Saukkonen JJ, Lim KG, Fine GD, Cruikshank WW and Center DM (1998) Identification of IL-16 as the lymphocyte chemotactic activity in the bronchoalveolar lavage fluid of histamine-challenged asthmatic subjects. *J Allergy Clin Immunol* **101:**786–792.

Massague J (1992) Receptors for the TGF-β family. *Cell* **69:**1067–1070.
Mastrianni DM, Eddy RL, Rosenberg HF, Corrette SE, Shows TB, Tenen DG and Ackerman SJ (1992) Localization of the human eosinophil Charcot-Leyden crystal protein (lysophospholipase) gene (CLC) to chromosome 19 and the human ribonuclease 2 (eosinophil-derived neurotoxin) and ribonuclease 3 (eosinophil cationic protein) genes (RNS2 and RNS3) to chromosome 14. *Genomics* **13:**240–242.
Masuyama K and Ishikawa T (1985) Direct interaction of guinea pig eosinophils and adrenergic agents. *Int Arch Allergy Appl Immunol* **78:**243–248.
Matsumoto K, Appiah-pippim J, Schleimer RP, Bickel C, Beck LA and Bochner BS (1998a) CD44 and CD69 represent different types of cell-surface activation markers for human eosinophils. *Am J Respir Crit Cell Mol Biol* **18:**860–866.
Matsumoto K, Schleimer RP, Saito H, Iikura Y and Bochner BS (1995) Induction of apoptosis in human eosinophils by anti-Fas antibody treatment *in vitro*. *Blood* **86:**1437–1443.
Matsumoto R, Matsumoto H, Seki M, Hata M, Asano Y, Kanegasaki S, Stevens RL and Hirashima M (1998b) Human ecalectin, a variant of human galectin-9, is a novel eosinophil chemoattractant produced by T lymphocytes. *J Biol Chem* **273:** 16976–16984.
Matthews AN, Friend DS, Zimmermann N, Sarafi MN, Luster AD, Pearlman E, Wert SE and Rothenberg ME (1998) Eotaxin is required for the baseline level of tissue eosinophils. *Proc Natl Acad Sci USA* **95:**6273–6278.
Maurer D, Fiebiger S, Ebner C, Reininger B, Fischer GF, Wichlas S, Jouvin MH, Schmitt Egenolf M, Kraft D, Kinet JP and Stingl G (1996) Peripheral blood dendritic cells express FcεRI as a complex composed of FcεRIα- and FcεRIγ chains and can use this receptor for IgE-mediated allergen presentation. *J Immunol* **157:**607–616.
Mawhorter SD, Kazura JW and Boom WH (1994) Human eosinophils as antigen-presenting cells: relative efficiency for superantigen- and antigen-induced $CD4^+$ T-cell proliferation. *Immunology* **81:**584–591.
Mawhorter SD, Pearlman E, Kazura JW and Boom WH (1993) Class II major histocompatibility complex molecule expression on murine eosinophils activated *in vivo* by *Brugia malayi*. *Infect Immun* **61:**5410–5412.
Mawhorter SD, Stephany DA, Ottesen EA and Nutman TB (1996) Identification of surface molecules associated with physiologic activation of eosinophils. Application of whole-blood flow cytometry to eosinophils. *J Immunol* **156:**4851–4858.
Mayeno AN, Curran AJ, Roberts RL and Foote CS (1989) Eosinophils preferentially use bromide to generate halogenating agents. *J Biol Chem* **264:**5660–5668.
McEvoy JD, Donald KJ, Edwards RL (1978) Immunoglobulin levels and electron microscopy in eosinophilic pneumonia. *Am J Med* **64:**529–536.
McEwen BJ (1992) Eosinophils: A review. *Vet Res Commun* **16:**11–44.
McEwen BJ, Wilcock BP and Eyre P (1990) The effect of leukotriene B_4, leukotriene C_4, zymosan-activated serum, histamine, tabanid extract and N-formyl-methionyl-leucyl-phenylalanine on the *in vitro* migration of equine eosinophils. *Can J Vet Res* **54:**400–404.
McGraw DW and Liggett SB (1997) Heterogeneity in β-adrenergic receptor kinase expression in the lung accounts for cell-specific desensitization of the β_2-adrenergic receptor. *J Biol Chem* **272:**7338–7344.
McGrogan M, Simonsen C, Scott R, Griffith J, Ellis N, Kennedy J, Campanelli D, Nathan C and Gabay J (1988) Isolation of a complementary DNA clone encoding a precursor to human eosinophil major basic protein. *J Exp Med* **168:**2295–2308.
McLaughlin MM, Cieslinski LB, Burman M, Torphy TJ and Livi GP (1993) A low-Km, rolipram-sensitive, cAMP-specific phosphodiesterase from human brain. Cloning and expression of cDNA, biochemical characterization of recombinant protein, and tissue distribution of mRNA. *J Biol Chem* **268:**6470–6476.
McPhail LC (1994) SH3-dependent assembly of the phagocyte NADPH oxidase. *J Exp Med* **180:**2011–2015.
Meacock SC, Brandon DR and Smith W (1991) Interleukin-2 receptors on rat eosinophils in adjuvant arthritis. *Immunology* **74:**169–171.
Meagher LC, Cousin JM, Seckl JR and Haslett C (1996) Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophil granulocytes. *J Immunol* **156:**4422–4428.
Meja KK, Barnes PJ and Giembycz MA (1997) Characterisation of the prostanoid receptors on human blood monocytes at which prostaglandin E_2 inhibits lipopolysaccharide-induced tumour necrosis factor-α generation. *Br J Pharmacol* **122:** 149–157.
Melani C, Mattia GF, Silvani A, Care A, Rivoltini L, Parmiani G and Colombo MP (1993) Interleukin-6 expression in human neutrophil and eosinophil peripheral blood granulocytes. *Blood* **81:**2744–2749.
Menegazzi R, Zabucchi G and Patriarca P (1986) A simple procedure for the purification of eosinophil peroxidase from normal human blood. *J Immunol Methods* **91:**283–288.
Mengelers HJ, Maikoe T, Brinkman L, Hooibrink B, Lammers JW and Koenderman L (1994) Immunophenotyping of eosinophils recovered from blood and BAL of allergic asthmatics. *Am J Respir Crit Care Med* **149:**345–351.
Metcalfe DD, Gadek JE, Raphael GD, Frank MM, Kaplan AP and Kaliner M (1977) Human eosinophil adherence to serum-treated Sepharose: granule-associated enzyme release and requirement for activation of the alternative complement pathway. *J Immunol* **119:**1744–1750.
Metwali A, Blum AM, Ferraris L, Klein JS, Fiocchi C and Weinstock JV (1994) Eosinophils within the healthy or inflamed human intestine produce substance P and vasoactive intestinal peptide. *J Neuroimmunol* **52:**69–78.
Metzger WJ, Richerson HB, Worden K, Monick M and Hunninghake GW (1986) Bronchoalveolar lavage of allergic asthmatic patients following allergen provocation. *Chest* **89:**477–483.
Meurer R, Van Riper G, Feeney W, Cunningham P, Hora D, Springer MS, MacIntyre DE and Rosen H (1993) Formation of eosinophilic and monocytic intradermal inflammatory sites in the dog by injection of human RANTES but not human monocyte chemoattractant protein 1, human macrophage inflammatory protein 1α, or human interleukin 8. *J Exp Med* **178:**1913–1921.
Michel L, De Vos C, Rihoux JP, Burtin C, Benveniste J and Dubertret L (1988) Inhibitory effect of oral cetirizine on *in vivo* antigen-induced histamine and PAF-acether release and eosinophil recruitment in human skin. *J Allergy Clin Immunol* **82:**101–109.
Michishita M, Videm V and Arnaout MA (1993) A novel distinct cation binding site in the A domain of the β_2 integrin CR3 (CD11b/CD18) is essential for ligand binding. *Cell* **72:**857–867.
Migita M, Yamaguchi N, Mita S, Higuchi S, Hitoshi Y, Yoshida Y, Tomonaga M, Matsuda I, Tominaga A and Takatsu K (1991) Characterization of the human IL-5 receptors on eosinophils. *Cell Immunol* **133:**484–497.
Minnicozzi M, Anthes JC, Siegel MI, Billah MM and Egan RW (1990) Activation of phospholipase D in normodense human eosinophils. *Biochem Biophys Res Commun* **170:**540–547.
Minshall EM, Cameron L, Lavigne, Leung DY, Hamilos D, Garcia-Zepada EA, Rothenberg M, Luster AD and Hamid Q (1997) Eotaxin mRNA and protein expression in chronic sinusitis and allergen-induced nasal responses in seasonal allergic rhinitis. *Am J Respir Cell Mol Biol* **17:**683–690.
Minshall EM, Johnson PR, Campbell AM, Brown TJ and Carter CM (1990) The influence of extracellular calcium and different loading concentrations of fura-2-AM on the calcium response to PAF in guinea pig eosinophils (Abstract). *Br J Pharmacol* **100:**448P.
Miyagawa H, Nabe M, Hopp RJ, Okada C, Bewtra AK and Townley G (1992) The effect of WEB 2086 on PAF-induced eosinophil chemotaxis and LTC_4 production from eosinophils. *Agents Actions* **37:**39–43.
Miyajima A, Hara T and Kitamura T (1992a) Common subunits of cytokine receptors and the functional redundancy of cytokines. *Trends Biochem Sci* **17:**378–382.
Miyajima A, Kitamura T, Harada N, Yokota T and Arai K (1992b) Cytokine receptors and signal transduction. *Annu Rev Immunol* **10:**295–331.
Miyake K, Underhill CB, Lesley J and Kincade PW (1990) Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. *J Exp Med* **172:**69–75.
Miyamasu M, Hirai K, Takahashi Y, Iida M, Yamaguchi M, Koshino T, Takaishi T, Morita Y, Ohta K, Kasahara T and Ito K (1995) Chemotactic agonists induce cytokine generation in eosinophils. *J Immunol* **154:**1339–1349.
Miyamasu M, Misaki Y, Hirai K, Izumi S, Takaishi T, Matsushima K, Morita Y and Kasahara T (1997) Eosinophilopoietic factors prime eosinophils for increased interleukin-8 generation. *Allergy* **52:**317–322.
Miyamasu M, Misaki Y, Izumi S, Takaishi T, Morita Y, Nakamura H, Matsushima K, Kasahara T and Kirai K (1998) Glucocorticoids inhibit chemokine generation by human eosinophils. *J Allergy Clin Immunol* **101:**75–83.
Miyasato M, Tsuda A, Kasada M, Iryo K and Sasai Y (1988) The effect of ketotifen on density distribution of eosinophils in patients with atopic dermatitis. *Jpn J Inflamm* **8:**260–262.
Molenaar P, Sarsero D and Kaumann AJ (1997) Proposal for the interaction of non-conventional partial agonists and catecholamines with the "putative" β_4-adrenoceptor" in mammalian heart. *Clin Exp Pharmacol Physiol* **24:**647–656.
Moller GM, de Jong TA, van der Kwast TH, Overbeek SE, Wierenga Wolf AF, Thepen T and Hoogsteden HC (1996a) Immunolocalization of interleukin-4 in eosinophils in the bronchial mucosa of atopic asthmatics. *Am J Respir Cell Mol Biol* **14:**439–443.
Moller GM, De Jong TA, Overbeek SE, van der Kwast TH, Postma DS and Hoogsteden HC (1996b) Ultrastructural immunogold localisation of interleukin 5 to the crystalloid core compartment of eosinophil secondary granules in patients with atopic asthma. *J Histochem Cytochem* **44:**67–69.
Monahan RA, Dvorak HF and Dvorak AM (1981) Ultrastructural localization of non-specific esterase activity in guinea-pig and human monocytes, macrophages and lymphocytes. *Blood* **58:**1089–1099.
Moncada S, Higgs A and Furchgott R (1997) International Union of Pharmacology nomenclature in nitric oxide research. *Pharmacol Rev* **49:**137–142.
Monteiro RC, Hostoffer RW, Cooper MD, Bonner JR, Gartland GL and Kubagawa H (1993) Definition of immunoglobulin A receptors on eosinophils and their enhanced expression in allergic individuals. *J Clin Invest* **92:**1681–1685.
Moqbel R, Cromwell O and Kay AB (1989) The effect of nedocromil sodium on human eosinophil activation. *Drugs* **37 (Suppl 1):**19–22.
Moqbel R, Cromwell O, Walsh GM, Wardlaw AJ, Kurlak L and Kay AB (1988) Effects of nedocromil sodium (Tilade) on the activation of human eosinophils and neutrophils and the release of histamine from mast cells. *Allergy* **43:**268–276.
Moqbel R, Hamid Q, Ying S, Barkans J, Hartnell A, Tsicopoulos A, Wardlaw AJ and Kay AB (1991) Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. *J Exp Med* **174:**749–752.
Moqbel R, Macdonald AJ, Cromwell O and Kay AB (1990a) Release of leukotriene C_4 (LTC_4) from human eosinophils following adherence to IgE- and IgG-coated schistosomula of *Schistosoma mansoni*. *Immunology* **69:**435–442.
Moqbel R, Walsh GM, Macdonald AJ and Kay B (1986) Effect of disodium cromoglycate on activation of human eosinophils and neutrophils following reversed (anti-IgE) anaphylaxis. *Clin Allergy* **16:**73–83.
Moqbel R, Walsh GM, Nagakura T, Macdonald AJ, Wardlaw AJ, Iikura Y and Kay AB (1990b) The effect of platelet-activating factor on IgE binding to, and IgE-dependent biological properties of, human eosinophils. *Immunology* **70:**251–257.
Moqbel R, Ying S, Barkans J, Newman TM, Kimmitt P, Wakelin M, Taborda Barata L, Meng Q, Corrigan CJ, Durham SR and Kay AB (1995) Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. *J Immunol* **155:**4939–4947.
Morita E, Schroder JM and Christophers E (1989a) Differential sensitivities of purified human eosinophils and neutrophils to defined chemotaxins. *Scand J Immunol* **29:**709–716.
Morita E, Schroder JM and Christophers E (1989b) Chemotactic responsiveness of eosinophils isolated from patients with inflammatory skin diseases. *J Dermatol* **16:**348–351.
Morita E, Schroder JM and Christophers E (1990a) Identification of a novel and highly potent eosinophil chemotactic lipid in human eosinophils treated with arachidonic acid. *J Immunol* **144:**1893–1900.

Morita M, Ohshima Y, Akutagawa H, Uenoyama Y, Nambu M, Mayumi M and Mikawa H (1993) Inhibitory effects of azelastine hydrochloride on Ca^{2+} influx, actin polymerization and release of eosinophil cationic protein of an eosinophilic leukaemia cell line EoL-1. *Curr Med Res Opin* **13:**163–174.
Morita M, Tsurata S, Mori KJ, Mayumi M and Mikawa H (1990b) Ketotifen inhibits PAF-induced actin polymerization in a human eosinophilic leukaemic cell line, EoL-1. *Eur Respir J* **3:**1173–1178.
Mosimann SC, Newton DL, Youle RJ and James MN (1996) X-ray crystallographic structure of recombinant eosinophil-derived neurotoxin at 1.83 A resolution. *J Mol Biol* **260:**540–552.
Mostov KE, Kraehenbuhl JP and Blobel GA (1980) Receptor-mediated transcellular transport of immunoglobulin: synthesis of secretory component as multiple and larger transmembrane forms. *Proc Natl Acad Sci USA* **77:**7257–7261.
Motegi Y and Kita H (1998) Interaction with secretory component stimulates effector functions of human eosinophils but not of neutrophils. *J Immunol* **161:**4340–4346.
Mousli M, Bueb J, Bronner C, Rouot B and Landry Y (1990) G-Protein activation: A receptor independent mode of action of cationic amphiphilic neuropeptides and venom peptides. *Trends Pharmacol Sci* **11:**358–362.
Mudde GC, Bheekha R and Bruijnzeel Koomen CA (1995) IgE-mediated antigen presentation. *Allergy* **50:**193–199.
Muller M and Renkawitz R (1991) The glucocorticoid receptor. *Biochim Biophys Acta* **1088:**177–182.
Mullol J, Lopez E, Roca-Ferrer J, Xaubet A, Pujols L, Fernandez-Morata JC, Fabra JM and Picado C (1997) Effects of topical anti-inflammatory drugs on eosinophil survival primed by epithelial cells. Additive effect of glucocorticoids on nedocromil sodium. *Clin Exp Allergy* **27:**1432–1441.
Mullol J, Xaubet A, Lopez E, Roca Ferrer J and Picado C (1995) Comparative study of the effects of different glucocorticosteroids on eosinophil survival primed by cultured epithelial cell supernatants obtained from nasal mucosa and nasal polyps. *Thorax* **50:**270–274.
Munoz NM, Douglas I, Mayer D, Herrnreiter A, Zhu XD and Leff AR (1997a) Eosinophil chemotaxis inhibited by 5-lipoxygenase blockade and leukotriene receptor antagonism. *Am J Respir Crit Care Med* **155:**1398–1403.
Munoz NM, Rabe KF, Neeley SP, Herrnreiter A, Zhu XD, McAllister K, Mayer D, Magnussen H, Galens S and Leff AR (1996) Eosinophil VLA-4 binding to fibronectin augments bronchial narrowing through 5-lipoxygenase activation. *Am J Physiol* **14:**L587–L594.
Munoz NM, Rabe KF, Vita AJ, McAllister K, Mayer D, Weiss M and Leff AR (1995) Paradoxical blockade of beta adrenergically mediated inhibition of stimulated eosinophil secretion by salmeterol. *J Pharmacol Exp Ther* **273:**850–854.
Munoz NM, Vita AJ, Neeley SP, McAllister K, Spaethe SM, White SR and Leff AR (1994) Beta adrenergic modulation of formyl-methionine-leucine-phenylalanine-stimulated secretion of eosinophil peroxidase and leukotriene C_4. *J Pharmacol Exp Ther* **268:**139–143.
Munoz NM, Zhu X, Rubio N, Douglas I and Leff AR (1997b) Localization and functional role of 85 kDa type IV and 14 kDa type II PLA_2 on LTC_4 generation by stimulated eosinophils (Abstract). *Am J Respir Crit Care Med* **155:**A837.
Murakami Y, Yamamoto T, Imanichi T and Nagasawa S (1993) Cellular responses of guinea-pig macrophages to C4a: Inhibition of C3a-induced O_2^- genertation by C4a. *Immunol Lett* **36:**301–304.
Murali PS, Kurup VP, Guo J and Fink JN (1993) Development of bone marrow eosinophilia in mice induced by *Aspergillus fumigatus* antigens. *Clin Immunol Immunopathol* **84:**216–220.
Murata Y, Takaki S, Migita M, Kikuchi Y, Tominaga A and Takatsu K (1992) Molecular cloning and expression of the human interleukin 5 receptor. *J Exp Med* **175:**341–351.
Muro S, Soussi-Gournni A, Nutku E, Hazcku A, Holroyd K, Nicolaides N, Levitt R and Hamid Q (1999) IL-9 causes up-regulation of the membrane bound IL-5 receptor on human eosinophils (Abstract). *Am J Respir Crit Care Med* **159:**A93.
Murphy PM (1994) The molecular biology of leukocyte chemoattractant receptors. *Annu Rev Immunol* **12:**593–633.
Murray JJ, Hagermara DD, Dworski R and Steller JR (1995) Effect of salmeterol and beclomethasone on the late phase response to segmental antigen challenge in man, in *Acute and Chronic Inflammation in the Respiratory Tract* (Johnson M ed) p 64, Colwood House, London.
Murray JJ, Tunnel AB, Brash AR, Roberts LJ, Gosset P, Workman MD, Capron A and Oates JA (1986) Release of prostaglandin D_2 in to human airways during acute antigen challenge. *N Engl J Med* **315:**800–804.
Muto A, Watanabe S, Miyajima A, Yokota T and Arai K (1996) The β subunit of human granulocyte/macrophage colony-stimulating factor receptor forms a homodimer and is activated via association with the α subunit. *J Exp Med* **183:**1911–1916.
Nabe M, Miyagawa H, Agrawal DK, Sugiyama H and Townley RG (1991) The effect of ketotifen on eosinophils as measured at LTC_4 release and by chemotaxis. *Allergy Proc* **12:**267–271.
Nadel JA, Conrad DJ, Ueki IF, Schuster A and Sigal E (1991) Immunocytochemical localization of arachidonate 15-lipoxygenase in erythrocytes, leukocytes, and airway cells. *J Clin Invest* **87:**1139–1145.
Nagai H, Yamaguchi S, Inagaki N, Tsuruoka N, Hitoshi Y and Takatsu K (1993) Effect of anti-IL-5 monoclonal antibody on allergic bronchial eosinophilia and airway hyperresponsiveness in mice. *Life Sci* **53:**PL243-PL247.
Nagai H, Yamaguchi S, Maeda Y and Tanaka H (1996) Role of mast cells, eosinophils and IL-5 in the development of airway hyperresponsiveness in sensitized mice. *Clin Exp Allergy* **26:**642–647.
Nagata M, Fukuda T, Numao T, Makino S and Dohi Y (1993) Tumor necrosis factor α induces migration of human eosinophils. *Arerugi* **42:**549–555.
Nagata M, Sedgwick JB, Bates ME, Kita H and Busse WW (1995a) Eosinophil adhesion to vascular cell adhesion molecule-1 activates superoxide anion generation. *J Immunol* **155:**2194–2202.
Nagata M, Sedgwick JB and Busse WW (1995b) Differential effects of granulocyte/macrophage colony-stimulating factor on eosinophil and neutrophil superoxide anion generation. *J Immunol* **155:**4948–4954.
Nagy L, Lee TH, Goetzl EJ, Pickett WC and Kay AB (1982) Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. *Clin Exp Immunol* **47:**541–547.
Nakagawa N, Obata T, Kobayashi T, Okada Y, Nambu F, Terawaki T, Furuya T, Muryobayashi K, Sawada M and Aishita H (1993) Effect of a peptide leukotriene receptor antagonist, ONO-1078, on guinea-pig models of asthma. *Eur J Pharmacol* **235:**211–219.
Nakahata T, Gross AJ and Ogawa M (1982) A stochastic model of self-renewal and commitment to differentiation of the primitive hemopoietic stem cells in culture. *J Cell Physiol* **113:**455–458.
Nakahata T and Ogawa M (1982) Identification in culture of a class of hemopoietic colony-forming units with extensive capability to self-renew and generate multipotential hemopoietic colonies. *Proc Natl Acad Sci USA* **79:**3843–3847.
Nakajima H, Gleich GJ and Kita H (1996) Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. *J Immunol* **156:**4859–4866.
Nakajima H, Sano H, Nishimura T, Yoshida S and Iwamoto I (1994) Role of vascular cell adhesion molecule 1/very late activation antigen 4 and intercellular adhesion molecule 1/lymphocyte function-associated antigen 1 interactions in antigen-induced eosinophil and T cell recruitment into the tissue. *J Exp Med* **179:**1145–1154.
Nakajima T, Yamada H, Iikura M, Miyamasu M, Izumi S, Shida H, Ohta K, Imai T, Yoshie O, Mochizuki M, Schroder JM, Morita Y, Yamamoto K and Hirai K (1998) Intracellular localization and release of eotaxin from normal eosinophils. *FEBS Lett* **434:**226–230.
Nakamura M, Honda Z, Izumi T, Sakanaka C, Mutoh H, Minami M, Bito H, Seyama Y, Matsumoto T, Noma M and Shimizu T (1991) Molecular cloning and expression of platelet-activating factor receptor from human leukocytes. *J Biol Chem* **266:**20400–20405.
Nakamura M, Ueda N, Kishimoto K, Yoshimoto T, Yamamoto S and Ishimura K (1995) Immunocytochemical localization of platelet-type arachidonate 12-lipoxygenase in mouse blood cells. *J Histochem Cytochem* **43:**237–244.
Namovic MT, Walsh RE, Goodfellow C, Harris RR, Carter GW and Bell RL (1996) Pharmacological modulation of eosinophil influx into the lungs of Brown Norway rats. *Eur J Pharmacol* **315:**81–88.
Neeley SP, Hamann KJ, Dowling TL, McAllister KT, White SR and Leff AR (1994) Augmentation of stimulated eosinophil degranulation by VLA-4 (CD49d)-mediated adhesion to fibronectin. *Am J Respir Cell Mol Biol* **11:**206–213.
Neeley SP, Hamann KJ, White SR, Baranowski SL, Burch RA and Leff AR (1993) Selective regulation of expression of surface adhesion molecules Mac-1, L-selectin, and VLA-4 on human eosinophils and neutrophils. *Am J Respir Cell Mol Biol* **8:**633–639.
Nepomuceno RR, Henschen-Edman AH, Burgess WH and Tenner AJ (1997) cDNA cloning and primary structure analysis of C1q-R(P), the human C1q/MBL/SPA receptor that mediates enhanced phagocytosis *in vitro*. *Immunity* **6:**119–129.
Newman TM, Tian M and Gomperts BD (1996) Ultrastructural characterization of tannic acid-arrested degranulation of permeabilized guinea pig eosinophils stimulated with GTPγS. *Eur J Cell Biol* **70:**209–220.
Newsholme SJ and Schwartz L (1993) cAMP-specific phosphodiesterase inhibitor, rolipram, reduces eosinophil infiltration evoked by leukotrienes or by histamine in guinea pig conjunctiva. *Inflammation* **17:**25–31.
Newton DL, Nicholls PJ, Rybak SM and Youle RJ (1994) Expression and characterization of recombinant human eosinophil-derived neurotoxin and eosinophil-derived neurotoxin-anti-transferrin receptor sFv. *J Biol Chem* **269:**26739–26745.
Ng CF, Sun FF, Taylor BM, Wolin MS and Wong PY (1991) Functional properties of guinea pig eosinophil leukotriene B_4 receptor. *J Immunol* **147:**3096–3103.
Nichol GM, Alton EWFW, Nix A, Geddes DM, Chung KF and Barnes PJ (1990) Effect of inhaled furosemide on metabisulfite- and methacholine-induced bronchoconstriction and on nasal potential difference in asthmatic subjects. *Am Rev Respir Dis* **142:**576–580.
Nicholson CD, Shahid M, Bruin J, Barron E, Spiers I, de Boer J, van Amsterdam RG, Zaagsma J, Kelly JJ, Dent G, Giembycz MA and Barnes PJ (1995) Characterization of ORG 20241, a combined phosphodiesterase IV/III cyclic nucleotide phosphodiesterase inhibitor for asthma. *J Pharmacol Exp Ther* **274:**678–687.
Nielson CP and Hadjokas NE (1998) Beta-adrenoceptor agonists block corticosteroid inhibition in eosinophils. *Am J Respir Crit Care Med* **157:**184–191.
Nishikawa K, Morii T, Ako H, Hamada K, Saito S and Narita N (1992) *In vivo* expression of CD69 on lung eosinophils in eosinophilic pneumonia: CD69 as a possible activation marker for eosinophils. *J Allergy Clin Immunol* **90:**169–174.
Nittoh T, Fujimori H, Kozumi Y, Ishihara K, Mue S and Ohuchi K (1998) Effects of glucocorticoids on apoptosis of infiltrated eosinophils and neutrophils in rats. *Eur J Pharmacol* **354:**73–81.
Nittoh T, Hirakata M, Mue S and Ohuchi K (1997) Identification of cDNA encoding rat eosinophil cationic protein/eosinophil-associated ribonuclease. *Biochim Biophys Acta* **1351:**42–46.
Nittoh T, Watanabe M, Okayama H, Misawa S, Isobe Y, Hayashi H, Mue S and Ohuchi K (1995) Cloning of a cDNA for rat eosinophil major basic protein. *Biochim Biophys Acta* **1264:**261–264.
Nonaka M, Nonaka R, Woolley K, Adelroth E, Miura K, Okhawara Y, Glibetic M, Nakano K, O'Byrne P, Dolovich J and Jordana M (1995) Distinct immunohistochemical localization of IL-4 in human inflamed airway tissues. IL-4 is localized to eosinophils *in vivo* and is released by peripheral blood eosinophils. *J Immunol* **155:**3234–3244.
Noso N, Proost P, Van Damme J and Schroder JM (1994) Human monocyte chemotactic proteins-2 and 3 (MCP-2 and MCP-3) attract human eosinophils and desensitize the chemotactic responses towards RANTES. *Biochem Biophys Res Commun* **200:**1470–1476.
Numao T and Agrawal DK (1992) Neuropeptides modulate human eosinophil chemotaxis. *J Immunol* **149:**3309–3315.

Nunez G, London L, Hockenbery D, Alexander M, McKearn JP and Korsmeyer SJ (1990) Deregulated Bcl-2 gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. *J Immunol* **144:**3602–3610.
Nusse O, Lindau M, Cromwell O, Kay AB and Gomperts BD (1990) Intracellular application of guanosine-5′-O-(3-thiotriphosphate) induces exocytotic granule fusion in guinea pig eosinophils. *J Exp Med* **171:**775–786.
Nutman TB, Ottesen EA and Cohen SG (1989a) The eosinophil, eosinophilia, and eosinophil-related disorders. IV. Eosinophil related disorders (continued). *Allergy Proc* **10:**47–62.
Nutman TB, Ottesen EA and Cohen SG (1989b) The eosinophil, eosinophilia, and eosinophil-related disorders. III. Clinical assessments and eosinophil related disorders. *Allergy Proc* **10:**33–46.
Oberhammer FA, Hochegger K, Froschl G, Tiefenbacher R and Pavelka M (1994) Chromatin condensation during apoptosis is accompanied by degradation of lamin A + B, without enhanced activation of cdc2 kinase. *J Cell Biol* **82:**349–352.
Obernolte R, Bhakta S, Alvarez R, Bach C, Zuppan P, Mulkins M, Jarnagin K and Shelton ER (1993) The cDNA of a human lymphocyte cyclic-AMP phosphodiesterase (PDE IV) reveals a multigene family. *Gene* **129:**239–247.
Ochiai K, Iwamoto I, Takahashi H, Yoshida S and Tomioka H (1995) Effect of IL-4 and interferon-γ (IFN-γ) on IL-3- and IL-5-induced eosinophil differentiation from human cord blood mononuclear cells. *Clin Exp Immunol* **99:**124–128.
Ochiai K, Kagami M, Matsumura R and Tomioka H (1997) IL-5 but not interferon-γ (IFN-γ) inhibits eosinophil apoptosis by up-regulation of bcl-2 expression. *Clin Exp Immunol* **107:**198–204.
O'Connor BJ, Chung KF, Chen-Worsdell YM, Fuller RW and Barnes PJ (1991) Effect of inhaled furosemide and bumetanide on adenosine 5′-monophosphate- and sodium metabisulfite-induced bronchoconstriction. *Am Rev Respir Dis* **143:**1329–1333.
O'Connor BJ, Ridge SM, Barnes PJ and Fuller RW (1992) Greater effect of inhaled budesonide on adenosine 5′-monophosphate-induced than on sodium-metabisulfite-induced bronchoconstriction in asthma. *Am Rev Respir Dis* **146:**560–564.
Oehm A, Behrmann I, Falk W, Pawlita M, Maier G, Klas C, Li-Weber M, Richards S, Dhein J, Trauth BC, Ponstingl H and Krammer PH (1992) Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily. Sequence identity with the Fas antigen. *J Biol Chem* **267:**10709–10715.
O'Flaherty JT, Kuroki M, Nixon AB, Wijkander J, Yee E, Lee SL, Smitherman PK, Wykle RL and Daniel LW (1996a) 5-Oxo-eicosanoids and hematopoietic cytokines cooperate in stimulating neutrophil function and the mitogen-activated protein kinase pathway. *J Biol Chem* **271:**17821–17828.
O'Flaherty JT, Kuroki M, Nixon AB, Wijkander J, Yee E, Lee SL, Smitherman PK, Wykle RL and Daniel LW (1996b) 5-Oxo-eicosatetraenoate is a broadly active, eosinophil-selective stimulus for human granulocytes. *J Immunol* **157:**336–342.
Ogawa M (1993) Differentiation and proliferation of hematopoietic stem cells. *Blood* **81:**2844–2853.
Ogawa M (1994) Hematopoiesis. *J Allergy Clin Immunol* **94:**645–650.
Ogawa H, Kunkel SL, Fantone JC and Ward PA (1981a) Digestion of the fifth component of complement by eosinophil lysosomal enzymes. Production of eosinophil-specific chemotactic activity. *Virchows Arch B Cell Pathol Incl Mol Pathol* **38:**149–157.
Ogawa H, Kunkel SL, Fantone JC and Ward PA (1981b) Comparative study of eosinophil and neutrophil chemotaxis and enzyme release. *Am J Pathol* **105:**149–155.
Ogawa M, Matsuzaki Y, Nishikawa S, Hayashi S, Kunisada T, Sudo T, Kina T and Nakauchi H (1991) Expression and function of *c-kit* in hemopoietic progenitor cells. *J Exp Med* **174:**63–71.
Ogushi F, Ozaki T, Kawano T and Yasuoka S (1987) PGE_2 and $PGF_{2\alpha}$ content in bronchoalveolar lavage fluid obtained from patients with eosinophilic pneumonia. *Chest* **91:**204–206.
Ohashi Y, Motojima S, Fukuda T and Makino S (1992) Airways hyperresponsiveness, increased intracellular spaces of bronchial epithelium, and increased infiltration of eosinophils and lymphocytes in bronchial mucosa in asthma. *Am Rev Respir Dis* **145:**1469–1476.
Ohkawara Y, Lim KG, Xing Z, Glibetic M, Nakano K, Dolovich J, Croitoru K, Weller PF and Jordana M (1996) CD40 expression by human peripheral blood eosinophils. *J Clin Invest* **97:**1761–1766.
Ohkawara Y, Yamauchi K, Maruyama N, Hoshi H, Ohno I, Honma M, Tanno Y, Tamura G, Shirato K and Ohtani H (1995) *In situ* expression of the cell adhesion molecules in bronchial tissues from asthmatics with air flow limitation: *In vivo* evidence of VCAM-1/VLA-4 interaction in selective eosinophil infiltration. *Am J Respir Cell Mol Biol* **12:**4–12.
Ohman JL, Lawrence M and Lowell FC (1972) Effect of propranolol on the eosinopenic responses to cortisol, isoproterenol and aminophylline. *J Allergy Clin Immunol* **50:**151–156.
Ohnishi T, Kita H, Mayeno AN, Okada S, Sur S, Broide DH and Gleich GJ (1996) Lidocaine in bronchoalveolar lavage fluid (BALF) is an inhibitor of eosinophil-active cytokines. *Clin Exp Immunol* **104:**325–331.
Ohno I, Lea R, Finotto S, Marshall J, Denburg J, Dolovich J, Gauldie J and Jordana M (1991) Granulocyte/macrophage colony-stimulating factor (GM-CSF) gene expression by eosinophils in nasal polyposis. *Am J Respir Cell Mol Biol* **5:**505–510.
Ohno I, Lea RG, Flanders KC, Clark DA, Banwatt D, Dolovich J, Denburg J, Harley CB, Gauldie J and Jordana M (1992) Eosinophils in chronically inflamed human upper airway tissues express transforming growth factor β_1 gene (TGF-β_1). *J Clin Invest* **89:**1662–1668.
Ohno I, Nitta Y, Suzuki J, Hoshi H, Horima M, Isoyama S, Tanno Y, Tamura G, Yamanchi K, Nagura H and Shirato K (1997) Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* **16:**212–219.
Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma M, Woolley K, O'Byrne P, Dolovich J, Jordana M, Tamura G, Tanno Y and Shirato K (1995) Eosinophils as a potential source of platelet-derived growth factor B-chain (PDGF-B) in nasal polyposis and bronchial asthma. *Am J Respir Cell Mol Biol* **13:**639–647.
Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma M, Woolley K, O'Byrne P, Tamura G, Jordana M and Shirato K (1996) Transforming growth factor β_1 (TGF-β_1) gene expression by eosinophils in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* **15:**404–409.
Ohta K, Sawamoto S, Nakajima M, Kubota S, Tanaka Y, Miyasaka T, Nagai A, Hirai K, Mano K and Miyashita H (1996) The prolonged survival of human eosinophils with interleukin-5 and its inhibition by theophylline via apoptosis. *Clin Exp Allergy* **26:**10–15.
Ojima Uchiyama A, Masuzawa Y, Sugiura T, Waku K, Fukuda T and Makino S (1991) Production of platelet-activating factor by human normodense and hypodense eosinophils. *Lipids* **26:**1200–1203.
Okada C, Eda R, Miyagawa H, Sugiyama H, Hopp RJ, Bewtra AK and Townley RG (1994) Effect of cetirizine on human eosinophil superoxide generation, eosinophil chemotaxis and eosinophil peroxidase *in vitro*. *Int Arch Allergy Immunol* **103:**384–390.
Okada K, Fujimoto K, Kubo K, Sekiguchi M and Sugane K (1996) Eosinophil chemotactic activity in bronchoalveolar lavage fluid obtained from *Toxocara canis*-infected rats. *Clin Immunol Immunopathol* **78:**256–262.
Okada S, Hagan JB, Kato M, Bankers-Filbright JL, Hunt LW, Gleich GJ and Kita H (1998) Lidocaine and its analogues inhibit IL-5-mediated survival and activation of human eosinophils. *J Immunol* **160:**4010–4017.
Okada S, Inoue H, Yamauchi K, Iijima H, Ohkawara Y, Takishima T and Shirato K (1995) Potential role of interleukin-1 in allergen-induced late asthmatic reactions in guinea pigs: suppressive effect of interleukin-1 receptor antagonist on late asthmatic reaction. *J Allergy Clin Immunol* **95:**1236–1245.
Okada S, Kita H, George TJ, Gleich GJ and Leiferman KM (1997) Transmigration of eosinophils through basement membrane components *in vitro*- synergistic effects of platelet-activating factor and eosinophil-active cytokines. *Am J Respir Cell Mol Biol* **16:**455–463.
Okuda M, Takenaka T, Kawabori S and Ogami Y (1981) Ultrastructural study of the specific granule of the human eosinophil. *J Submicrosc Cytol* **13:**465–471.
Oliveira SH, Fonseca SG, Romao PR, Figueiredo F, Ferreira SH and Cunha FQ (1998) Microbicidal activity of eosinophils is associated with activation of the arginine-NO pathway. *Parasite Immunol* **20:**405–412.
Olsen RL and Little C (1983) Purification and some properties of myeloperoxidase and eosinophil peroxidase from human blood. *Biochem J* **209:**781–787.
Olsen RL, Syse K, Little C and Christensen TB (1985) Further characterization of human eosinophil peroxidase. *Biochem J* **229:**779–784.
Olsson I, Egesten A, Gullberg U, Lantz M, Stromberg K and Winqvist I (1986) The biosynthesis of neutrophil and eosinophil granule proteins. *Folia Histochem Cytobiol* **24:**89–97.
Olsson I, Persson AM, Stromberg K, Winqvist I, Tai PC and Spry CJ (1985) Purification of eosinophil peroxidase and studies of biosynthesis and processing in human marrow cells. *Blood* **66:**1143–1148.
Oltvai ZN, Milliman CL and Korsmeyer SJ (1993) Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* **74:**609–619.
Onorato JJ, Kiley L, Gillis ME, Bates ME and Busse W (1995) Identification of the β-adrenergic receptor kinase in human peripheral eosinophils (Abstract). *Am J Respir Crit Care Med* **151:**A238.
O'Prey J and Harrison PR (1995) Tissue-specific regulation of the rabbit 15-lipoxygenase gene in erythroid cells by a transcriptional silencer. *Nucleic Acids Res* **23:**3664–3672.
Ortiz JL, Valles JM, Marticabrera M, Cortijo J and Morcillo EJ (1996) Effects of selective phosphodiesterase inhibitors on platelet-activating factor- and antigen-induced airway hyperreactivity, eosinophil accumulation, and microvascular leakage in guinea pigs. *Naunyn-Schmiedeberg's Arch Pharmacol* **353:**200–206.
Osborn L, Vassallo C and Benjamin CD (1992) Activated endothelium binds lymphocytes through a novel binding site in the alternately spliced domain of vascular cell adhesion molecule-1. *J Exp Med* **176:**99–107.
O'Sullivan AJ, Brown AM, Freeman HN and Gomperts BD (1996) Purification and identification of FOAD-II, a cytosolic protein that regulates secretion in streptolysin-O-permeabilized mast cells, as a rac/rhoGDI complex. *Mol Cell Biol* **7:**397–408.
Ottesen EA, Stanley AM, Gelfand JA, Gadek JE, Frank MM, Nash TE and Cheever AW (1977) Immunoglobulin and complement receptors on human eosinophils and their role in cellular adherence to schistosomules. *Am J Trop Med Hyg* **26:**134–141.
Owen WF (1993) Eosinophil heterogeneity, in *Immunopharmacology of Eosinophils* (Smith H and Cook RM eds) pp 57–72, Academic Press, London.
Owen WF, Petersen J and Austen KF (1991) Eosinophils altered phenotypically and primed by culture with granulocyte/macrophage colony-stimulating factor and 3T3 fibroblasts generate leukotriene C_4 in response to fMLP. *J Clin Invest* **87:**1958–1963.
Owen WF, Petersen J, Sheff DM, Folkerth RD, Anderson RJ, Corson JM, Sheffer AL and Austen KF (1990) Hypodense eosinophils and interleukin 5 activity in the blood of patients with the eosinophilia-myalgia syndrome. *Proc Natl Acad Sci USA* **87:**8647–8651.
Owen WF, Soberman RJ, Yoshimoto T, Sheffer AL, Lewis RA and Austen KF (1987) Synthesis and release of leukotriene C_4 by human eosinophils. *J Immunol* **138:**532–538.
Page CP (1988) The role of platelet activating factor in asthma. *J Allergy Clin Immunol* **81:**144–152.
Palczynski C, Gorski P and Grzegorczyk J (1989) Effect of disodium cromoglycate on deuterium oxide-induced degranulation of human eosinophils in allergic asthmatic patients *in vitro*. *Arch Immunol Ther Exp Warsz* **37:**157–160.
Palframan RT, Collins PD, Severs NJ, Rothery S, Williams TJ and Rankin SM (1998a) Mechanisms of acute eosinophil mobilization from the bone marrow stim-

ulated by interleukin 5: The role of specific adhesion molecules and phosphatidylinositol 3-kinase. *J Exp Med* **188:**1621–1632.
Palframan RT, Collins PD, Williams TJ and Rankin SM (1998b) Eotaxin induces a rapid release of eosinophils and their progenitors from the bone marrow. *Blood* **91:**2240–2248.
Palmblad J, Gyllenhammar H, Lindgren JA and Malmsten CL (1984) Effects of leukotrienes and f-Met-Leu-Phe on oxidative metabolism of neutrophils and eosinophils. *J Immunol* **132:**3041–3045.
Palmqvist M, Balder B, Lowhagen O, Melander B, Svedmyr N and Wahlander L (1992) Late asthmatic reaction decreased after pre-treatment with salbutamol and formoterol, a new long-acting β_2-agonist. *J Allergy Clin Immunol* **89:**844–849.
Pardi R, Zocchi M, Ferrero E, Ciboddo GF, Inverardi L and Rugarli C (1984) *In vivo* effects of a single infusion of theophylline on human peripheral blood lymphocytes. *Clin Exp Immunol* **57:**722–728.
Park JW and Ahn SM (1995) Translocation of recombinant $p47^{phox}$ cytosolic component of the phagocyte oxidase by *in vitro* phosphorylation. *Biochem Biophys Res Commun* **211:**410–416.
Park JW, Benna JE, Scott KE, Christensen BL, Chanock SJ and Babior BM (1994) Isolation of a complex of respiratory burst oxidase components from resting neutrophil cytosol. *Biochemistry* **33:**2907–2911.
Park JW, Ma M, Ruedi JM, Smith RM and Babior BM (1992) The cytosolic components of the respiratory burst oxidase exist as a M(r) approximately 240,000 complex that acquires a membrane-binding site during activation of the oxidase in a cell-free system. *J Biol Chem* **267:**17327–17332.
Parker SD, Brown RH and Hirshman CA (1991) Differential effect of glucocorticoids on pulmonary responses and eosinophils. *Respir Physiol* **83:**323–331.
Parmley RT and Spicer SS (1974) Cytochemical and ultrastructural identification of a small type granule in human late eosinophils. *Lab Invest* **30:**557–567.
Parsons WG and Roberts LJ (1988) Transformation of prostaglandin D_2 to isomeric prostaglandin F_2 compounds by human eosinophils. A potential mast cell-eosinophil interaction. *J Immunol* **141:**2413–2419.
Parwaresch MR, Walle AJ and Arndt D (1976) The peripheral kinetics of human radiolabelled eosinophils. *Virchows Arch B Cell Pathol* **1:**57–66.
Pasquale CP, de Silva PM, Lima MC, Diaz BL, Rihoux JP, Vargaftig BB, Cordeiro RS and Martins MA (1992) Suppression by cetirizine of pleurisy triggered by antigen in actively sensitized rats. *Eur J Pharmacol* **223:**9–14.
Pasquale CP, Martins MA, Bozza PT, de Silva PM, Faria Neto HC, Pires AL and Cordeiro RS (1991) Bradykinin induces eosinophil accumulation in the rat pleural cavity. *Int Arch Allergy Appl Immunol* **95:**244–247.
Patel KD and McEver RP (1997) Comparison of tethering and rolling of eosinophils and neutrophils through selectins and P-selectin glycoprotein ligand-1. *J Immunol* **159:**4555–4565.
Patry C, Sibille Y, Lehuen A and Monteiro RC (1996) Identification of Fcα receptor (CD89) isoforms generated by alternate splicing that are differentially expressed between blood monocytes and alveolar macrophages. *J Immunol* **156:**4442–4448.
Pauwels RA, Brusselle G and Kips JC (1997) Cytokine manipulation in animal models of asthma. *Am J Respir Crit Care Med* **156:**S78–S81.
Pazdrak K, Adachi T and Alam R (1997) Src homology 2 protein tyrosine phosphatase (SHPTP2)/src homology 2 phosphatase 2 (SHP2) tyrosine phosphatase is a positive regulator of the interleukin 5 receptor signal transduction pathways leading to the prolongation of eosinophil survival. *J Exp Med* **186:**561–568.
Pazdrak K, Justement L and Alam R (1995a) Mechanism of inhibition of eosinophil activation by transforming growth factor-β. Inhibition of Lyn, MAP, Jak2 kinases and STAT1 nuclear factor. *J Immunol* **155:**4454–4458.
Pazdrak K, Olszewska-Pazdrak B, Stafford S, Garofalo RP and Alam R (1998) Lyn, jak2, and raf-1 kinases are critical for the antiapoptotic effect of interleukin 5, whereas only raf-1 kinase is essential for eosinophil activation and degranulation. *J Exp Med* **188:**421–429.
Pazdrak K, Schreiber D, Forsythe P, Justement L and Alam R (1995b) The intracellular signal transduction mechanism of interleukin 5 in eosinophils: The involvement of lyn tyrosine kinase and the Ras-Raf-1-MEK-microtubule-associated protein kinase pathway. *J Exp Med* **181:**1827–1834.
Pazdrak K, Stafford S and Alam R (1995c) The activation of the Jak-STAT 1 signaling pathway by IL-5 in eosinophils. *J Immunol* **155:**397–402.
Pearlman E, Lass JH, Bardenstein DS, Diaconu E, Hazlett FE, Albright J, Higgins AW and Kazura JW (1997) IL-12 exacerbates helmith-mediated corneal pathology by augmenting inflammatory cell recruitment and chemokine expression. *J Immunol* **158:**827–833.
Pedersen B, Dahl R, Karlstrom R, Peterson CG and Venge P (1996) Eosinophil and neutrophil activity in asthma in a one-year trial with inhaled budesonide. The impact of smoking. *Am J Respir Crit Care Med* **153:**1519–1529.
Pedersen B, Dahl R, Larsen BB and Venge P (1993) The effect of salmeterol on the early- and late-phase reaction to bronchial allergen and postchallenge variation in bronchial reactivity, blood eosinophils, serum eosinophil cationic protein, and serum eosinophil protein. X. *Allergy* **48:**377–382.
Peerschke EI, Reid KB and Ghebrehiwet B (1994) Identification of a novel 33-kDa C1q-binding site on human blood platelets. *J Immunol* **152:**5896–5901.
Peled A, Gonzalo JA, Lloyd C and Gutierrez-Ramos JC (1998) The chemotactic cytokine eotaxin acts as a granulocyte/macrophage colony-stimulating factor during lung inflammation. *Blood* **91:**1909–1916.
Pemrick SM, Lucas DA and Grippo JF (1994) The retinoid receptors. *Leukemia* **8** (**Suppl 3):**S1–S10.
Penrose JF, Spector J, Lam BK, Friend DS, Xu K, Jack RM and Austen KF (1995) Purification of human lung leukotriene C_4 synthase and preparation of a polyclonal antibody. *Am J Respir Crit Care Med* **152:**283–289.
Perkins RS (1993) Signal transduction pathways involved in activation of the respiratory burst oxidase by leukotriene B_4 in guinea-pig eosinophils. Ph.D. Thesis. University of London, London.
Perkins RS, Dent G, Chung KF and Barnes PJ (1992) The effect of anion transport inhibitors and extracellular Cl^- concentration on eosinophil respiratory burst activity. *Biochem Pharmacol* **43:**2480–2483.
Perkins RS, Lindsay MA, Barnes PJ and Giembycz MA (1995) Early signalling events implicated in leukotriene B_4-induced activation of the NADPH oxidase in eosinophils: Role of Ca^{2+}, protein kinase C and phospholipases C and D. *Biochem J* **310:**795–806.
Perruchoud AP, Yerger L and Abraham WM (1984) Differential effects of aminophylline on the early and late antigen-induced bronchial obstruction in allergic sheep. *Respiration* **46:**44–52.
Persson CG and Erjefalt JS (1997a) Eosinophil lysis and free granules: an *in vivo* paradigm for cell activation and drug development. *Trends Pharmacol Sci* **18:**117–123.
Persson CG and Erjefalt JS (1997b) "Ultimate activation" of eosinophils *in vivo*: Lysis and release of clusters of free eosinophil granules (Cfegs). *Thorax* **52:**569–574.
Petering H, Hochstetter R, Kimmig D, Smolarski R, Kapp A and Elsner J (1998) Detection of MCP-4 in dermal fibroblasts and its activation of the respiratory burst in human eosinophils. *J Immunol* **160:**555–558.
Peters MJ, Adcock IM, Brown CA and Barnes PJ (1995) β-Adrenoceptor agonists interfere with glucocorticoid receptor DNA binding in rat lung. *Eur J Pharmacol* **289:**275–281.
Peters MJ, Mak JCW, Virdee H, Adcock IM, Barnes PJ and Chung KF (1993) Demonstration of β_2-receptor (β_2-R) and β_2-R mRNA in human blood eosinophils (Abstract). *Am Rev Respir Dis* **147:**A825.
Peters MS, Gleich GJ, Dunnette SL and Fukuda T (1988) Ultrastructural study of eosinophils from patients with the hypereosinophilic syndrome: A morphological basis of hypodense eosinophils. *Blood* **71:**780–785.
Peters MS, Rodriguez M and Gleich GJ (1986) Localization of human eosinophil granule major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin by immunoelectron microscopy. *Lab Invest* **54:**656–662.
Peterson AP, Altman LC, Hill JS, Gosney K and Kadin ME (1981) Glucocorticoid receptors in normal human eosinophils: Comparison with neutrophils. *J Allergy Clin Immunol* **68:**212–217.
Peterson CG, Jornvall H and Venge P (1988) Purification and characterization of eosinophil cationic protein from normal human eosinophils. *Eur J Haematol* **40:**415–423.
Peterson CG and Venge P (1983) Purification and characterization of a new cationic protein-eosinophil protein-X (EPX)-from granules of human eosinophils. *Immunology* **50:**19–26.
Petreccia DC, Nauseef WM and Clark RA (1987) Respiratory burst of normal human eosinophils. *J Leukoc Biol* **41:**283–288.
Pettipher ER, Salter ED and Showell HJ (1994) Effect of *in vivo* desensitization to leukotriene B_4 on eosinophil infiltration in response to C5a in guinea-pig skin. *Br J Pharmacol* **113:**117–120.
Pfahl M (1993) Signal transduction by retinoid receptors. *Skin Pharmacol* **6** (**Suppl 1):**8–16.
Piacentini GL, Mazzi P, Vinco S, Peroni DG, Ferro I, Vicentini L and Boner AL (1996) The effect of cetirizine on the integrin-dependent respiratory burst of normodense eosinophils. *J Asthma* **33:**417–423.
Pieroni JP, Jacobowitz O, Chen J and Iyengar R (1993) Signal recognition and integration by Gs-stimulated adenylyl cyclases. *Curr Opin Neurobiol* **3:**345–351.
Pincus SH, DiNapoli AM and Schooley WR (1982) Superoxide production by eosinophils: Activation by histamine. *J Invest Dermatol* **79:**53–57.
Pincus SH, Schooley WR, DiNapoli AM and Broder S (1981) Metabolic heterogeneity of eosinophils from normal and hypereosinophilic patients. *Blood* **58:**1175–1181.
Pincus SH, Whitcomb EA and Dinarello CA (1986) Interaction of IL 1 and TPA in modulation of eosinophil function. *J Immunol* **137:**3509–3514.
Pinto A, Aldinucci D, Gloghini A, Zagonel V, Degan M, Improta S, Juzbasic S, Todesco M, Perin V, Gattei V, Herrmann F, Gruss HJ and Carbone A (1996) Human eosinophils express functional CD30 ligand and stimulate proliferation of a Hodgkin's disease cell line. *Blood* **88:**3299–3305.
Pinto A, Aldinucci D, Gloghini A, Zagonel V, Degan M, Perin V, Todesco M, De Iuliis A, Improta S, Sacco C, Gattei V, Gruss HJ and Carbone A (1997) The role of eosinophils in the pathobiology of Hodgkin's disease. *Ann Oncol* **8** (**Suppl 2):**89–96.
Piotrowski W and Foreman JC (1986) Some effects of calcitonin gene-related peptide in human skin and on histamine release. *Br J Dermatol* **114:**37–46.
Pipkorn U, Proud D, Lichtenstein LM, Kagey Sobotka A, Norman P and Naclerio RM (1987a) Inhibition of mediator release in allergic rhinitis by pre-treatment with topical glucocorticosteroids. *N Engl J Med* **316:**1506–1510.
Pipkorn U, Proud D, Lichtenstein LM, Schleimer RP, Peters SP, Adkinson NJ, Kagey Sobotka A, Norman PS and Naclerio RM (1987b) Effect of short-term systemic glucocorticoid treatment on human nasal mediator release after antigen challenge. *J Clin Invest* **80:**957–961.
Pires AL, de Silva PM, Pasquale C, Castro-Faria-Neto HC, Bozza PT, Cordeiro RS, Rae GA, Braquet P, Lagente V and Martins MA (1994) Long-lasting inhibitory activity of the tetrazepinic BN 50730 on exudation and cellular alterations evoked by PAF and LPS. *Br J Pharmacol* **113:**994–1000.
Pizzichini E, Kidney JC, Wong BJO, Morris MM, Efthimiadis A, Dolovich J and Hargreave FE (1995) Effect of salmeterol compared to beclomethasone in allergen-induced asthmatic and inflammatory responses. *Eur Respir J* **9:**449–455.
Plager DA, Stuart S and Gleich GJ (1998) Human eosinophil granule major basic protein and its novel homolog. *Allergy* **53** (**Suppl 45):**33–40.
Pleass RJ, Andrews PD, Kerr MA and Woof JM (1996) Alternative splicing of the human IgA Fc receptor CD89 in neutrophils and eosinophils. *Biochem J* **318:**771–777.
Pleass RJ, Dunlop JI and Woof JM (1997) Multiple transcripts of human IgA Fc receptor CD89 in neutrophils, eosinophils and the monocyte-like cell line THP-1. *Biochem Soc Trans* **25:**327S.
Ploemacher RE, van Soest PL, Boudewijn A and Neben S (1993) Interleukin-12 enhances interleukin-3 dependent multilineage hematopoietic colony formation stimulated by interleukin-11 or Steel factor. *Leukemia (Baltimore)* **7:**1374–1380.
Podleski WK, Panaszek BA, Schmidt JL and Burns RB (1984) Inhibition of eosino-

phils degranulation by Ketotifen in a patient with milk allergy, manifested as bronchial asthma - an electron microscopic study. *Agents Actions* **15:**177–181.
Polosa R, Rajakularingam K, Prosperini G, Magri S, Mastruzzo C and Holgate ST (1995) Inhaled loop diuretics and basal airway responsiveness in man: Evidence of a role for cyclo-oxygenase products. *Eur Resp J* **8:**593–599.
Ponath PD, Qin S, Post TW, Wang J, Wu L, Gerard NP, Newman W, Gerard C and Mackay CR (1996a) Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. *J Exp Med* **183:**2437–2448.
Ponath PD, Qin S, Ringler DJ, Clark Lewis I, Wang J, Kassam N, Smith H, Shi X, Gonzalo JA, Newman W, Gutierrez Ramos JC and Mackay CR (1996b) Cloning of the human eosinophil chemoattractant, eotaxin. Expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J Clin Invest* **97:**604–612.
Popken-Harris P, Checkel J, Loegering D, Madden B, Springett M, Kephart G and Gleich GJ (1998) Regulation and processing of a precursor form of eosinophil granule major basic protein (ProMBP) in differentiating eosinophils. *Blood* **92:**623–631.
Popken-Harris P, McGrogan M, Loegering DA, Checkel JL, Kubo H, Thomas LL, Moy JN, Sottrup Jensen L, Snable JL, Kikuchi MT and Gleich GJ (1995) Expression, purification, and characterization of the recombinant proform of eosinophil granule major basic protein. *J Immunol* **155:**1472–1480.
Popken-Harris P, Thomas L, Oxvig C, Sottrup Jensen L, Kubo H, Klein JS and Gleich GJ (1994) Biochemical properties, activities, and presence in biologic fluids of eosinophil granule major basic protein. *J Allergy Clin Immunol* **94:**1282–1289.
Popper H, Knipping G, Czarnetzki BM, Steiner R, Helleis G and Auer H (1989) Activation and release of enzymes and major basic protein from guinea pig eosinophil granulocytes induced by different inflammatory stimuli and other substances. A histochemical, biochemical, and electron microscopic study. *Inflammation* **13:**147–162.
Porges AJ, Redecha PB, Doebele R, Pan LC, Salmon JE and Kimberly RP (1992) Novel Fcγ receptor I family gene products in human mononuclear cells. *J Clin Invest* **90:**2102–2109.
Porteu F, Mir A and Halbwachs-Mecarelli L (1987) Modulation of neutrophil expression of C3b receptors (CR1) by soluble monomeric human C3b. *Eur J Immunol* **17:**629–635.
Postigo AA, Sanchez Mateos P, Lazarovits AI, Sanchez Madrid F and de Landazuri MO (1993) $\alpha_4\beta_7$ integrin mediates B cell binding to fibronectin and vascular cell adhesion molecule-1. Expression and function of α_4 integrins on human B lymphocytes. *J Immunol* **151:**2471–2483.
Powell PP, Klagsbrun M, Abraham JA and Jones RC (1993) Eosinophils expressing heparin-binding EGF-like growth factor mRNA localize around lung microvessels in pulmonary hypertension. *Am J Pathol* **143:**784–793.
Powell WS, Chung D and Gravel S (1995) 5-Oxo-6,8,11,14-eicosatetraenoic acid is a potent stimulator of human eosinophil migration. *J Immunol* **154:**4123–4132.
Powell WS, Gravel S, Halwani F (1999) 5-oxo-6,8,11,14-eicosatetraenoic acid is a potent stimulator of L-selectin shedding, surface expression of CD11b, actin polymerization, and calcium mobilization in human eosinophils. *Am J Respir Cell Mol Biol* **20:**163–170.
Power CA, Meyer A, Nemeth K, Bacon KB, Hoogewerf AJ, Proudfoot AE and Wells TN (1995) Molecular cloning and functional expression of a novel CC chemokine receptor cDNA from a human basophilic cell line. *J Biol Chem* **270:**19495–19500.
Power CA and Wells TN (1996) Cloning and characterization of human chemokine receptors. *Trends Pharmacol Sci* **17:**209–213.
Presentey B, Jerushalmy Z, Ben Bassat M and Perk K (1980) Genesis, ultrastructure and cytochemical study of the cat eosinophil. *Anat Rec* **196:**119–127.
Pretolani M, Lefort J, Silva P, Malachere E, Dumarey C, Bachelet M and Vargaftig BB (1990) Protection by nedocromil sodium of active immunization-induced bronchopulmonary alterations in the guinea-pig. *Am Rev Respir Dis* **141:**1259–1265.
Pretolani M and Goldman M (1997) IL-10: A potential therapy for allergic inflammation? *Immunol Today* **18:**277–280.
Prin L, Capron M, Gosset P, Wallaert B, Kusnierz JP, Bletry O, Tonnel AB and Capron A (1986) Eosinophilic lung disease: Immunological studies of blood and alveolar eosinophils. *Clin Exp Immunol* **63:**249–257.
Prin L, Capron M, Tonnel AB, Bletry O and Capron A (1983) Heterogeneity of human peripheral blood eosinophils: variability in cell density and cytotoxic ability in relation to the level and the origin of hypereosinophilia. *Int Arch Allergy Appl Immunol* **72:**336–346.
Prin L, Charon J, Capron M, Gosset P, Taelman H, Tonnel AB and Capron A (1984) Heterogeneity of human eosinophils. II. Variability of respiratory burst activity related to cell density. *Clin Exp Immunol* **57:**735–742.
Prin L, Lefebvre P, Gruart V, Capron M, Storme L, Formstecher P, Loiseau S and Capron A (1989) Heterogeneity of human eosinophil glucocorticoid receptor expression in hypereosinophilic patients: Absence of detectable receptor correlates with resistance to corticotherapy. *Clin Exp Immunol* **78:**383–389.
Rabe KF, Giembycz MA, Dent G and Barnes PJ (1992) Activation of guinea pig eosinophil respiratory burst by leukotriene B_4: Role of protein kinase C. *Fund Clin Pharmacol* **6:**353–358.
Rabe KF, Giembycz MA, Dent G, Perkins RS, Evans P and Barnes PJ (1993) Salmeterol is a competitive antagonist at β-adrenoceptors mediating inhibition of respiratory burst in guinea-pig eosinophils. *Eur J Pharmacol* **231:**305–308.
Raeburn D, Souness JE, Tomkinson A and Karlsson J-A (1993) Isoenzyme-selective cyclic nucleotide phosphodiesterase inhibitors: biochemistry, pharmacology and therapeutic potential in asthma. *Prog Drug Res* **40:**9–31.
Raeburn D, Underwood SL, Lewis SA, Woodman VR, Battram CH, Tomkinson A, Sharma S, Jordan R, Souness JE, Webber SE and Karlsson J-A (1994) Anti-inflammatory and bronchodilator properties of RP 73401, a novel and selective phosphodiesterase type IV inhibitor. *Br J Pharmacol* **113:**1423–1431.
Raible DG, Lenahan T, Fayvilevich Y, Kosinski R and Schulman ES (1994) Pharmacologic characterization of a novel histamine receptor on human eosinophils. *Am J Respir Crit Care Med* **149:**1506–1511.
Raible DG, Mohanty JG, McDermott LJ, Wang Y, Pelleg A and Schulman ES (1999) Pharmacology and signal transduction pathways of ATP-induced activation of human eosinophils (Abstract). *Am J Respir Crit Care Med* **159:**A466.
Raible DG, Schulman ES, DiMuzio J, Cardillo R and Post TJ (1992) Mast cell mediators prostaglandin-D_2 and histamine activate human eosinophils. *J Immunol* **148:**3536–3542.
Rajakulasingam K, Durham SR, O'Brien F, Humbert M, Barata LT, Reece L, Kay AB and Grant JA (1997) Enhanced expression of high-affinity IgE receptor (FcεRI) α chain in human allergen-induced rhinitis with co-localization to mast cells, macrophages, eosinophils, and dendritic cells. *J Allergy Clin Immunol* **100:**78–86.
Rajakulasingam K, Till S, Ying S, Humbert M, Barkans J, Sullivan M, Meng Q, Corrigan CJ, Bungre J, Grant JA, Kay AB and Durham SR (1998) Increased expression of high affinity IgE (FcεRI) receptor-α chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma. *Am J Respir Crit Care Med* **158:**233–240.
Rampton DS, Brown MJ, Causon R and Sahib M (1982) The effect of disodium cromoglycate on rectal mucosal histamine release, eosinophil exudation and disease activity in active ulcerative colitis. *Clin Allergy* **12:**243–248.
Rand TH, Cruikshank WW, Center DM and Weller PF (1991b) CD4-mediated stimulation of human eosinophils: Lymphocyte chemoattractant factor and other CD4-binding ligands elicit eosinophil migration. *J Exp Med* **173:**1521–1528.
Rand TH, Silberstein DS, Kornfeld H and Weller PF (1991a) Human eosinophils express functional interleukin 2 receptors. *J Clin Invest* **88:**825–832.
Rand TH, Turk J, Maas RL and Colley DG (1982) Arachidonic acid metabolism of the murine eosinophil. II. Involvement of the lipoxygenase pathway in the response to the lymphokine eosinophil stimulation promoter. *J Immunol* **129:**1239–1244.
Rao A, Luo C and Hogan PG (1997) Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* **15:**707–747.
Raport CJ, Gosling J, Schweickart VL, Gray PW and Charo IF (1996b) Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1β, and MIP-1α. *J Biol Chem* **271:**17161–17166.
Raport CJ, Schweickart VL, Chantry D, Eddy RL, Shows TB, Godiska R and Gray PW (1996a) New members of the chemokine receptor gene family. *J Leukoc Biol* **59:**18–23.
Ravetch JV (1994) Fc receptors: rubor redux. *Cell* **78:**553–560.
Ravetch JV (1997) Fc receptors. *Curr Opin Immunol* **9:**121–125.
Ravetch JV and Kinet JP (1991) Fc receptors. *Annu Rev Immunol* **9:**457–492.
Ravetch JV and Perussia B (1989) Alternative membrane forms of FcγRIII (CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. *J Exp Med* **170:**481–497.
Rebuck JW and Mellinger RC (1953) Interruption by topical cortisone of leukocytic cycles in acute inflammation in man *in vivo*. *Ann NY Acad Sci* **56:**715–751.
Regan JW, Bailey TJ, Donello JE, Pierce KL, Pepperl DJ, Zhang D, Kedzie KM, Fairbairn CE, Bogardus AM, Woodward DF and Gil DW (1994a) Molecular cloning and expression of human EP_3 receptors: Evidence of three variants with differing carboxyl termini. *Br J Pharmacol* **112:**377–385.
Regan JW, Bailey TJ, Pepperl DJ, Pierce KL, Bogardus AM, Donello JE, Fairbairn CE, Kedzie KM, Woodward DF and Gil DW (1994b) Cloning of a novel human prostaglandin receptor with characteristics of the pharmacologically defined EP_2 subtype. *Mol Pharmacol* **46:**213–220.
Regoli D, Boudon A and Fauchere JL (1994) Receptors and antagonists for substance P and related peptides. *Pharmacol Rev* **46:**551–599.
Reiss TF, Altman LC, Chervinsky P, Bewtra A, Stricker WE, Noonan GP, Kundu S and Zhang J (1996) Effects of montelukast (MK-0476), a new potent cysteinyl leukotriene (LTD_4) receptor antagonist, in patients with chronic asthma. *J Allergy Clin Immunol* **98:**528–534.
Renzi PM, Sapienza S, Waserman S, Du T, Olivenstein R, Wang NS and Martin JG (1992) Effect of interleukin-2 on the airway response to antigen in the rat. *Am Rev Respir Dis* **146:**163–169.
Resler B, Sedgwick JB and Busse WW (1992) Inhibition of interleukin-5 effects on human eosinophils by nedocromil sodium. *J Allergy Clin Immunol* **89:**235.
Resnick MB, Colgan SP, Parkos CA, Delp Archer C, McGuirk D, Weller PF and Madara JL (1995) Human eosinophils migrate across an intestinal epithelium in response to platelet-activating factor. *Gastroenterology* **108:**409–416.
Richards IM, Shields SK, Bienkowski MJ, Dunn CJ and Jacobsen EJ (1991a) Novel inhibitors of pulmonary eosinophil accumulation. *Agents Actions* **34 (Suppl 34):**359–368.
Richards IM, Shields SK, Griffin RL, Fidler SF and Dunn CJ (1992) Novel steroid-based inhibitors of lung inflammation. *Clin Exp Allergy* **22:**432–439.
Richards IM, Shields SK, Johnson RA and Smith HW (1989) Effect of the selective leukotriene B_4 antagonists U-75302 and LY255283 on bronchoalveolar eosinophilia induced byinhalation of leukotriene B_4 or allergen in Brown-Norway rats. *Ann NY Acad Sci* **629:**428–429.
Richards IM, Sun FF, Taylor BM, Shields SK, Griffin RL, Morris J, Wishka DG, Smith HW, Johnson RA and Dunn CJ (1991b) Contribution of leukotriene B_4 to airway inflammation and the effect of antagonists. *Ann NY Acad Sci* **629:**274–287.
Riedel D, Lindemann A, Brach M, Mertelsmann R and Herrmann F (1990) Granulocyte/macrophage colony-stimulating factor and interleukin-3 induce surface expression of interleukin-2 receptor p55-chain and CD4 by human eosinophils. *Immunology* **70:**258–261.
Rihoux JP, Melac M and Henocq E (1990) Anti-IgE- and anti-IgA-induced eosinophil migration in atopics and healthy volunteers. *Clin Exp Allergy* **20 (Suppl 4):**11–18.
Rioux JD, Stone VA, Daly MJ, Cargill M, Green T, Nguyen H, Nutman T, Zimmerman PA, Tucker MA, Hudson T, Goldstein AM, Lander E and Lin AY (1998) Familial eosinophilia maps to the cytokine gene cluster on human chromosomal region 5q31–q33. *Am J Hum Genet* **63:**1086–1094.
Robbins RA, Romero FA, Nelson KJ, Hill GE and Townley RG (1995) Inhibitors of nitric oxide attenuate human eosinophil chemotaxis (Abstract). *Am J Respir Crit Care Med* **151:**A238.
Roberge CJ, Laviolette M, Boulet LP and Poubelle PE (1990) *In vitro* leukotriene (LT) C_4 synthesis by blood eosinophils from atopic asthmatics: Predominance of

eosinophil subpopulations with high potency for LTC_4 generation. *Prostaglandins Leukotr Essent Fatty Acids* **41:**243–249.
Robuschi M, Gamboro G, Spagnotto S, Vaghi A and Bianco S (1988) Inhaled frusemide is highly effective in protecting ultrasonically nebulised water-induced bronchoconstriction. *Pulm Pharmacol* **1:**187–191.
Rodaway AR, Teahan CG, Casimir CM, Segal AW and Bentley DL (1990) Characterization of the 47-kilodalton autosomal chronic granulomatous disease protein: tissue-specific expression and transcriptional control by retinoic acid. *Mol Cell Biol* **10**: 5388–5396.
Rolfe FG, Hughes JM, Armour CL and Sewell WA (1992) Inhibition of interleukin-5 gene expression by dexamethasone. *Immunology* **77:**494–499.
Rooney TA, Hager R and Thomas AP (1991) Beta-adrenergic receptor-mediated phospholipase C activation independent of cAMP formation in turkey erythrocyte membranes. *J Biol Chem* **266:**15068–15074.
Rosenberg HF (1995) Recombinant human eosinophil cationic protein: Ribonuclease activity is not essential for cytotoxicity. *J Biol Chem* **270:**7876–7881.
Rosenberg HF, Ackerman SJ and Tenen DG (1989a) Human eosinophil cationic protein. Molecular cloning of a cytotoxin and helminthotoxin with ribonuclease activity. *J Exp Med* **170:**163–176.
Rosenberg HF and Dyer KD (1997) Diversity among the primate eosinophil-derived neurotoxin genes: A specific C-terminal sequence is necessary for enhanced ribonuclease activity. *Nucleic Acids Res* **25:**3532–3536.
Rosenberg HF, Dyer KD, Tiffany HL and Gonzalez M (1995) Rapid evolution of a unique family of primate ribonuclease genes. *Nat Genet* **10:**219–223.
Rosenberg HF, Tenen DG and Ackerman SJ (1989b) Molecular cloning of the human eosinophil-derived neurotoxin: a member of the ribonuclease gene family. *Proc Natl Acad Sci USA* **86**: 4460–4464.
Ross GD (1989) Complement and complement receptors. *Curr Opin Immunol* **2:**50–62.
Ross GD (1994) Membrane complement receptors, in *Clinical Aspects of Immunology* (Lachman PL, Peters DK, Rosen FS and Walport MJ eds) pp 241–264, Blackwell Scientific, Boston.
Ross GD and Lambris JD (1982) Identification of a C3bi-specific membrane complement receptor that is expressed on lymphocytes, monocytes, neutrophils and erythrocytes. *J Exp Med* **155:**96–110.
Ross GD, Newman SL, Lambris JD, Devery Pocius JE, Cain JA and Lachmann PJ (1983) Generation of three different fragments of bound C3 with purified factor I or serum. II. Location of binding sites in the C3 fragments for factors B and H, complement receptors, and bovine conglutinin. *J Exp Med* **158:**334–352.
Ross GD, Reed W, Dalzell JG, Becker SE and Hogg N (1992) Macrophage cytoskeleton association with CR3 and CR4 regulates receptor mobility and phagocytosis of iC3b-opsonized particles. *J Leukoc Biol* **51:**109–117.
Ross JS and Camp RD (1990) Cyclosporin A in atopic dermatitis. *Br J Dermatol* **122:**41–45.
Rossi AG, Cousin JM, Dransfield I, Lawson MF, Chilvers ER and Haslett C (1995) Agents that elevate cAMP inhibit human neutrophil apoptosis. *Biochem Biophys Res Commun* **217:**892–899.
Rossi AG, Haslett C, Hirani N, Greening AP, Rahman I, Metz CN, Bucala R and Donnelly SC (1998) Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF). Potential role in asthma. *J Clin Invest* **101:**2869–2874.
Rossi GA, Sacco O, Vassallo F, Lantero S, Morelli A, Benatti U and Damiani G (1989) Oxidative metabolism of human peripheral blood eosinophils and neutrophils: H_2O_2 production after stimulation with phorbol myristate acetate and immune complexes. *Eur Respir J* **(6 Suppl):**435s–440s.
Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ and Dahinden CA (1992) RANTES and macrophage inflammatory protein 1α induce the migration and activation of normal human eosinophil granulocytes. *J Exp Med* **176:**1489–1495.
Rothenberg ME (1998) Eosinophilia. *N Engl J Med* **338:**1592–1600.
Rothenberg ME, Owen WF, Silberstein DS, Soberman RJ, Austen KF and Stevens RL (1987) Eosinophils cocultured with endothelial cells have increased survival and functional properties. *Science (Wash DC)* **237:**645–647.
Rothenberg ME, Owen WF, Silberstein DS, Woods J, Soberman RJ, Austen KF and Stevens RL (1988) Human eosinophils have prolonged survival, enhanced functional properties, and become hypodense when exposed to human interleukin 3. *J Clin Invest* **81:**1986–1992.
Rothenberg ME, Ownbey R, Mehlhop PD, Loiselle PM, Vanderijn M, Bonventre JV, Oettgen HC, Leder P and Luster AD (1996) Eotaxin triggers eosinophil-selective chemotaxis and calcium flux via a distinct receptor and induces pulmonary eosinophilia in the presence of interleukin 5 in mice. *Mol Med* **2:**334–348.
Rothenberg ME, Petersen J, Stevens RL, Silberstein DS, McKenzie DT, Austen KF and Owen WF (1989) IL-5-dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability, accelerated hypodensity, and sustained antibody-dependent cytotoxicity. *J Immunol* **143:**2311–2316.
Roubin R, Elsas PP, Fiers w and Dessein AJ (1987) Recombinant human tumour necrosis factor (rTNF)2 enhances leukotriene biosynthesis in neutrophils and eosinophils stimulated with the Ca^{2+} ionophore A23187. *Clin Exp Immunol* **70:**484–490.
Rouzer CA and Kargman S (1988) Translocation of 5-lipoxygenase to the membrane in human leukocytes challenged with ionophore A 23187. *J Biol Chem* **263:**10980–10988.
Rouzer CA and Samuelsson B (1985) On the nature of the 5-lipoxygenase reaction in human leukocytes: enzyme purification and requirement for multiple stimulatory factors. *Proc Natl Acad Sci USA* **82:**6040–6044.
Rozell MD, Erger RA and Casale TB (1996) Isolation technique alters eosinophil migration response to IL-8. *J Immunol Methods* **197:**97–107.
Ruegg C, Postigo AA, Sikorski EE, Butcher EC, Pytela R and Erle DJ (1992) Role of integrin $\alpha_4\beta_7/\alpha_4\beta_P$ in lymphocyte adherence to fibronectin and VCAM-1 and in homotypic cell clustering. *J Cell Biol* **117:**179–189.
Rumi C, Rutella S, Leone G and Bonini S (1998) Fc-RII/CD23 receptor on circulating human eosinophils. *Blood* **91:**2621–2622.
Russell SM, Keegan AD, Harada N,Nakamura Y, Noguchi M, Leland P, Friedmann MC, Miyajima A, Puri RK, Paul WE and Leonard WE (1993) Interleukin-2 receptor gamma chain: A functional component of the interleukin-4 receptor. *Science (Wash DC)* **262:**1880–1883.
Rytomaa T (1960) Organ distribution and histochemical properties of eosinophil granulocytes in the rat. *Acta Pathol Microbiol Scand* **50** (**Suppl 140):**12–18.
Sabag N, Castrillon MA and Tchernitchin A (1978) Cortisol-induced migration of eosinophil leukocytes to lymphoid organs. *Experientia* **34:**666–667.
Sabin EA, Kopf MA and Pearce EJ (1996) *Schistosoma mansoni* egg-induced early IL-4 production is dependent upon IL-5 and eosinophils. *J Exp Med* **184:**1871–1878.
Sabroe I, Conroy DM, Gerard NP, Li Y, Collins PD, Post TW, Jose PJ, Williams TJ, Gerard CJ and Ponath PD (1998) Cloning and characterization of the guinea pig eosinophil eotaxin receptor, C-C chemokine receptor-3: blockade using a monoclonal antibody *in vivo*. *J Immunol* **161:**6139–6147.
Sagara H, Fukuda T, Okada T, Ishikawa A and Makino S (1996) Theophylline at therapeutic concentration suppresses PAF-induced upregulation of Mac-1 on human eosinophils. *Clin Exp Allergy* **26:**16–21.
Sagara H, Matsuda H, Wada N, Yagita H, Fukuda T, Okumura K, Makino S and Ra C (1997) A monoclonal antibody against very late activation antigen-4 inhibits eosinophil accumulation and late asthmatic response in a guinea pig model of asthma. *Int Arch Allergy Immunol* **112:**287–294.
Saito M, Hisatome I, Nakajima S and Sato R (1995) Possible mechanism of oxygen radical production by human eosinophils mediated by K^+ channel activation. *Eur J Pharmacol* **291:**217–219.
Saito M, Sato R, Hisatome I and Narahashi T (1996) RANTES and platelet-activating factor open Ca^{2+}-activated K^+ channels in eosinophils. *FASEB J* **10:**792–798.
Saito M, Sato R, Munoz NM, Herrnreiter A, Oyaizu M, Kasugai H, Narahashi T and Leff AR (1997) Association of granular exocytosis with Ca^{2+}-activated K^+ channels in human eosinophils. *Am J Physiol* **273:**L16–L21.
Sakakibara M, Shima K, Takamatsu J and Said S (1990) Vasoactive intestinal peptide (VIP) binds to guinea-pig peritoneal eosinophils: A single class of binding sites with low affinity and high capacity (Abstract). *FASEB J* **4:**A639.
Sakamaki K, Tomonaga M, Tsukui K and Nagata S (1989) Molecular cloning and characterization of a chromosomal gene for human eosinophil peroxidase. *J Biol Chem* **264:**16828–16836.
Sako D, Chang XJ, Barone KM, Vachino G, White HM, Shaw G, Veldman GM, Bean KM, Ahern TJ, Furie B, Cumming DA and Larsen GR (1993) Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell* **75:**1179–1186.
Sakuma R, Elsas PP, Fiers W and Dessein AJ (1987) Recombinant human tumour necrosis factor (rTNF)2 enhances leukotriene biosynthesis in neutrophils and eosinophils stimulated with the Ca^{2+} ionophore A23187. *Clin Exp Immunol* **70:**484–490.
Sakuma Y, Underwood H, Katayama S, Harada K, Obaishi H, Shirato M, Yamada K, Miyazawa S, Okano K and Machida Y (1990) Effect of a novel PAF antagonist, E6123, on passive anaphylaxis. *Agents Actions Suppl* **31:**255–258.
Saleh TS, Calixto J and Medeiros Y (1996) Anti-inflammatory effects of theophylline, cromolyn and salbutamol in a murine model of pleurisy. *Br J Pharmacol* **118:**811–819.
Salek MS, Finlay AY, Luscombe DK, Allen BR, Berth-Jones J, Camp RD, Graham-Brown RA, Kahn GK, Marks R, Motley RJ, Ross JS and Sowden JM (1993) Cyclosporin greatly improves the quality of life of adults with severe atopic dermatitis: A randomized, double-blind, placebo-controlled trial. *Br J Dermatol* **129:**422–430.
Samson M, Labbe O, Mollereau C, Vassart G and Parmentier M (1996) Molecular cloning and functional expression of a new human CC-chemokine receptor gene. *Biochemistry* **35:**3362–3367.
Sanchez Madrid F, Nagy JA, Robbins E, Simon P and Springer TA (1983) A human leukocyte differentiation antigen family with distinct α-subunits and a common β-subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. *J Exp Med* **158:**1785–1803.
Sanderson CJ (1993) Interleukin-5 and the regulation of eosinophil production, in *Immunopharmacology of Eosinophils* (Smith H and Cook RM eds) pp 10–24, Academic Press, London.
Sanderson CJ, Warren DJ and Strath M (1985) Identification of a lymphokine that stimulates eosinophil differentiation *in vitro*. Its relationship to interleukin 3, and functional properties of eosinophils produced in cultures. *J Exp Med* **162:**60–74.
Sanjar S, Aoki S, Boubekeur K, Burrows L, Colditz I, Chapman I and Morley J (1989) Inhibition of PAF-induced eosinophil accumulation in pulmonary airways of guinea pigs by anti-asthma drugs. *Jpn J Pharmacol* **51:**167–172.
Sanjar S, Aoki S, Boubekeur K, Chapman ID, Smith D, Kings MA and Morley J (1990a) Eosinophil accumulation in pulmonary airways of guinea-pigs induced by exposure to an aerosol of platelet-activating factor: Effect of anti-asthma drugs. *Br J Pharmacol* **99:**267–272.
Sanjar S, Aoki S, Kristersson A, Smith D and Morley J (1990b) Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitized guinea-pigs: The effect of anti-asthma drugs. *Br J Pharmacol* **99:**679–686.
Sanjar S, McCabe PJ, Reynolds LH and Johnson M (1991) Salmeterol inhibits antigen-induced eosinophil accumulation in the guinea-pig lung. *Fund Clin Pharmacol* **5:**402 (Abstract).
Sanjar S, Smith D, Kings MA and Morley J (1990c) Pretreatment with rh-GMCSF, but not rh-IL3, enhances PAF-induced eosinophil accumulation in guinea-pig airways. *Br J Pharmacol* **100:**399–400.
Santamaria LF, Palacios JM and Beleta J (1997) Inhibition of eotaxin-mediated human eosinophil activation and migration by the selective cyclic nucleotide phosphodiesterase type 4 inhibitor rolipram. *Br J Pharmacol* **121:**1150–1154.
Santing RE, Olymulder CG, Van der Molen K, Meurs H and Zaagsma J (1995)

Phosphodiesterase inhibitors reduce bronchial hyperreactivity and airway inflammation in unrestrained guinea pigs. *Eur J Pharmacol* **275:**75–82.
Sanz MJ, Ponath PD, Mackay CR, Newman W, Miyasaka M, Tamatani T, Flanagan BF, Lobb RR, Williams TJ, Nourshargh S and Jose PJ (1998) Human eotaxin induces α_4 and β_2 intergrin-dependent eosinophil accumulation in rat skin *in vivo*: Delayed generation of eotaxin in response to IL-4. *J Immunol* **160:**3569–3576.
Sanz MJ, Weg VB, Bolanowski MA and Nourshargh S (1995) IL-1 is a potent inducer of eosinophil accumulation in rat skin. Inhibition of response by a platelet-activating factor antagonist and an anti-human IL-8 antibody. *J Immunol* **154:** 1364–1373.
Sanz MJ, Weg VB, Walsh DT, Williams TJ and Nourshargh S (1994) Differential effects of the PAF receptor antagonist UK-74,505 on neutrophil and eosinophil accumulation in guinea-pig skin. *Br J Pharmacol* **113:**513–521.
Sarafi MN, Garcia Zepeda EA, MacLean JA, Charo IF and Luster AD (1997) Murine monocyte chemoattractant protein (MCP)-5: A novel CC chemokine that is a structural and functional homologue of human MCP-1. *J Exp Med* **185:**99–109.
Sasaki O, Sugaya H, Ishida K and Yoshimura K (1993) Ablation of eosinophils with anti-IL-5 antibody enhances the survival of intracranial worms of *Angiostrongylus cantonensis* in the mouse. *Parasite Immunol* **15:**349–354.
Sato N, Sakamaki K, Terada N, Arai K and Miyajima A (1993) Signal transduction by the high-affinity GM-CSF receptor: Two distinct cytoplasmic regions of the common β subunit responsible for different signaling. *EMBO J* **12:**4181–4189.
Satoh T, Chen QJ, Sasaki G, Yokozeki H, Katayama I and Nishioka K (1997) Cyclophosphamide-induced blood and tissue eosinophilia in contact sensitivity—Mechanism of hapten-induced eosinophil recruitment into the skin. *Eur J Immunol* **27:**85–91.
Saunders RH and Adams E (1950) Changes in circulating leukocytes following the administration of adrenal cortex extract (ACE) and adrenocorticotropic hormone (ACTH) in infectious mononucleosis and chronic lymphatic leukemia. *Blood* **5:**732–741.
Scallon BJ, Scigliano E, Freedman VH, Miedel MC, Pan YC, Unkeless JC and Kochan JP (1989) A human immunoglobulin G receptor exists in both polypeptide-anchored and phosphatidylinositol-glycan-anchored forms. *Proc Natl Acad Sci USA* **86:**5079–5083.
Scepek S, Coorsen JR and Lindau M (1998) Fusion pore expansion in horse eosinophils is modulated by Ca^{2+} and protein kinase C via distinct mechanisms. *EMBO J* **17:**4340–4345.
Scepek S and Lindau M (1993) Focal exocytosis by eosinophils—Compound exocytosis and cumulative fusion. *EMBO J* **12:**1811–1817.
Schaller M and Parsons J (1994) Focal adhesion kinase and associated proteins. *Curr Opin Cell Biol* **6:**705–710.
Schauer U, Daume U, Muller R, Riedel F, Gemsa D and Rieger CH (1990) Relationship between LTC_4 generation of hypodense eosinophils and bronchial hyperreactivity in asthmatic children. *Int Arch Allergy Appl Immunol* **92:**82–87.
Schauer U, Eckhart A, Muller R, Gemsa D and Rieger CH (1989) Enhanced leukotriene C_4 production by peripheral eosinophilic granulocytes from children with asthma. *Int Arch Allergy Appl Immunol* **90:**201–206.
Schauer U, Trube M, Jager R, Gieler U and Rieger CH (1995) Blood eosinophils, eosinophil-derived proteins, and leukotriene C_4 generation in relation to bronchial hyperreactivity in children with atopic dermatitis. *Allergy* **50:**126–132.
Schellenberg RR, Ishida K and Thomson RJ (1991) Nedocromil sodium inhibits airway hyperresponsiveness and eosinophilic infiltration induced by repeated antigen challenge in guinea-pigs. *Br J Pharmacol* **103:**1842–1846.
Schleimer RP (1988) Glucocorticosteroids: their mechanisms of action and use in allergic diseases, in *Allergy: Principals and Practice* (Middleton EJ, Reed CE, Ellis EF, Adkinson NFJ and Yunginger JW eds) pp 739–765, Mosby, St. Louis.
Schleimer RP and Bochner BS (1994) The effects of glucocorticoids on human eosinophils. *J Allergy Clin Immunol* **94:**1202–1213.
Schleimer RP, Davidson DA, Lichtenstein LM and Adkinson NF (1986) Selective inhibition of arachidonic acid metabolite release from human lung tissue by anti-inflammatory steroids. *J Immunol* **136:**3006–3011.
Schleimer RP, Freeland HS, Peters SP, Brown KE and Derse CP (1989) An assessment of the effects of glucocorticoids on degranulation, chemotaxis, binding to vascular endothelial cells and formation of leukotriene B_4 by purified human eosinophils. *J Pharmacol Exp Ther* **250:**598–605.
Schleimer RP, Lichtenstein LM and Gillespie E (1981) Inhibition of basophil histamine release by anti-inflammatory steroids. *Nature (Lond)* **292:**454–455.
Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, Newman W, Luscinskas FW, Gimbrone MA, McIntyre BW and Bochner BS (1992) IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. *J Immunol* **148:**1086–1092.
Schreiber SL (1991) Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science (Wash DC)* **251:**283–287.
Schrier DJ and Imre RM (1986) The effect of adenosine antagonists on human neutrophil function. *J Immunol* **137:**3284–3289.
Schudt C, Winder S, Litze M, Kilian U and Beume R (1991) Zardaverine: A cyclic AMP specific PDE III/IV inhibitor. *Agents Actions* **34:**161–177.
Schuler GD, Boguski MS, Stewart EA, Stein LD, Gyapay G, Rice K, White RE, Rodriguez-Tome P, Aggarwal A, Bajorek E, Bentolila S, Birren BB, Butler A, Castle AB, Chiannilkulchai N, Chu A, Clee C, Cowles S, Day PJ, Dibling T, Drouot N, Dunham I, Duprat S, East C, Fan J-B, Fang N, Fizames C, Garrett C, Green L, Hadley D, Harris M, Harrison P, Brady S, Hicks A, Holloway E, Hui L, Hussain S, Louis-Dit-Sulley C, Ma J, MaxGilvery A, Mader C, Maratukulam A, Matise TC, McKusick KB, Piercy M, Morissette J, Mungall A, Muselet D, Nusbaum HC, Page DC, Peck A, Perkins S, Piercy M, Qin F, Quackenbush J, Ranby S, Reif T, Rozen S, Sanders C, She X, Silva J, Slonim DK, Soderlund C, Sun W-L, Tabar P, Thangarajah T, Vega-Czarny N, Vollrath D, Voyticky S, Wilmer T, Wu X, Adams MD, Auffray C, Walter NAR, Brandon R, Dehejia A, Goodfellow PN, Houlgatte R, Hudson JR, Ide SE, Iorio KR, Lee WY, Seki N,Nagase T, Ishikawa K, Nomura N, Phillips C, Polymeropoulos MH, Sandusky M, Schmitt, K, Berry R, Swanson K, Torres R, Venter JC, Sikela JM, Beckmann JS, Weissenbach J, Myers RM, Cox DR, James MR, Bentley D, Deloukas P, Lamder ES and Hudson TJ (1996) A gene map of the human genome. *Science (Wash DC)* **274:**540–546.
Schweizer RC, van Kessel Welmers BA, Warringa RA, Maikoe T, Raaijmakers JA, Lammers JW and Koenderman L (1996) Mechanisms involved in eosinophil migration. Platelet-activating factor-induced chemotaxis and interleukin-5-induced chemokinesis are mediated by different signals. *J Leukoc Biol* **59:**347–356.
Schweizer RC, Welmers BA, Raaijmakers JA, Zanen P, Lammers JW and Koenderman L (1994) RANTES- and interleukin-8-induced responses in normal human eosinophils: effects of priming with interleukin-5. *Blood* **83:**3697–3704.
Schwenk U, Morita E, Engel R and Schroder JM (1992) Identification of 5-oxo-15-hydroxy-6,8,11,13-eicosatetraenoic acid as a novel and potent human eosinophil chemotactic eicosanoid. *J Biol Chem* **267:**12482–12488.
Schwenk U and Schroder JM (1995) 5-Oxo-eicosanoids are potent eosinophil chemotactic factors. Functional characterization and structural requirements. *J Biol Chem* **270:**15029–15036.
Scotland G and Houslay MD (1995) Chimeric constructs show that the unique N-terminal domain of the cyclic AMP phosphodiesterase RD1 (RNPDE4A1A; rPDE-IVA1) can confer membrane association upon the normally cytosolic protein chloramphenicol acetyltransferase. *Biochem J* **308:**673–681.
Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ and Holdaway MD (1991) Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N Engl J Med* **325:**1067–1071.
Sears MR and Taylor DR (1994) The β_2-agonist controversy: Observations, explanations and relationship to asthma epidemiology. *Drug Safety* **11:**259–283.
Sears MR, Taylor DR, Print CG, Lake DC, Li QQ, Flannery EM, Yates DM, Lucas MK and Herbison GP (1990) Regular inhaled β-agonist treatment in bronchial asthma. *Lancet* **336:**1391–1396.
Sedgwick JB, Berube ML and Zurier RB (1985) Stimulus-dependent inhibition of superoxide generation by prostaglandins. *Clin Immunol Immunopathol* **34:**205–215.
Sedgwick JB, Bjornsdottir U, Geiger KM, Busse WW (1992a) Inhibition of eosinophil density change and leukotriene C_4 generation by nedocromil sodium. *J Allergy Clin Immunol* **90:**202–209.
Sedgwick JB, Calhoun WJ, Vrtis RF, Bates ME, McAllister PK and Busse WW (1992b) Comparison of airway and blood eosinophil function after *in vivo* antigen challenge. *J Immunol* **149:**3710–3718.
Sedgwick JB, Frick WE, Sondel PM, Hank JA, Borden E and Busse WW (1990a) The appearance of hypodense eosinophils during interleukin-2 treatment. *J Allergy Clin Immunol* **85:**557–566.
Sedgwick JB, Geiger KM and Busse WW (1990b) Superoxide generation by hypodense eosinophils from patients with asthma. *Am Rev Respir Dis* **142:**120–125.
Sedgwick JB, Quan SF, Calhoun WJ and Busse WW (1995) Effect of interleukin-5 and granulocyte/macrophage colony-stimulating factor on *in vitro* eosinophil function: Comparison with airway eosinophils. *J Allergy Clin Immunol* **96:**375–385.
Sedgwick JB, Vrtis RF, Gourley MF and Busse WW (1988) Stimulus-dependent differences in superoxide anion generation by normal human eosinophils and neutrophils. *J Allergy Clin Immunol* **81:**876–883.
Seeds EA, Coyle AJ and Page CP (1991) The effect of the selective PAF antagonist WEB 2170 on PAF and antigen induced airway hyperresponsiveness and eosinophil infiltration. *J Lipid Mediat* **4:**111–121.
Seeds EAM, Kilfeather S, Okiji S, Donigi-Gale D and Page CP (1995) Role of lipoxygenase metabolites in platelet-activating factor and antigen-induced bronchial hyperresponsiveness and eosinophil infiltration. *Eur J Pharmacol Environ Toxicol Pharmacol Sect* **293:**369–376.
Segal AW and Abo A (1993) The biochemical basis of the NADPH oxidase of phagocytes. *Trends Biochem Sci* **18:**43–47.
Segal AW, Garcia R, Goldstone H, Cross AR and Jones OT (1981) Cytochrome b_{245} of neutrophils is also present in human monocytes, macrophages and eosinophils. *Biochem J* **196:**363–367.
Seguchi M and Nakajima S (1995) Induction of ICAM-1 expression on the eosinophilic leukemia cell line-3 (EoL-3) by platelet-activating factor and platelet factor 4. *Ann Allergy Asthma Immunol* **74:**255–257.
Sehmi R, Cromwell O, Taylor GW and Kay AB (1991) Identification of guinea pig eosinophil chemotactic factor of anaphylaxis as leukotriene B_4 and 8(*S*),15(*S*)-dihydroxy-5,9,11,13(Z, E, Z, E)-eicosatetraenoic acid. *J Immunol* **147:**2276–2283.
Sehmi R, Rossi AG, Kay AB and Cromwell O (1992a) Identification of receptors for leukotriene B_4 expressed on guinea-pig peritoneal eosinophils. *Immunology* **77:** 129–135.
Sehmi R, Walsh GM, Hartnell A, Barkans J, North J, Kay AB and Moqbel R (1993) Modulation of human eosinophil chemotaxis and adhesion by anti-allergic drugs *in vitro*. *Pediatr Allergy Immunol* **4:**13–18.
Sehmi R, Wardlaw AJ, Cromwell O, Kurihara K, Waltmann P and Kay AB (1992b) Interluukin-5 selectively enhances the chemotactic response of eosinophils obtained from normal but not eosinophilic subjects. *Blood* **79:**2952–2959.
Seminario MC and Bochner BS (1997) Expression and function of β_1 integrins on human eosinophils. *Mem Inst Oswaldo Cruz* **92:**157–164.
Sengelov H (1995) Complement receptors in neutrophils. *Crit Rev Immunol* **15:**107–131.
Sensi LG, Seri A, Siracusa A, Pertici L and Marcucci F (1997) Allergic rhinitis in children—Effects of flunisolide and disodium cromoglycate on nasal eosinophil cationic protein. *Clin Exp Allergy* **27:**270–276.
Sentman CL, Shutter JR, Hockenbery D, Kanagawa O and Korsmeyer SJ (1991) bcl-2 Inhibits multiple forms of apoptosis but not negative selection in thymocytes. *Cell* **67:**879–888.
Serhan CN (1991) Lipoxins: eicosanoids carrying intra- and intercellular messages. *J Bioenerg Biomembr* **23:**105–122.
Serhan CN (1996) Inflammation. Signalling the fat controller. *Nature (Lond)* **384:** 23–24.
Serhan CN, Fiore S and Levy BD (1994) Cell-cell interactions in lipoxin generation and characterization of lipoxin A_4 receptors. *Ann NY Acad Sci* **744:**166–180.
Serhan CN, Hamberg M and Samuelsson B (1984a) Lipoxins: Novel series of biolog-

ically active compounds formed from arachidonic acid in human leukocytes. *Proc Natl Acad Sci USA* **81:**5335–5339.
Serhan CN, Hamberg M and Samuelsson B (1984b) Trihydroxytetraenes: A novel series of compounds formed from arachidonic acid in human leukocytes. *Biochem Biophys Res Commun* **118:**943–949.
Serhan CN, Hirsch U, Palmblad J and Samuelsson B (1987) Formation of lipoxin A by granulocytes from eosinophilic donors. *FEBS Letts* **217:**242–246.
Serhan CN and Samuelsson B (1988) Lipoxins: a new series of eicosanoids (biosynthesis, stereochemistry, and biological activities). *Adv Exp Med Biol* **229:**1–14.
Serra MF, Diaz BL, Barreto EO, Pereira AP, Lima MC, Barbosa-Filho JM, Cordeiro RS, Martins MA and de Silva PM (1997) Anti-allergic properties of the natural PAF antagonist yangambin. *Planta Med* **63:**207–212.
Seybold J, Newton R, Wright L, Finney P, Suttorp N, Barnes PJ, Adcock IM and Giembycz MA (1998) Induction of cyclic AMP phosphodiesterases 3B, 4A4, 4D1, 4D2 and 4D3 in Jurkat T-cells and human T-lymphocytes: Potential role in β_2-adrenoceptor desensitization. *J Biol Chem* **273:**20575–20588.
Shakur Y, Pryde JG and Houslay MD (1993) Engineered deletion of the unique N-terminal domain of the cyclic AMP-specific phosphodiesterase RD1 prevents plasma membrane association and the attainment of enhanced thermostability without altering its sensitivity to inhibition by rolipram. *Biochem J* **292:**677–686.
Shalit M, Sekhsaria S and Malech HL (1995) Modulation of growth and differentiation of eosinophils from human peripheral blood $CD34^+$ cells by IL5 and other growth factors. *Cell Immunol* **160:**50–57.
Shattil SJ, Ginsberg M and Brugge JS (1994) Adhesive signaling in platelets. *Curr Opin Cell Biol* **6:**695–704.
Shaw RJ, Cromwell O and Kay AB (1984) Preferential generation of leukotriene C_4 by human eosinophils. *Clin Exp Immunol* **56:**716–722.
Shaw RJ, Walsh GM, Cromwell O, Moqbel R, Spry CJ and Kay AB (1985) Activated human eosinophils generate SRS-A leukotrienes following IgG-dependent stimulation. *Nature (Lond)* **316:**150–152.
Shearman MS, Naor Z, Sekiguchi K, Kishimoto A and Nishizuka Y (1989) Selective activation of the γ-subspecies of protein kinase C from bovine cerebellum by arachidonic acid and its lipoxygenase metabolites. *FEBS Lett* **243:**177–182.
Sher A, Coffman RL, Hieny S and Cheever AW (1990) Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. *J Immunol* **145:**3911–3916.
Sher R and Wadee AA (1981) Eosinophil degranulation. Monitoring by interference contrast microscopy. *Inflammation* **5:**37–53.
Sherr CJ (1993) Mammalian G1 cyclins. *Cell* **73:**1059–1065.
Shi H, Qin S, Huang G, Chen Y, Xiao C, Xu H, Liang G, Xie Z, Qin X, Wu J, Li G and Zhang C (1997) Infiltration of eosinophils into the asthmatic airways caused by interleukin 5. *Am J Respir Cell Mol Biol* **16:**220–224.
Shi H, Xiao C, Zhong D, Qin SM, Lui Y, Liang G, Xu H, Chen Y, Long X and Xie Z (1998) Effect of inhaled interleukin-5 on airway hyper-reaactivity and eosinophilia in asthmatics. *Am J Respir Crit Care Med* **157:**204–209.
Shi L, Nishioka WK, Th'ng T, Bradbury EM, Litchfield DW and Greenberg AH (1994) Premature $p34^{cdc2}$ activation required for apoptosis. *Science (Wash DC)* **263:**1143–1145.
Shi Y, Glynn JM, Guilbert LJ, Cotter TG, Bissonette RP and Green DR (1992) Role for c-*myc* in activation-induced apoptotic cell death in T cell hybridomas. *Science (Wash DC)* **257:**212–214.
Shikata Y, Hayashi Y, Yoshimatsu K, Ohya Y, Seto T, Fukushima K and Yoshida Y (1993) Pro-major basic protein has three types of sugar chains at the pro portion. *Biochim Biophys Acta* **1163:**243–249.
Shimizu Y and Shaw S (1993) Cell adhesion. Mucins in the mainstream. *Nature (Lond)* **366:**630–631.
Shindo K and Fukumura M (1996) Azelastine hydrochloride inhibits platelet activating factor-like activity in human eosinophils. *Prostaglandins Leukotr Essent Fatty Acids* **55:**217–221.
Shindo K, Koide K and Fukumura M (1997) Paf-induced eosinophil chemotaxis increases during an asthmatic attack and is inhibited by prednisolone *in vivo* and *in vitro. Biochem Biophys Res Commun* **237:**146–151.
Shindo K, Koide K, Hirai Y, Sumitomo M and Fukumura M (1996) Priming effect of platelet activating factor on leukotriene C_4 from stimulated eosinophils of asthmatic patients. *Thorax* **51:**155–158.
Shlopov BV and Hasty KA (1998) Collagenase expression by normal human eosinophils. *J Leukoc Biol* **63:**451–455.
Shult PA, Graziano FM, Wallow IH and Busse WW (1985) Comparison of superoxide generation and luminol-dependent chemiluminescence with eosinophils and neutrophils from normal individuals. *J Lab Clin Med* **106:**638–645.
Shult PA, Lega M, Jadidi S, Vrtis R, Warner T, Graziano FM and Busse WW (1988) The presence of hypodense eosinophils and diminished chemiluminescence response in asthma. *J Allergy Clin Immunol* **81:**429–437.
Shupack JL, Kenny C, Jondreau L, Eckman I, Gropper C and Stiller MJ (1992) Decreased peripheral blood eosinophil counts in severe psoriatic patients treated with low-dose cyclosporin A. *Dermatology* **185:**202–204.
Shute JK, Rimmer SJ, Akerman CL, Church MK and Holgate ST (1990) Studies of cellular mechanisms for the generation of superoxide by guinea-pig eosinophils and its dissociation from granule peroxidase release. *Biochem Pharmacol* **40:**2013–2021.
Sigal CE, Valone FH, Holtzman MJ and Goetzl EJ (1987) Preferential human eosinophil chemotactic activity of the platelet-activating factor (PAF) 1–0-hexadecyl-2-acetyl-sn-glyceryl-3-phosphocholine (AGEPC). *J Clin Immunol* **7:**179–184.
Sigal E, Craik CS, Highland E, Grunberger D, Costello LL, Dixon RAF and Nadel JA (1988a) Molecular cloning and primary structure of human 15-lipoxygenase. *Biochem Biophy Res Commun* **157:**457–464.
Sigal E, Dicharry S, Highland E, Finkbeiner WE (1992) Cloning of human airway 15-lipoxygenase: idenity to the reticulocyte enyzme and expression in epithelium. *Am J Physiol* **262:**L392–L398.
Sigal E, Grunberger D, Cashman JR, Craik CS, Caughey GH and Nadel JA (1988b) Arachidonate 15-lipoxygenase from human eosinophil-enriched leukocytes: partial purification and properties. *Biochem Biophys Res Commun* **150:**376–383.
Sigal E, Grunberger D, Craik CS, Caughey GH and Nadel JA (1988c) Arachidonate 15-lipoxygenase (ω-6 lipoxygenase) from human leukocytes. Purification and structural homology to other mammalian lipoxygenases. *J Biol Chem* **263:**5328–5332.
Sihra BS, Kon OM, Grant JA and Kay AB (1997) Expression of high-affinity IgE receptors (FcεRI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects—Relationship to total serum IgE concentrations. *J Allergy Clin Immunol* **99:**699–706.
Silbaugh SA, Stengel PW, Williams GD, Herron DK, Gallagher P and Baker SR (1987) Effects of leukotriene B_4 inhalation. Airway sensitization and lung granulocyte infiltration in the guinea pig. *Am Rev Respir Dis* **136:**930–934.
Silberstein DS and David JR (1986) Tumor necrosis factor enhances eosinophil toxicity to *Schistosoma mansoni* larvae. *Proc Natl Acad Sci USA* **83:**1055–1059.
Silberstein DS, Owen WF, Gasson JC, DiPersio JF, Golde DW, Bina JC, Soberman R, Austen KF and David JR (1986) Enhancement of human eosinophil cytotoxicity and leukotriene synthesis by biosynthetic (recombinant) granulocyte/macrophage colony-stimulating factor. *J Immunol* **137:**3290–3294.
Sillaber C, Geissler K, Scherrer R, Kaltenbrunner R, Bettelheim P, Lechner K and Valent P (1992) Type beta transforming growth factors promote interleukin-3 (IL-3)-dependent differentiation of human basophils but inhibit IL-3-dependent differentiation of human eosinophils. *Blood* **80:**634–641.
Silva H, Tchernitchin AN and Tchernitchin NN (1997) Low doses of estradiol-17-alpha degranulate blood eosinophil leucocytes and high doses alter homeostatic mechanisms. *Med Sci Res* **25:**201–204.
Silva PM, Martins MA, Cordeiro RS and Vargaftig BB (1989) Late eosinophil mobilization induced by PAF-acether in the pleural cavity of rats. *Braz J Med Biol Res* **22:**1281–1285.
Simon HU (1997) Molecular mechanisms of defective eosinophil apoptosis in diseases associated with eosinophilia. *Int Arch Allergy Immunol* **113:**206–208.
Simon HU and Blaser K (1995) Inhibition of programmed eosinophil death: A key pathogenic event for eosinophilia? *Immunol Today* **16**: 53–55.
Simon HU, Yousefi S and Blaser K (1995a) Tyrosine phosphorylation regulates activation and inhibition of apoptosis in human eosinophils and neutrophils. *Int Arch Allergy Immunol* **107:**338–339.
Simon HU, Yousefi S, Dibbert B, Hebestreit H, Weber M, Branch DR, Blaser K, Levi-Schaffer F and Anderson GP (1998) Role for tyrosine phosphorylation and Lyn tyrosine kinase in fas receptor-mediated apoptosis in eosinophils. *Blood* **92:**547–557.
Simon HU, Yousefi S, Dibbert B, Levi-Schaffer F, Blaser K (1997a) Anti-apoptotic signals of granulocyte/macrophage colony-stimulating factor are transduced via Jak2 tyrosine kinase in eosinophils. *Eur J Immunol* **27:**3536–3539.
Simon HU, Yousefi S, Schranz C, Schapowal A, Bachert C and Blaser K (1997b) Direct demonstration of delayed eosinophil apoptosis as a mechanism causing tissue eosinophilia. *J Immunol* **158:**3902–3908.
Simon HU, Yousefi S, Weber M, Simon D, Holzer C, Hartung K and Blaser K (1995b) Human peripheral blood eosinophils express and release interleukin-8. *Int Arch Allergy Immunol* **107:**124–126.
Simmons D and Seed B (1988) The Fcγ receptor of natural killer cells is a phospholipid-linked membrane protein. *Nature (Lond)* **333:**568–570.
Sironi M, Sciacca FL, Matteucci C, Conni M, Vecchi A, Bernasconi S, Minty A, Caput D, Ferrara P, Colotta F and Mantovani A (1994) Regulation of endothelial and mesothelial cell function by interleukin-13: Selective induction of vascular cell adhesion molecule-1 and amplification of interleukin-6 production. *Blood* **84:**1913–1921.
Skoner DP, Page R, Asman B, Gillen L and Fireman P (1988) Plasma elevations of histamine and a prostaglandin metabolite in acute asthma. *Am Rev Respir Dis* **137:**1009–1014.
Slifman NR, Loegering DA, McKean DJ and Gleich GJ (1986) Ribonuclease activity associated with human eosinophil-derived neurotoxin and eosinophil cationic protein. *J Immunol* **137:**2913–2917.
Slifman NR, Venge P, Peterson CG, McKean DJ and Gleich GJ (1989) Human eosinophil-derived neurotoxin and eosinophil protein X are likely the same protein. *J Immunol* **143:**2317–2322.
Slovick FT, Abboud CN, Brennan JK and Lichtman MA (1985) Modulation of *in vitro* eosinophil progenitors by hydrocortisone: Role of accessory cells and interleukins. *Blood* **66:**1072–1079.
Slungaard A, Vercellotti GM, Walker G, Nelson RD and Jacob HS (1990) Tumor necrosis factor α/cachectin stimulates eosinophil oxidant production and toxicity towards human endothelium. *J Exp Med* **171:**2025–2041.
Smith CA, Farrah T and Goodwin RG (1994) The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. *Cell* **76:**959–962.
Smith DM, Gerrard JM and White JG (1987) Comparison of arachidonic acid metabolism in nasal polyps and eosinophils. *Int Arch Allergy Appl Immunol* **82:**83–88.
Smith JB, Kunjummen RD, Kishimoto TK and Anderson DC (1992) Expression and regulation of L-selectin on eosinophils from human adults and neonates. *Pediatr Res* **32:**465–471.
Smith KJ, Scotland G, Beattie J, Trayer IP and Houslay MD (1996b) Determination of the structure of the N-terminal splice region of the cyclic AMP-specific phosphodiesterase RD1 (RNPDE4A1) by ^{1}H NMR and identification of the membrane association domain using chimeric constructs. *J Biol Chem* **271:**16703–16711.
Smith WL, Garavito RM and DeWitt DL (1996a) Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J Biol Chem* **271:**33157–33160.
Snyder F (1987) The significance of dual pathways for the biosynthesis of platelet-activating factor: 1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, in *New Horizons in Platelet-Activating Factor Research* (Winslow CM and Lee ML eds) pp 13–25, John Wiley, New York.
Snyder SH and Snowman AM (1987) Receptor effects of cetirizine? *Ann Allergy* **59:**4–8.

Snyman JR, Sommers DK, Gregorowski MD and Boraine H (1992) Effect of cetirizine, ketotifen and chlorpheniramine on the dynamics of the cutaneous hypersensitivity reaction: A comparative study. *Eur J Clin Pharmacol* **42:**359–362.
Soderling SH, Bayuga SJ and Beavo JA (1998) Identification and characterization of a novel family of cyclic nucleotide phosphodiesterases. *J Biol Chem* **273:**15553–15558.
Sokol RJ, Hudson G, Brown MJ, Wales J and James NT (1988) Hypereosinophilic syndrome: A case report and morphometric study. *Acta Haematol* **79:**107–111.
Sokol RJ, James NT, Wales J and Hudson G (1987) Morphometry of eosinophils in human blood. *Acta Anat* **129:**211–213.
Solley GO, Maldonado JE, Gleich GJ, Giulani ER, Hoagland HC, Pierre RV and Brown AL (1976) Endomyocardiopathy with eosinophilia. *Mayo Clin Proc* **51:**697–708.
Solomon A, Aloe L, Pe'er J, Frucht-Pery J, Bonini S, Bonini S and Levi-Schaffer F (1998) Nerve growth factor is preformed in and activates human peripheral blood eosinophils. *J Allergy Clin Immunol* **102:**454–460.
Someya A, Nagaoka I, Iwabuchi K and Yamashita T (1991) Comparison of O_2^--producing activity of guinea-pig eosinophils and neutrophils in a cell-free system. *Comp Biochem Physiol B* **100:**25–30.
Sorice F and De Simone C (1986) Human eosinophil heterogeneity. *Ric Clin Lab* **16:**429–434.
Sorrentino S and Glitz DG (1991) Ribonuclease activity and substrate preference of human eosinophil cationic protein (ECP). *FEBS Lett* **288:**23–26.
Sorrentino S, Glitz DG, Hamann KJ, Loegering DA, Checkel JL and Gleich GJ (1992) Eosinophil-derived neurotoxin and human liver ribonuclease. Identity of structure and linkage of neurotoxicity to nuclease activity. *J Biol Chem* **267:**14859–14865.
Soto J, Tchernitchin AN, Poloni P, Voigt G, Caro B and Agurto M (1989) Effect of ketotifen on the distribution and degranulation of uterine eosinophils in estrogen-treated rats. *Agents Actions* **28:**198–203.
Souness JE, Carter CM, Diocee BK, Hassall GA, Wood LJ and Turner NC (1991) Characterization of guinea-pig eosinophil phosphodiesterase activity. Assessment of its involvement in regulating superoxide generation. *Biochem Pharmacol* **42:**937–945.
Souness JE, Griffin M, Maslen C, Ebsworth K, Scott LC, Pollock K, Palfreyman MN and Karlsson JA (1996) Evidence that cyclic AMP phosphodiesterase inhibitors suppress TNFα generation from human monocytes by interacting with a "low-affinity" phosphodiesterase 4 conformer. *Br J Pharmacol* **118:**649–658.
Souness JE, Houghton C, Sardar N and Withnall MT (1997) Evidence that cyclic AMP phosphodiesterase inhibitors suppress interleukin-2 release from murine splenocytes by interacting with a "low affinity" phosphodiesterase 4 conformer. *Br J Pharmacol* **121:**743–750.
Souness JE, Maslen C and Scott LC (1992) Effects of solubilization and vanadate/glutathione complex on inhibitor potencies against eosinophil cyclic AMP-specific phosphodiesterase. *FEBS Lett* **302:**181–184.
Souness JE, Maslen C, Webber S, Foster M, Raeburn D, Palfreyman MN, Ashton MJ and Karlsson JA (1995) Suppression of eosinophil function by RP 73401, a potent and selective inhibitor of cyclic AMP-specific phosphodiesterase: comparison with rolipram. *Br J Pharmacol* **115:**39–46.
Souness JE and Rao S (1997) Proposal for pharmacologically distinct conformers of PDE4 cyclic AMP phosphodiesterases. *Cell Signalling* **9:**227–236.
Souness JE and Scott LC (1993) Stereospecificity of rolipram actions on eosinophil cyclic AMP-specific phosphodiesterase. *Biochem J* **291:**389–395.
Souness JE, Thompson WJ and Strada SJ (1985) Adipocyte cyclic nucleotide phosphodiesterase activation by vanadate. *J Cyclic Nucleotide Res* **10:**383–396.
Souness JE, Villamil ME, Scott LC, Tomkinson A, Giembycz MA and Raeburn D (1994) Possible role of cyclic AMP phosphodiesterases in the actions of ibudilast on eosinophil thromboxane generation and airways smooth muscle tone. *Br J Pharmacol* **111:**1081–1088.
Sowden JM, Berth-Jones J, Ross JS, Motley RJ, Marks R, Finlay AY, Salek MS, Graham-Brown RA, Allen BR and Camp RD (1991) Double-blind, controlled, cross-over study of cyclosporin in adults with severe refractory atopic dermatitis. *Lancet* **338:**137–140.
Soyombo O, Spur BW and Lee TH (1994) Effects of lipoxin A_4 on chemotaxis and degranulation of human eosinophils stimulated by platelet-activating factor and N-formyl-L-methionyl-L-leucyl-L-phenylalanine. *Allergy* **49:**230–234.
Spada CS, Krauss AH, Nieves AL and Woodward DF (1997) Effects of leukotrienes B_4 (LTB_4) and D_4 (LTD_4) on motility of isolated normodense human eosinophils and eosinophils. *Adv Exp Biol Med* **400B:**699–706.
Spada CS, Nieves AL, Krauss AH and Woodward DF (1994) Comparison of leukotriene B_4 and D_4 effects on human eosinophil and neutrophil motility *in vitro*. *J Leukoc Biol* **55:**183–191.
Spada CS, Woodward DF, Hawley SB and Nieves AL (1986) Leukotrienes cause eosinophil emigration into conjunctival tissue. *Prostaglandins* **31:**795–809.
Spada CS, Woodward DF, Hawley SB, Nieves AL, Williams LS and Feldman BJ (1988) Synergistic effects of LTB_4 and LTD_4 on leukocyte emigration in to the guinea-pig conjunctiva. *Am J Pathol* **130:**354–368.
Spector AA, Gordon JA and Moore SA (1988) Hydroxyeicosatetraenoic acids (HETEs). *Prog Lipid Res* **27:**271–323.
Spector SL, Wangaard CH and Farr RS (1979) Aspirin and concomitant idiosyncrasies in adult asthmatic patients. *J Allergy Clin Immunol* **64:**500–506.
Spence DPS, Johnson SL, Calverley PMA, Dhillon P, Higgins C, Ramhamadany E, Turner S, Winning A, Winter J and Holgate ST (1994) The effect of orally active platelet activating factor antagonist WEB 2086 in the treatemt of asthma. *Am J Respir Crit Care Med* **149:**1142–1148.
Spencer FER, Faulds D and Peters DH (1993) Cetirizine: A reapraissal of its pharmacological properties and therapeutic use in selected allergic disorders. *Drugs* **46:**1055–1080.
Springer TA and Lasky LA (1991) Cell adhesion. Sticky sugars for selectins. *Nature (Lond)* **349:**196–197.
Spry CJ (1988) *Eosinophils. A Comprehensive Review, and Guide to the Scientific and Medical Literature*, Oxford University Press, Oxford.
Spry CJ and Tai PC (1976) Studies on blood eosinophils. II. Patients with Loffler's cardiomyopathy. *Clin Exp Immunol* **24:**423–434.
Spry CJF (1993) The natural history of eosinophils, in *Immunopharmacology of Eosinophils* (Smith H and Cook RM eds) pp 1–9, Academic Press, London.
Spry CJF, Kumaraswami V and Tai PC (1986) The effect of nedocromil sodium on secretion from human eosinophils. *Eur Respir J* **69:**241–243.
Sriramarao P, Norton CR, Borgstrom P, Discipio RG, Wolitzky BA and Broide DH (1996) E-selectin preferentially supports neutrophil but not eosinophil rolling under conditions of flow *in vitro* and *in vivo*. *J Immunol* **157:**4672–4680.
Sriramarao P, von Andrian UH, Butcher EC, Bourdon MA and Broide DH (1994) L-selectin and very late antigen-4 integrin promote eosinophil rolling at physiological shear rates *in vivo*. *J Immunol* **153:**4238–4246.
Stamatiou P, Hamid Q, Taha R, Yu W, Issekutz TB, Rokach J, Khanapure SP and Powell WS (1998) 5-Oxo-ETE induces pulmonary eosinophilia in an integrin-dependent manner in Brown Norway rats. *J Clin Invest* **102:**2165–2172.
Staunton DE, Dustin ML, Erickson HP and Springer TA (1990) The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and rhinovirus. *Cell* **61:**243–254.
Steinbach KH, Schick P, Trepel F, Raffler H, Dohrmann J, Heilgeist G, Heltzel W, Li K, Past W, van der Woerd de Lange JA, Themel H, Fliedner TM and Begemann H (1979) Estimation of kinetic parameters of neutrophilic, eosinophilic, and basophilic granulocytes in human blood. *Blut* **39:**27–38.
Steinhilber D and Roth HJ (1989) New series of lipoxins isolated from human eosinophils. *FEBS Lett* **255:**143–148.
Stellato C, Collins P, Ponath PD, Soler D, Newman W, La Rosa G, Li H, White J, Schwiebert LM, Bickel C, Liu M, Bochner BS, Williams T and Schleimer RP (1997) Production of the novel C-C chemokine MCP-4 by airway cells and comparison of its biological activity to other C-C chemokines. *J Clin Invest* **99:**926–936.
Stern M, Meagher L, Savill J and Haslett C (1992) Apoptosis in human eosinophils. Programmed cell death in the eosinophil leads to phagocytosis by macrophages and is modulated by IL-5. *J Immunol* **148:**3543–3549.
Stern M, Savill J and Haslett C (1996) Human monocyte-derived macrophage phagocytosis of senescent eosinophils undergoing apoptosis—Mediation by $\alpha_v\beta_3$/CD36/thrombospondin recognition mechanism and lack of phlogistic response. *Am J Pathol* **149:**911–921.
Sug JW (1989) Ultrastructural localization of lysozyme in human colon eosinophils using the protein A-gold technique: Effects of processing on probe distribution. *J Histochem Cytochem* **37:**709–714.
Stuart SG, Trounstine ML, Vaux DJ, Koch T, Martens CL, Mellman I and Moore KW (1987) Isolation and expression of cDNA clones encoding a human receptor for IgG (FcγRII). *J Exp Med* **166:**1668–1684.
Sturton G and Norman P (1991) Prostanoid receptors on human PNM and eosinophils (Abstract). *Am Rev Respir Dis* **143:**A641.
Subramanian N (1992) Leukotriene B_4 induced steady state calcium rise and superoxide anion generation in guinea pig eosinophils are not related events. *Biochem Biophys Res Commun* **187:**670–676.
Sugasawa T, Imanishi N and Morooka S (1991) Effect of the selective PAF antagonist SM 10661 on an asthmatic model. 2. Effect on antigen-induced dual asthmatic response and infiltration of leukocytes into airways in actively sensitized conscious guinea-pigs. *Lipids* **26:**1305–1309.
Sugasawa T and Morooka S (1992) Effect of BRL-35135 on LTB_4-induced guinea pig eosinophil chemotaxis. *Agents Actions* **37:**232–237.
Sugiyama H, Okada C, Bewtra AK, Hopp RJ and Townley RG (1992) The effect of formoterol on the late asthmatic phenomenon in guinea-pigs. *J Allergy Clin Immunol* **89:**858–866.
Sullivan M, Egerton M, Shakur Y, Marquardsen A and Houslay MD (1994a) Molecular cloning and expression, in both COS-1 cells and S. cerevisiae, of a human cytosolic type-IVA, cyclic AMP specific phosphodiesterase (hPDE-IVA-h6.1). *Cell Signal* **6:**793–812.
Sullivan PJ, Bekir S, Jaffar Z, Page CP and Costello JF (1994b) The effect of low dose theophylline on the bronchial wall infiltrate after antigen challenge. *Lancet* **343:**1006–1008.
Sullivan S and Broide DH (1996) Compartmentalization of eosinophil granulocyte/macrophage colony-stimulating factor expression in patients with asthma. *J Allergy Clin Immunol* **97:**966–976.
Sun FF, Crittenden NJ, Czuk CI, Taylor BM, Stout BK and Johnson HG (1991) Biochemical and functional differences between eosinophils from animal species and man. *J Leukoc Biol* **50:**140–150.
Sun FF, Czuk CI and Taylor BM (1989) Arachidonic acid metabolism in guinea pig eosinophils: Synthesis of thromboxane B_2 and leukotriene B_4 in response to soluble or particulate activators. *J Leukoc Biol* **46:**152–160.
Sun L, Li MS, Fisher LM and Spry CJ (1995a) Expression in *Escherichia coli* and purification of human eosinophil-derived neurotoxin with ribonuclease activity. *Protein Expr Purif* **6:**685–692.
Sun Z, Yergeau DA, Tuypens T, Tavernier J, Paul CC, Baumann MA, Tenen DG and Ackerman SJ (1995b) Identification and characterization of a functional promoter region in the human eosinophil IL-5 receptor-α subunit gene. *J Biol Chem* **270:**1462–1471.
Sun Z, Yergeau DA, Wong IC, Tuypens T, Tavernier J, Paul CC, Baumann MA, Auron PE, Tenen DG and Ackerman SJ (1996) Interleukin-5 receptor-α subunit gene regulation in human eosinophil development: Identification of a unique *cis*-element that acts like an enhancer in regulating activity of the IL-5R-α promoter. *Curr Top Microbiol Immunol* **211:**173–187.
Sung KLP, Yang L, Elices M, Jin G, Sriramarao P and Broide DH (1997) Granulocyte/macrophage colony-stimulating factor regulates the functional adhesive state of very late antigen-4 expressed by eosinophils. *J Immunol* **158:**919–927.
Sur S, Lam J, Bouchard P, Sigounas A, Holbert D and Metzger WJ (1996) Immunomodulatory effects of IL-12 on allergic lung inflammation depends on timing and doses. *J Immunol* **157:**4173–4180.
Swinnen JV, Joseph DR and Conti M (1989a) The mRNA encoding a high-affinity

cAMP phosphodiesterase is regulated by hormones and cAMP. *Proc Natl Acad Sci USA* **86:**8197–8201.
Swinnen JV, Joseph DR and Conti M (1989b) Molecular cloning of rat homologues of the *Drosophila melanogaster* dunce cAMP phosphodiesterase: evidence for a family of genes The mRNA encoding a high-affinity cAMP phosphodiesterase is regulated by hormones and cAMP. *Proc Natl Acad Sci USA* **86:**8197–8201.
Symon FA, Lawrence MB, Williamson ML, Walsh GM, Watson SR and Wardlaw AJ (1996) Functional and structural characterization of the eosinophil P-selectin ligand. *J Immunol* **157:**1711–1719.
Symon FA, Walsh GM, Watson SR and Wardlaw AJ (1994) Eosinophil adhesion to nasal polyp endothelium is P-selectin-dependent. *J Exp Med* **180:**371–376.
Szeczelik A, Nikankowska E, Dworski R, Domagala B and Pinis G (1991) Cyclosporin for steroid-dependent asthma. *Allergy* **46:**312–315.
Taborda Barata L, Jacobson M, Walker S, Njuki F, Ying S, Durham SR and Kay AB (1996) Effect of cetirizine and prednisolone on cellular infiltration and cytokine mRNA expression during allergen-induced late cutaneous responses. *Clin Exp Allergy* **26:**69–78.
Tagari P, Pecheur EI, Scheid M, Brown P, Ford Hutchinson AW and Nicholson D (1993) Activation of human eosinophils and differentiated HL-60 cells by interleukin-5. *Int Arch Allergy Immunol* **101:**227–233.
Tai PC and Spry CJ (1976) Studies on blood eosinophils. I. Patients with a transient eosinophilia. *Clin Exp Immunol* **24:**415–422.
Tai PC and Spry CJ (1981) The mechanisms which produce vacuolated and degranulated eosinophils. *Br J Haematol* **49:**219–226.
Tai PC and Spry CJ (1990) The effects of recombinant granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin-3 on the secretory capacity of human blood eosinophils. *Clin Exp Immunol* **80:**426–434.
Tai PC, Spry CJ, Peterson C, Venge P and Olsson I (1984) Monoclonal antibodies distinguish between storage and secreted forms of eosinophil cationic protein. *Nature (Lond)* **309:**182–184.
Tai PC, Sun L and Spry CJ (1991) Effects of IL-5, granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-3 on the survival of human blood eosinophils *in vitro*. *Clin Exp Immunol* **85:**312–316.
Takafuji S, Bischoff SC, De Weck AL and Dahinden CA (1991) IL-3 and IL-5 prime normal human eosinophils to produce leukotriene C_4 in response to soluble agonists. *J Immunol* **147:**3855–3861.
Takafuji S, Bischoff SC, De Weck AL and Dahinden CA (1992) Opposing effects of tumor necrosis factor-α and nerve growth factor upon leukotriene C_4 production by human eosinophils triggered with N-formyl-methionyl-leucyl-phenylalanine. *Eur J Immunol* **22:**969–974.
Takafuji S, Tadokoro K and Ito K (1996) Effects of interleukin (IL)-3 and IL-5 on human eosinophil degranulation induced by complement components C3a and C5a. *Allergy* **51:**563–568.
Takafuji S, Tadokoro K, Ito K and Dahinden CA (1994) Degranulation from human eosinophils stimulated with C3a and C5a. *Int Arch Allergy Immunol* **104** (**Suppl 1):**27–29.
Takafuji S, Tadokoro K, Ito K and Dahinden CA (1995) Effects of physiologic soluble agonists on leukotriene C_4 production and degranulation by human eosinophils. *Int Arch Allergy Immunol* **108** (**Suppl 1):**36–38.
Takaki S, Kanazawa H, Shiiba M and Takatsu K (1994) A critical cytoplasmic domain of the interleukin-5 (IL-5) receptor α chain and its function in IL-5-mediated growth signal transduction. *Mol Cell Biol* **14:**7404–7413.
Takanaski S, Nonaka R, Xing Z, O'Byrne P, Dolovich J and Jordana M (1994) Interleukin 10 inhibits lipopolysaccharide-induced survival and cytokine production by human peripheral blood eosinophils. *J Exp Med* **180:**711–715.
Takano T, Fiore S, Maddox JF, Brady HR, Petasis NA and Serhan CN (1997) Aspirin-triggered 15-epi-lipoxin A_4 (LXA_4) and LXA_4 stable analogues are potent inhibitors of acute inflammation: Evidence for anti-inflammatory receptors. *J Exp Med* **185:**1693–1704.
Tamura N, Agrawal DK, Suliaman FA and Townley RG (1987) Effects of platelet activating factor on the chemotaxis of normodense eosinophils from normal subjects. *Biochem Biophys Res Commun* **142:**638–644.
Tamura N, Agrawal DK and Townley RG (1988) Leukotriene C_4 production from human eosinophils *in vitro*. Role of eosinophil chemotactic factors on eosinophil activation. *J Immunol* **141:**4291–4297.
Tamura N, Ishii N,Nakazawa M, Nagoya M, Yoshinari M, Amano T, Nakazima H and Minami M (1996) Requirement of CD80 and CD86 molecules for antigen presentation by eosinophils. *Scand J Immunol* **44:**229–238.
Tan WC and Bethel RA (1992) The effect of platelet activating factor antagonist on ozone-induced airway inflammation and bronchial hyperresponsiveness in guinea-pigs. *Am Rev Respir Dis* **146:**916–922.
Tan X, Hsueh W and Gonzalez Crussi F (1993) Cellular localization of tumor necrosis factor (TNF)-α transcripts in normal bowel and in necrotizing enterocolitis. Tnf gene expression by Paneth cells, intestinal eosinophils, and macrophages. *Am J Pathol* **142:**1858–1865.
Tanabe K, Takahashi K, Maeda M and Kimura I (1993) Formation of Charcot-Leyden crystals by human basophils in sputum and peripheral blood. *Acta Med Okayama* **47:**85–90.
Tanaka Y, Delaporte E, Dubucquoi S, Gounni AS, Porchet E, Capron A and Capron M (1994) Interleukin-5 messenger RNA and immunoreactive protein expression by activated eosinophils in lesional atopic dermatitis skin. *J Invest Dermatol* **103:**589–592.
Taniguchi N, Mita H, Saito H, Yui Y, Kajita T and Shida T (1985) Increased generation of leukotriene C_4 from eosinophils in asthmatic patients. *Allergy* **40:**571–573.
Tarayre JP, Aliaga M, Barbara M, Malfetes N, Vieu S and Tisne-Versaille J (1991a) Theophylline reduces pulmonary eosinophilia after various types of active anaphylactic shock in guinea-pigs. *J Pharm Pharmacol* **43:**877–879.
Tarayre JP, Aliaga M, Barbara M, Tisseyre N, Vieu S and Tisne-Versaille J (1991b) Pharmacological modulation of a model of bronchial inflammation after aerosol-induced active anaphylactic shock in conscious guinea-pigs. *Int J Immunopharmacol* **13:**349–356.
Tarayre JP, Aliaga M, Barbara M, Tisseyre N, Vieu S and Tisne-Versaille J (1992) Model of bronchial allergic inflammation in the Brown Norway rat: Pharmacological modulation. *Int J Immunopharmacol* **14:**847–855.
Tavassoli M (1981) Eosinophils, eosinophilia, and eosinophilic disorders. *Crit Rev Clin Lab Sci* **16:**35–83.
Tavernier J, Devos R, Cornelis S, Tuypens T, Van der Heyden J, Fiers W and Plaetinck G (1991) A human high affinity interleukin-5 receptor (IL5R) is composed of an IL5-specific α-chain and a β-chain shared with the receptor for GM-CSF. *Cell* **66:**1175–1184.
Tavernier J, Tuypens T, Plaetinck G, Verhee A, Fiers W and Devos R (1992) Molecular basis of the membrane-anchored and two soluble isoforms of the human interleukin 5 receptor α-subunit. *Proc Natl Acad Sci USA* **89:**7041–7045.
Taylor BM, Crittenden NJ, Bruden MN, Wishka DG, Morris J, Richards IM and Sun FF (1991) Biological activity of leukotriene B_4 analogs: Inhibition of guinea pig eosinophil migration *in vitro* by the 2,6-disubstituted pyridine analogs U-75,302 and U-75,485. *Prostaglandins* **42:**211–224.
Taylor BM, Czuk CI, Brunden MN, Wishka DG, Morris J and Sun FF (1989) Inhibition of *in vitro* guinea pig eosinophil chemotaxis by leukotriene B_4 antagonist U-75,302. *Adv Prostaglandin Thromboxane Leukotr Res* **19:**195–198.
Tchernitchin A (1979) The role of eosinophil receptors in the non-genomic response to oestrogens in the uterus. *J Steroid Biochem* **11:**417–424.
Tchernitchin A, Roorijck J, Tchernitchin X, Vandenhende J and Galand F (1974) Dramatic early increase in uterine eosinophils after oestrogen administration. *Nature (Lond)* **248:**142–143.
Tchernitchin A, Tchernitchin X and Galand P (1976) New concepts on the action of oestrogens in the uterus and the role of the eosinophil receptor system. *Differentiation* **5:**145–150.
Tchernitchin AN, Barrera J, Arroyo P, Mena MA, Vilches K and Grunert G (1985) Degranulatory action of estradiol on blood eosinophil leukocytes *in vivo* and *in vitro*. *Agents Actions* **17:**60–66.
Tchernitchin X, Tchernitchin A and Galand P (1976) Dynamics of eosinophils in the uterus after oestrogen administration. *Differentiation* **5:**151–154.
Tecoma ES, Motulsky HJ, Traynor AE, Omann GM, Muller H and Sklar LA (1986) Transient catecholamine modulation of neutrophil activation: Kinetic and intracellular aspects of isoproterenol action. *J Leukoc Biol* **40:**629–644.
Tedeschi A, Milazzo N and Miadonna A (1994) Nasal eosinophilia induced by PAF-acether is accompanied by the release of eosinophil cationic protein. *Eur Respir J* **7:**1445–1451.
Teixeira MM, Al-Rashed A, Rossi AG and Hellewell PG (1997a) Characterization of the prostanoid receptors mediating inhibition of PAF-induced aggregation of guinea-pig eosinophils. *Br J Pharmacol* **121:**77–82.
Teixeira MM, Das AM, Miotla JM, Perretti M and Hellewell PG (1998) The role of lipocortin-1 in the inhibitory action of dexamethasone on eosinophil trafficking in cutaneous inflammatory reactions in the mouse. *Br J Pharmacol* **123:**538–544.
Teixeira MM, Giembycz MA, Lindsay MA and Hellewell PG (1997b) Pertussis toxin shows distincts early signalling events in platelet-activating factor-, leukotriene B_4-, and C5a-induced eosinophil homotypic aggregation *in vitro* and recruitment *in vivo*. *Blood* **89:**4566–4573.
Teixeira MM and Hellewell PG (1997a) The effect of the selectin binding polysaccharide fucoidin on eosinophil recruitment *in vivo*. *Br J Pharmacol* **120:**1059–1066.
Teixeira MM and Hellewell PG (1997b) Evidence that the eosinophil is a cellular target for the inhibitory action of salmeterol on eosinophil recruitment *in vivo*. *Eur J Pharmacol* **323:**255–260.
Teixeira MM, Reynia S, Robinson M, Shock A, Williams TJ, Williams FM, Rossi AG and Hellewell PG (1994a) Role of CD18 in the accumulation of eosinophils and neutrophils and local oedema formation in inflammatory reactions in guinea-pig skin. *Br J Pharmacol* **111:**811–818.
Teixeira MM, Rossi AG, Giembycz MA and Hellewell PG (1996a) Effects of agents which elevate cyclic AMP on guinea-pig eosinophil homotypic aggregation. *Br J Pharmacol* **118:**2099–2106.
Teixeira MM, Rossi AG and Hellewell PG (1996b) Adhesion mechanisms involved in C5a-induced eosinophil homotypic aggregation. *J Leukoc Biol* **59:**389–396.
Teixeira MM, Rossi A, Williams TJ and Hellewell PG (1994b) Effect of phosphodiesterase isoenzyme inhibitors on cutaneous inflammation in the guinea-pig. *Br J Pharmacol* **112:**332–340.
Teixeira MM, Williams TJ, Au BT, Hellewell PG and Rossi AG (1995c) Characterization of eosinophil homotypic aggregation. *J Leukoc Biol* **57:**226–234.
Teixeira MM, Williams TJ and Hellewell PG (1993) E-Type prostaglandins enhance local oedema formation and neutrophil accumulation but suppress eosinophil accumulation in guinea-pig skin. *Br J Pharmacol* **110:**416–422.
Teixeira MM, Williams TJ and Hellewell PG (1995a) Anti-inflammatory effects of short-acting and long-acting β_2-adrenoceptor agonists in guinea pig skin. *Eur J Pharmacol* **272:**185–193.
Teixeira MM, Williams TJ and Hellewell PG (1995b) Mechanisms and pharmacological manipulation of eosinophil accumulation *in vivo*. *Trends Pharmacol Sci* **16:**418–423.
Teixeira MM, Williams TJ and Hellewell PG (1996c) Effects of dexamethasone and cyclosporin A on the accumulation of eosinophils in acute cutaneous inflammation in the guinea-pig. *Br J Pharmacol* **118:**317–324.
Ten RM, Butterfield JH, Kita H, Weiler DA, Fischkoff S, Ishizaka T, Sanderson CJ and Gleich GJ (1991) Eosinophil differentiation of human umbilical cord mononuclear cells and prolonged survival of mature eosinophils by murine EL-4 thymoma cell conditioned medium. *Cytokine* **3:**350–359.
Ten RM, Pease LR, McKean DJ, Bell MP and Gleich GJ (1989) Molecular cloning of the human eosinophil peroxidase. Evidence for the existence of a peroxidase multigene family. *J Exp Med* **169:**1757–1769.
Tenner AJ (1993) Functional aspects of the C1q receptors. *Behring Inst Mitt* **93:**241–253.

Tenor H, Hatzelmann A, Church MK, Schudt C and Shute JK (1996) Effects of theophylline and rolipram on leukotriene C_4 (LTC_4) synthesis and chemotaxis of human eosinophils from normal and atopic subjects. *Br J Pharmacol* **118:**1727–1735.

Tenscher K, Metzner B, Hofmann C, Schopf E, Norgauer J (1997) The monocyte chemoattractant protein-4 induces oxygen radical production, actin polymerisation, and CD11b up-regulation via a pertussis toxin-sensitive G-protein in human eosinophils. *Biochem Biophys Res Commun* **240:**32–35.

Tenscher K, Metzner B, Schopf E, Norgauer J and Czech W (1996) Recombinant human eotaxin induces oxygen radical production, Ca^{2+}-mobilization, actin reorganization, and CD11b upregulation in human eosinophils via a pertussis toxin-sensitive heterotrimeric guanine nucleotide-binding protein. *Blood* **88:**3195–3199.

Tepper CG, Jayadev S, Liu B, Bielawska A, Wolff R, Yonehara S, Hannun YA and Seldin MF (1995) Role of ceramide as an endogenous mediator of Fas-induced cytotoxicity. *Proc Natl Acad Sci USA* **92:**8443–8447.

Terada N, Konno A, Terada Y, Fukuda S, Yamashita T, Abe T, Shimada H, Ishida K, Yoshimura K, Tanaka Y, Ra C, Ishikawa K and Togawa K (1995) IL-4 upregulates FcεRI α-chain messenger RNA in eosinophils. *J Allergy Clin Immunol* **96:**1161–1169.

Terada N, Sagara H, Yagita H, Okumura K, Makino S, Konno A, Yamashita T, Togawa K and Ra C (1996) The effect of anti-VLA-4 monoclonal antibody on eosinophil accumulation and leukotriene production in nasal mucosa. *Acta Oto-Laryngologica* **116:**883–887.

Testi R, D'Ambrosio D, De Maria R and Santoni A (1994) The CD69 receptor: A multipurpose cell-surface trigger for hematopoietic cells. *Immunol Today* **15:**479–483.

Thomas EL, Bozeman PM, Jefferson MM and King CC (1995) Oxidation of bromide by the human leukocyte enzymes myeloperoxidase and eosinophil peroxidase. Formation of bromamines. *J Biol Chem* **270:**2906–2913.

Thompson AW (1993) Immunological effect of cyclosporin A, in *T-Cell-Directed Immunosuppresion* (Bach J ed) pp 26–50, Blackwell Scientific, Oxford.

Thompson AW, Woo J and Zeevi A (1994) The influence of FK506 on lymphocyte responses *in vitro* and *in vivo,* in *Immunosuppressive Drugs: Developments in Anti-Rejection Therapy* (Thompson AW and Starzl TE eds) pp 95–111, Edward Arnold, London.

Thorne KJ, Richardson BA, Mazza G and Butterworth AE (1990) A new method for measuring eosinophil activating factors, based on the increased expression of CR3 α-chain (CD11b) on the surface of activated eosinophils. *J Immunol Methods* **133:**47–54.

Thorne KJ, Richardson BA, Taverne J, Williamson DJ, Vadas MA and Butterworth AE (1986) A comparison of eosinophil-activating factor (EAF) with other monokines and lymphokines. *Eur J Immunol* **16:**1143–1149.

Tiffany HL, Alkhatib G, Combadiere C, Berger EA and Murphy PM (1998) CC Chemokine receptors 1 and 3 are differentially regulated by IL-5 during maturation of eosinophilic HL-60 cells. *J Immunol* **160:**1385–1392.

Tiffany HL, Handen JS and Rosenberg HF (1996) Enhanced expression of the eosinophil-derived neurotoxin ribonuclease (*RNS2*) gene requires interaction between the promoter and intron. *J Biol Chem* **271:**12387–12393.

Till JE and McCulloch FA (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiol Res* **14:**213–219.

Till JE, McCulloch FA and Siminovitch L (1964) A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. *Proc Natl Acad Sci USA* **51:**29–33.

Ting S, Zweiman B and Lavker R (1983) Terbutaline modulation of human allergic skin reactions. *J Allergy Clin Immunol* **71:**437–441.

Ting S, Zweiman B, Lavker R and Dunsky EH (1981) Histamine suppression of *in vivo* eosinophil accumulation and histamine release in human allergic reactions. *J Allergy Clin Immunol* **68:**65–71.

Todd R, Donoff BR, Chiang T, Chou MY, Elovic A, Gallagher GT and Wong DT (1991) The eosinophil as a cellular source of transforming growth factor-α in healing cutaneous wounds. *Am J Pathol* **138:**1307–1313.

Toh H, Ichikawa A and Narumiya S (1995) Molecular evolution of receptors for eicosanoids. *FEBS Lett* **361:**17–21.

Tohda Y, Muraki M, Iwanaga T, Kubo H, Fukuoka M and Nakajima S (1998) The effect of theophylline on blood and sputum eosinophils and ECP in patients with bronchial asthma. *Int J Immunopharmacol* **20:**173–181.

Tohda Y, Nakajima S, Shizawa T, Maeda K, Ohmori S, Satoh H, Ishii T and Kamitani T (1997) The inhibitory effect of TMK688, a novel anti-allergic drug having both 5-lipoxygenase inhibitory activity and anti-histamine activity, against bronchoconstriction, leukotriene production and inflammatory cell infiltration in sensitized guinea pigs. *Clin Exp Allergy* **27:**110–118.

Tomassini M, Tsicopoulos A, Tai PC, Gruart V, Tonnel AB, Prin L, Capron A and Capron M (1991) Release of granule proteins by eosinophils from allergic and nonallergic patients with eosinophilia on immunoglobulin-dependent activation. *J Allergy Clin Immunol* **88:**365–375.

Tominaga T, Watanabe A, Tsuji J, Haruki H and Koda A (1995) A similarity and a difference between two models of late eosinophil accumulation into the airway induced by antigen exposure in actively sensitized Brown Norway (BN) rats. *Gen Pharmacol* **26:**353–356.

Tomioka K, MacGlashan DW, Lichtenstein LM, Bochner BS and Schleimer RP (1993) GM-CSF regulates human eosinophil responses to F-Met peptide and platelet activating factor. *J Immunol* **151:**4989–4997.

Tool AT, Koenderman L, Kok PT, Blom M, Roos D and Verhoeven AJ (1992) Release of platelet-activating factor is important for the respiratory burst induced in human eosinophils by opsonized particles. *Blood* **79:**2729–2732.

Tool ATJ, Mul FPJ, Knol EF, Verhoeven AJ and Roos D (1996) The effect of salmeterol and nimesulide on chemotaxis and synthesis of PAF and LTC_4 by human eosinophils. *Eur Respir J* **9:**S141–S145.

Tooze SA, Flatmark T, Tooze J and Huttner WB (1991) Characterization of the immature secretory granule, an intermediate in granule biogenesis. *J Cell Biol* **115:**1491–1503.

Tornetta MA, Foley JJ, Sarau HM and Ames RS (1997) The mouse anaphylatoxin C3a receptor: Molecular cloning, genomic organization, and functional expression. *J Immunol* **158:**5277–5282.

Torphy TJ, Barnette MS, Hay DW and Underwood DC (1994) Phosphodiesterase IV inhibitors as therapy for eosinophil-induced lung injury in asthma. *Environ Health Perspect* **102 (Suppl 10):**79–84.

Torphy TJ, Zhou HL, Foley JJ, Sarau HM, Manning CD and Barnette MS (1995) Salbutamol up-regulates PDE4 activity and induces a heterologous desensitization of U937 cells to prostaglandin E_2: Implications for the therapeutic use of β-adrenoceptor agonists. *J Biol Chem* **270:**23598–23604.

Torpier G, Colombel JF, Mathieu Chandelier C, Capron M, Dessaint JP, Cortot A, Paris JC and Capron A (1988) Eosinophilic gastroenteritis: Ultrastructural evidence for a selective release of eosinophil major basic protein. *Clin Exp Immunol* **74:**404–408.

Tourkin A, Anderson T, LeRoy EC and Hoffman S (1993) Eosinophil adhesion and maturation is modulated by laminin. *Cell Adhes Commun* **1:**161–176.

Townley RG, Eda R, Hopp RJ, Bewtra AK and Gillen MS (1994) The effect of RP 59227, a platelet-activating factor antagonist, against antigen challenge and eosinophil and neutrophil chemotaxis in asthmatics. *J Lipid Mediat Cell Signal* **10:**345–353.

Triggiani M, Schleimer RP, Tomioka K, Hubbard WC and Chilton FH (1992) Characterization of platelet-activating factor synthesized by normal and granulocyte/macrophage colony-stimulating factor-primed human eosinophils. *Immunology* **77:**500–504.

Truong MJ, Gruart V, Liu FT, Prin L, Capron A and Capron M (1993) IgE-binding molecules (Mac-2/εBP) expressed by human eosinophils. Implication in IgE-dependent eosinophil cytotoxicity. *Eur J Immunol* **23:**3230–3235.

Tsai JJ, Yu LN and Wang SR (1993) Enhanced eosinophil luminol-dependent chemiluminescence and complement receptor expression by platelet-activating factor and interleukin-5. *Asian Pac J Allergy Immunol* **11:**5–11.

Tsunawaki S, Mizunari H, Nagata M, Tatsuzawa O and Kuratsuji T (1994) A novel cytosolic component, $p40^{phox}$, of respiratory burst oxidase associates with $p67^{phox}$ and is absent in patients with chronic granulomatous disease who lack $p67^{phox}$. *Biochem Biophys Res Commun* **199:**1378–1387.

Tsunoda H, Sakuma Y, Shirato M, Obaishi H, Harada K, Yamada K, Shimomura N, Machida Y, Yamatsu I and Katayama K (1991) Activity of a novel thienodiazepine derivative as a platelet-activating factor antagonist in guinea pig lungs. Effects on platelet-activating factor and allergen induced eosinophil accumulation. *Arzneimittelforschung* **41:**224–227.

Tsuyuki S, Bertrand C, Erard F, Trifilieff A, Tsuyuki J, Wesp M, Anderson GP and Coyle AJ (1995) Activation of the Fas receptor on lung eosinophils leads to apoptosis and the resolution of eosinophilic inflammation of the airways. *J Clin Invest* **96:**2924–2931.

Turk J, Maas RL, Brash AR, Roberts LJ and Oates JA (1982) Arachidonic acid 15-lipoxygenase products from human eosinophils. *J Biol Chem* **257:**7068–7076.

Turk J, Rand TH, Maas RL, Lawson JA, Brash AR, Roberts LJ, Colley DG and Oates JA (1983) Identification of lipoxygenase products from arachidonic acid metabolism in stimulated murine eosinophils. *Biochim Biophys Acta* **750:**78–90.

Turner CR, Andreson CJ, Smith WB and Watson JW (1994) Effects of rolipram on responses to acute and chronic antigen exposure in monkeys. *Am J Respir Crit Care Med* **149:**1153–1159.

Turner CR, Cohan VL, Cheng JB, Showell HJ, Pazoles CJ and Watson JW (1996) The *in vivo* pharmacology of CP-80,633, a selective inhibitor of phosphodiesterase 4. *J Pharmacol Exp Ther* **278:**1349–1355.

Twentyman OP, Finnerty JP, Harris A, Palmer J and Holgate ST (1990) Protection against allergen-induced asthma by salmeterol. *Lancet* **236:**1338–1342.

Twentyman OP, Finnerty JP and Holgate ST (1991) The inhibitory effect of nebulised albuterol on the early and late phase reactions and increase in airway responsiveness provoked by inhaled allergen in asthma. *Am Rev Respir Dis* **144:**782–787.

Twentyman OP, Sams VR and Holgate ST (1993) Albuterol and nedocromil sodium affect airway and leukocyte responses to allergen. *Am Rev Respir Dis* **147:**1425–1430.

Uehara M, Sawai T and Aeba K (1988) Clinical effect of ketotifen on atopic dermatitis with special attention to the influence of patient background factors. *Acta Dermatol (Kyoto)* **83:**211–214.

Ukena D, Krogel C, Dent G, Yukawa T, Sybrecht G and Barnes PJ (1989) PAF-receptors on eosinophils: Identification with a novel ligand, [^{3}H]WEB 2086. *Biochem Pharmacol* **38:**1702–1705.

Underwood DC, Kotzer CJ, Bochnowicz S, Osborn RR, Luttmann MA, Hay DW and Torphy TJ (1994) Comparison of phosphodiesterase III, IV and dual III/IV inhibitors on bronchospasm and pulmonary eosinophil influx in guinea pigs. *J Pharmacol Exp Ther* **270:**250–259.

Underwood DC, Osborn RR, Newsholme SJ, Torphy TJ and Hay DWP (1996) Persistent airway eosinophilia after leukotriene (LT) D_4 administration in the guinea-pig: modulation by the LTD_4 receptor antagonist, pranlikast, or an interleukin-5 monoclonal antibody. *Am J Respir Crit Care Med* **154:**850–857.

Underwood DC, Osborn RR, Novak LB, Matthews JK, Newsholme SJ, Undem BJ, Hand JM and Torphy TJ (1993) Inhibition of antigen-induced bronchoconstriction and eosinophil infiltration in the guinea pig by the cyclic AMP-specific phosphodiesterase inhibitor, rolipram. *J Pharmacol Exp Ther* **266:**306–313.

Underwood SL, Lewis SA and Raeburn D (1992) RP 59227, a novel PAF receptor antagonist: Effects in guinea-pig models of airway hyperreactivity. *Eur J Pharmacol* **210:**97–102.

Undritz E, Lang M and Vanoyi E (1956) La reaction peroxydasique des eosinophiles comme moyen de taxonomie. *Le Sang* **27:**513–515.

Valerius T, Repp R, Kalden JR and Platzer E (1990) Effects of IFN on human eosinophils in comparison with other cytokines. A novel class of eosinophil activators with delayed onset of action. *J Immunol* **145:**2950–2958.

van den Berg RH, Prins F, Faber-Krol M, Lynch NJ, Schwaeble W, van Es LA and

Daha MR (1997) Intracellular localization of the human receptor for the globular domains of C1q. *J Immunol* **158:**3909–3916.
van der Bruggen T, Caldenhoven E, Kanters D, Coffer P, Raaijmakers JA, Lammers JW and Koenderman L (1995) Interleukin-5 signaling in human eosinophils involves JAK2 tyrosine kinase and STAT-1α. *Blood* **85:**1442–1448.
van der Bruggen T, Kanters D, Tool AT, Raaijmakers JA, Lammers JW, Verhoven AJ, Koenderman L (1998) Cytokine-induced protein tyrosine phosphorylation is essential for cytokine priming of human eosinophils. *J Allergy Clin Immunol* **101:**103–109.
van der Bruggen T, Kok PT, Blom M, Verhoeven AJ, Raaijmakers JA, Lammers JW and Koenderman L (1993b) Transient exposure of human eosinophils to the protein kinase C inhibitors CGP 39–360, CGP 41–251, and CGP 44–800 leads to priming of the respiratory burst induced by opsonized particles. *J Leukoc Biol* **54:**552–557.
van der Bruggen T, Kok PT, Raaijmakers JA, Verhoeven AJ, Kessels RG, Lammers JW and Koenderman L (1993a) Cytokine priming of the respiratory burst in human eosinophils is Ca^{2+} independent and accompanied by induction of tyrosine kinase activity. *J Leukoc Biol* **53:**347–353.
van der Bruggen T and Koenderman L (1996) Signal transduction in eosinophils. *Clin Exp Allergy* **26:**880–891.
van Dijk TB, Bracke M, Caldenhoven E, Raaijmakers JAM, Lammers JWJ, Koenderman L and Degroot RP (1996) Cloning and characterization of Fcα-rb, a novel Fcα receptor (CD89) isoform expressed in eosinophils and neutrophils. *Blood* **88:**4229–4238.
van Dijk TB, Caldenhoven E, Raaijmakers JA, Lammers JW, Koenderman L and De Groot RP (1998) The role of transcription factor PU.1 in the activity of the intronic enhancer of the eosinophil-derived neurotoxin (*RNS2*) gene. *Blood* **91:**2126–2132.
Van Metre TE (1969) Adverse effects of inhalation of excessive amounts of nebulized isoproterenol in status asthmaticus. *J Allergy* **43:**101–113.
Van Oosterhout AJ, Ladenius AR, Savelkoul HF, Van Ark I, Delsman KC and Nijkamp FP (1993) Effect of anti-IL-5 and IL-5 on airway hyperreactivity and eosinophils in guinea pigs. *Am Rev Respir Dis* **147:**548–552.
Vancheri C, Gauldie J, Bienenstock J, Cox G, Scicchitano R, Stanisz A and Jordana M (1989) Human lung fibroblast-derived granulocyte/macrophage colony-stimulating factor (GM-CSF) mediates eosinophil survival *in vitro*. *Am J Respir Cell Mol Biol* **1:**289–295.
Varney V, Gaga M, Frew AJ, De vos R and Kay AB (1992) The effect of a single oral dose of prednisolone or cetirizine on inflammatory cells infiltrating allergen-induced cutaneous late-phase reactions in atopic subjects. *Clin Exp Allergy* **22:**43–49.
Vaux DL, Haecker G and Strasser A (1994) An evolutionary perspective on apoptosis. *Cell* **76:**777–779.
Vaziri C and Downes CP (1992) G-protein-mediated activation of turkey erythrocyte phospholipase C by β-adrenergic and P_{2Y}-purinergic receptors. *Biochem J* **284:** 917–922.
Vegad JL and Lancaster MC (1972) Eosinophil leucocyte-attracting effect of histamine in the sheep skin. *Indian J Exp Biol* **10:**147–148.
Venge J, Lampinen M, Hakansson L, Rak S and Venge P (1996) Identification of IL-5 and RANTES as the major eosinophil chemoattractants in the asthmatic lung. *J Allergy Clin Immunol* **97:**1110–1115.
Venge P (1993) Human eosinophil granule proteins: structure, function and release, in *Immunopharmacology of Eosinophils* (Smith H and Cook RM eds) pp 43–55, Academic Press, London.
Venge P and Dahl R (1989) Are blood eosinophil number and activity important for the development of the late asthmatic reaction after allergen challenge? *Eur Respir J Suppl* **6**:430s–434s.
Verhagen J, Bruynzeel PL, Koedam JA, Wassink GA, De Boer M, Terpstra GK, Kreukniet J, Veldink GA and Vliegenthart JF (1984) Specific leukotriene formation by purified human eosinophils and neutrophils. *FEBS Lett* **168:**23–28.
Vitale N, Mukai H, Rouot B, Thierse D, Aunis D and Bader MF (1993) Exocytosis in chromaffin cells. Possible involvement of the heterotrimeric GTP-binding protein Go. *J Biol Chem* **268:**14715–14723.
Vonderheide RH, Tedder TF, Springer TA and Staunton DE (1994) Residues within a conserved amino acid motif of domains 1 and 4 of VCAM-1 are required for binding to VLA-4. *J Cell Biol* **125:**215–222.
Wadee AA, Anderson R and Sher R (1980) *In vitro* effects of histamine on eosinophil migration. *Int Arch Allergy Appl Immunol* **63:**322–329.
Wahlgren CF, Sheynius A and Hagermark O (1990) Antipruritic effect of oral cyclosporin A in atopic dermatitis. *Acta Dermato-Venereol* **70:**323–329.
Walker BAM (1996) Effects of adenosine on guinea pig pulmonary eosinophils. *Inflammation* **20:**11–21.
Walker BAM, Jacobson MA, Knight DA, Salvatore CA, Weir T, Zhou DY and Bai TR (1997) Adenosine A_3 receptor expression and function in eosinophils. *Am J Respir Cell Mol Biol* **16:**531–537.
Walker JW, Gilbert SH, Drummond RM, Yamada M, Sreekumar R, Carraway RE, Ikebe M and Fay FS (1998) Signaling pathways underlying eosinophil cell motility revealed by using caged peptides. *Proc Natl Acad Sci USA* **95:**1568–1573.
Walle AJ and Parwaresch MR (1979) Estimation of effective eosinopoiesis and bone marrow eosinophil reserve capacity in normal man. *Cell Tissue Kinet* **12:**249–255.
Wallen N, Kita H, Weiler D and Gleich GJ (1991) Glucocorticoids inhibit cytokine-mediated eosinophil survival. *J Immunol* **147:**3490–3495.
Walsh DT, Weg VB, Williams TJ and Nourshargh S (1995) Substance P-induced inflammatory responses in guinea-pig skin: The effect of specific NK_1 receptor antagonists and the role of endogenous mediators. *Br J Pharmacol* **114**: 1343–1350.
Walsh GM (1993) The anti-inflammatory effects of cetirizine. *Clin Exp Allergy* **24:**81–85.
Walsh GM (1997a) Mechanisms of human eosinophil survival and apoptosis. *Clin Exp Allergy* **27:**482–487.
Walsh GM (1997b) The effects of cetirizine on the function on inflammatory cells involved in the allergic response. *Clin Exp Allergy* **27:**47–53.
Walsh GM, Hartnell A, Moqbel R, Cromwell O, Nagy L, Bradley B, Furitsu T, Ishizaka T and Kay AB (1990a) Receptor expression and functional status of cultured human eosinophils derived from umbilical cord blood mononuclear cells. *Blood* **76:**105–111.
Walsh GM, Hartnell A, Wardlaw AJ, Kurihara K, Sanderson CJ and Kay AB (1990b) IL-5 enhances the *in vitro* adhesion of human eosinophils, but not neutrophils, in a leucocyte integrin (CD11/18)-dependent manner. *Immunology* **71:**258–265.
Walsh GM and Kay AB (1986) Binding of immunoglobulin classes and subclasses to human neutrophils and eosinophils. *Clin Exp Immunol* **63:**466–472.
Walsh GM, Mermod JJ, Hartnell A, Kay AB and Wardlaw AJ (1991a) Human eosinophil, but not neutrophil, adherence to IL-1-stimulated human umbilical vascular endothelial cells is $\alpha_4\beta^1$ (very late antigen-4) dependent. *J Immunol* **146:**3419–3423.
Walsh GM, Moqbel R, Hartnell A and Kay AB (1991b) Effects of cetirizine on human eosinophil and neutrophil activation *in vitro*. *Int Arch Allergy Appl Immunol* **95:**158–162.
Walsh GM, Moqbel R, Nagakura T, Iikura Y and Kay AB (1990c) Enhancement of the expression of eosinophil IgE receptor (FcεR2) and its function by platelet-activating factor. *J Lipid Mediat* **2** (**Suppl):**S177–S186.
Walsh GM, Nagakura T and Iikura Y (1989) Flow-cytometric analysis of increased IgE uptake by normal eosinophils following activation with PAF-acether and other inflammatory mediators. *Int Arch Allergy Appl Immunol* **88:**194–196.
Walsh GM, Symon FA, Lazarovits AI and Wardlaw AJ (1996a) Integrin $\alpha_4\beta_7$ mediates human eosinophil interaction with MADCAM-1, VCAM-1 and fibronectin. *Immunology* **89:**112–119.
Walsh GM, Symon FA and Wardlaw AJ (1995) Human eosinophils preferentially survive on tissue fibronectin compared with plasma fibronectin. *Clin Exp Allergy* **25:**1128–1136.
Walsh GM, Williamson ML, Symon FA, Willars GB and Wardlaw AJ (1996b) Ligation of CD69 induces apoptosis and cell death in human eosinophils cultured with granulocyte/macrophage colony-stimulating factor. *Blood* **87:**2815–2821.
Walz TM, Nishikawa BK, Malm C, Briheim K and Wasteson A (1994) Transforming growth factor α expression in normal human blood eosinophils: Differential regulation by granulocyte/macrophage colony-stimulating factor and interleukin-3. *Leukemia* **8:**612–619.
Walz TM, Nishikawa BK, Malm C and Wasteson A (1993) Production of transforming growth factor α by normal human blood eosinophils. *Leukemia* **7:**1531–1537.
Wang XF, Lin HY, Ng-Eaton E, Downward J, Lodish HF and Weinberg RA (1991) Expression cloning and characterization of the TGF-β type III receptor. *Cell* **67:**797–805.
Wang J, Palmer K, Lotvall J, Milan S, Lei XF, Matthaei KI, Gauldie J, Inman MD, Jordana M and Xing Z (1998) Circulating, but not local lung, IL-5 is required for the development of antigen-induced airways eosinophilia. *J Clin Invest* **102:**1132–1141.
Wang JH, Trigg CJ, Devalia JL, Jordan S and Davies RJ (1994) Effect of inhaled beclomethasone dipropionate on expression of proinflammatory cytokines and activated eosinophils in the bronchial epithelium of patients with mild asthma. *J Allergy Clin Immunol* **94:**1025–1034.
Wang JM, Rambaldi A, Biondi A, Chen ZG, Sanderson CJ and Mantovani A (1989) Recombinant human interleukin 5 is a selective eosinophil chemoattractant. *Eur J Immunol* **19:**701–705.
Wang P, Myers JG, Wu P, Cheewatrakoolpong B, Egan RW and Billah MM (1997) Expression, purification, and characterization of human cAMP-specific phosphodiesterase (PDE4) subtypes A, B, C and D. *Biochem Biophys Res Commun* **234:** 320–324.
Wangemann P, Wittner M, Di Stefano A, Englert HC, Lang HJ, Schlatter E and Greger R (1986) Cl^--Channel blockers in the thick ascending limb of the loop of Henle. Structure activity relationship. *Pflugers Arch Eur J Physiol* **407:**S128–S141.
Ward AJM, McKenniff M, Evans JM, Page CP and Costello JF (1993) Theophylline—An immunomodulatory role in asthma. *Am Rev Respir Dis* **147:**518–523.
Wardlaw A (1995) Eosinophil density—What does it mean ? *Clin Exp Allergy* **25:** 1145–1149.
Wardlaw AJ, Hibbs ML, Stacker SA and Springer TA (1990) Distinct mutations in two patients with leukocyte adhesion deficiency and their functional correlates. *J Exp Med* **172:**335–345.
Wardlaw AJ, Moqbel R, Cromwell O and Kay AB (1986) Platelet-activating factor. A potent chemotactic and chemokinetic factor for human eosinophils. *J Clin Invest* **78:**1701–1706.
Wardlaw AJ, Moqbel R and Kay AB (1995) Eosinophils: Biology and role in disease. *Adv Immunol* **60:**151–266.
Warren DJ (1988) The biology of murine eosinophil differentiation factor (an overview). *Behring Inst Mitt* **83:**98–101.
Warringa RA, Koenderman L, Kok PT, Kreukniet J and Bruijnzeel PL (1991) Modulation and induction of eosinophil chemotaxis by granulocyte/macrophage colony-stimulating factor and interleukin-3. *Blood* **77:**2694–2700.
Warringa RA, Mengelers HJ, Kuijper PH, Raaijmakers JA, Bruijnzeel PL and Koenderman L (1992a) *In vivo* priming of platelet-activating factor-induced eosinophil chemotaxis in allergic asthmatic individuals. *Blood* **79:**1836–1841.
Warringa RA, Mengelers HJ, Maikoe T, Bruijnzeel PL and Koenderman L (1993a) Inhibition of cytokine-primed eosinophil chemotaxis by nedocromil sodium. *J Allergy Clin Immunol* **91:**802–809.
Warringa RA, Mengelers HJ, Raaijmakers JA, Bruijnzeel PL, Koenderman L (1993b) Upregulation of formyl-peptide and interleukin-8-induced chemotaxis in patients with allergic asthma. *J Allergy Clin Immunol* **91:**1198–1205.
Warringa RA, Schweizer RC, Maikoe T, Kuijper PH, Bruijnzeel PL and Koendermann L (1992b) Modulation of eosinophil chemotaxis by interleukin-5. *Am J Respir Cell Mol Biol* **7:**631–636.
Wasmoen TL, Bell MP, Loegering DA, Gleich GJ, Prendergast FG and McKean DJ (1988) Biochemical and amino acid sequence analysis of human eosinophil granule major basic protein. *J Biol Chem* **263:**12559–12563.

Watanabe M, Nittoh T, Suzuki T, Kitoh A, Mue S and Ohuchi K (1995) Isolation and partial characterization of eosinophil granule proteins in rats—Eosinophil cationic protein and major basic protein. *Int Arch Allergy Immunol* **108:**11–18.
Watanabe S, Itoh T and Arai K (1997) Roles of JAK kinase in human GM-CSF receptor signals. *Leukemia* **11** (**Suppl 3):**76–78.
Watkins AD, Hatfield CA, Fidler SF, Winterrowd GE, Brashler JR, Sun FF, Taylor BM, Vonderfecht SL, Conder GA, Holgate ST, Chin JE and Richards IM (1996) Phenotypic analysis of airway eosinophils and lymphocytes in a Th-2-driven murine model of pulmonary inflammation. *Am J Respir Cell Mol Biol* **15:**20–34.
Watson ML, Smith D, Bourne AD, Thompson RC and Westwick J (1993) Cytokines contribute to airway dysfunction in antigen-challenged guinea pigs: inhibition of airway hyperreactivity, pulmonary eosinophil accumulation, and tumor necrosis factor generation by pretreatment with an interleukin-1 receptor antagonist. *Am J Respir Cell Mol Biol* **8:**365–369.
Weber C, Kitayama J and Springer TA (1996) Differential regulation of β_1 and β_2 integrin avidity by chemoattractants in eosinophils. *Proc Natl Acad Sci USA* **93:**10939–10944.
Weber M and Dahinden CA (1995) Basophil and eosinophil activation by CC chemokines. *Int Arch Allergy Immunol* **107:**148–150.
Wedi B, Raap U, Lewrick H and Kapp A (1998) IL-4-induced apoptosis in peripheral blood eosinophils. *J Allergy Clin Immunol* **102:**1013–1020.
Weg VB, Watson ML, Faccioli LH and Williams TJ (1994) Investigation of the endogenous chemoattractants involved in ^{111}In-eosinophil accumulation in passive cutaneous anaphylactic reactions in the guinea-pig. *Br J Pharmacol* **113:**35–42.
Wegner CD, Clarke CC, Torcellini CA, Letts LG and Gundel RH (1992) Effects of single and multiple inhalations of platelet activating factor on airway composition and responsiveness in monkeys. *Clin Exp Allergy* **22:**51–57.
Wegner CD, Gundel RH, Abraham WM, Schulman ES, Kontny MJ, Lazer ES, Homon CA, Graham AG, Torcellini CA, Clarke CC, Jager P, Wolyniec WW, Letts G and Farina PR (1993) The role of 5-lipoxygenase products in preclinical models of asthma. *J Allergy Clin Immunol* **91:**917–929.
Weiler CR, Kita H, Hukee M and Gleich GJ (1996) Eosinophil viability during immunoglobulin-induced degranulation. *J Leukoc Biol* **60:**493–501.
Wein M, Sterbinsky SA, Bickel CA, Schleimer RP and Bochner BS (1995) Comparison of human eosinophil and neutrophil ligands for P-selectin: Ligands for P-selectin differ from those for E-selectin. *Am J Respir Cell Mol Biol* **12**: 315–319.
Weinstock JV (1991) Production of neuropeptides by inflammatory cells within the granulomas of murine schistosomiasis mansoni. *Eur J Clin Invest* **21:**145–153.
Weinstock JV and Blum AM (1989) Tachykinin production in granulomas of murine schistosomiasis mansoni. *J Immunol* **142:**3256–3261.
Weinstock JV and Blum AM (1990a) Detection of vasoactive intestinal peptide and localization of its mRNA within granulomas of murine schistosomiasis. *Cell Immunol* **125:**291–300.
Weinstock JV and Blum AM (1990b) Release of substance P by granuloma eosinophils in response to secretagogues in murine schistosomiasis mansoni. *Cell Immunol* **125:**380–385.
Weinstock JV, Blum A, Walder J and Walder R (1988) Eosinophils from granulomas in murine schistosomiasis mansoni produce substance P. *J Immunol* **141:**961–966.
Weiss SJ, Test ST, Eckmann CM, Roos D and Regiani S (1986) Brominating oxidants generated by human eosinophils. *Science (Wash DC)* **234:**200–203.
Weller PF (1991) The immunobiology of eosinophils. *N Engl J Med* **324:**1110–1118.
Weller PF (1993) Lipid, peptide and cytokine mediators elaborated by eosinophils, in *Immunopharmacology of Eosinophils* (Smith H and Cook RM eds) pp 25–42, Academic Press, London.
Weller PF (1994) Eosinophils: structure and functions. *Curr Opin Immunol* **6:**85–90.
Weller PF, Ackerman SJ, Nicholson Weller A and Dvorak AM (1989) Cytoplasmic lipid bodies of human neutrophilic leukocytes. *Am J Pathol* **135:**947–959.
Weller PF, Bach D and Austen KF (1981) Expression of lysophospholipase activity by intact human eosinophils and their Charcot-Leyden crystals. *Trans Assoc Am Physicians* **94:**165–171.
Weller PF, Bach D and Austen KF (1982) Human eosinophil lysophospholipase: The sole protein component of Charcot-Leyden crystals. *J Immunol* **128:**1346–1349.
Weller PF, Bach DS and Austen KF (1984) Biochemical characterization of human eosinophil Charcot-Leyden crystal protein (lysophospholipase). *J Biol Chem* **259:** 15100–15105.
Weller PF and Dvorak AM (1985) Arachidonic acid incorporation by cytoplasmic lipid bodies of human eosinophils. *Blood* **65:**1269–1274.
Weller PF and Dvorak AM (1994) Lipid bodies: intracellular sites for eicosanoid formation. *J Allergy Clin Immunol* **94:**1151–1156.
Weller PF, Goetzl EJ and Austen KF (1980) Identification of human eosinophil lysophospholipase as the constituent of Charcot-Leyden crystals. *Proc Natl Acad Sci USA* **77:**7440–7443.
Weller PF, Lee CW, Foster DW, Corey EJ, Austen KF and Lewis RA (1983) Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of leukotriene C_4. *Proc Natl Acad Sci USA* **80:** 7626–7630.
Weller PF, Monahan Earley RA, Dvorak HF and Dvorak AM (1991a) Cytoplasmic lipid bodies of human eosinophils. Subcellular isolation and analysis of arachidonate incorporation. *Am J Pathol* **138:**141–148.
Weller PF, Rand TH, Barrett T, Elovic A, Wong DT and Finberg RW (1993) Accessory cell function of human eosinophils. Hla-DR-dependent, MHC-restricted antigen-presentation and IL-1α expression. *J Immunol* **150:**2554–2562.
Weller PF, Rand TH, Goelz SE, Chi Rosso G and Lobb RR (1991b) Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecule-1 and endothelial leukocyte adhesion molecule-1. *Proc Natl Acad Sci USA* **88:**7430–7433.
Wenzel SE, Trudeau JB, Kaminsky DA, Cohn J, Martin RJ and Westcott JY (1995) Effect of 5-lipoxygenase inhibition on bronchoconstriction and airway inflammation in nocturnal asthma. *Am J Respir Crit Care Med* **152:**897–905.
Wetsel RA (1995) Structure, function and cellular expression of complement anaphylatoxin receptors. *Curr Opin Immunol* **7:**48–53.
Whelan CJ and Johnson M (1990) The anti-inflammatory effects of inhaled salmeterol and salbutamol in guinea-pig lung (Abstract). *Br J Pharmacol* **101:**528P.
Whelan CJ and Johnson M (1992) Inhibition by salmeterol of increased vascular permeability and granulocyte accumulation in guinea-pig lung and skin. *Br J Pharmacol* **105:**831–838.
Whelan CJ, Johnson M and Vardey CJ (1993) Comparison of the anti-inflammatory properties of formoterol, salbutamol and salmeterol in guinea-pig skin and lung. *Br J Pharmacol* **110:**613–619.
Whitcomb EA, Dinarello CA and Pincus SH (1989) Differential effects of interleukin-1α and interleukin-1β on human peripheral blood eosinophils. *Blood* **73:**1904–1908.
White SR, Strek ME, Kulp GV, Spaethe SM, Burch RA, Neeley SP and Leff AR (1993) Regulation of human eosinophil degranulation and activation by endogenous phospholipase A_2. *J Clin Invest* **91:**2118–2125.
Wiedermann FJ, Kahler CM, Reinisch N and Wiedermann CJ (1993) Induction of normal human eosinophil migration *in vitro* by substance P. *Acta Haematol* **89:**213–215.
Wiederrecht G, Lam E, Hung S, Martin M and Sigal N (1993) The mechanism of action of FK506 and cyclosporin A. *Ann NY Acad Sci* **696:**9–19.
Wiegers GJ and Reul JMHM (1998) Induction of cytokine receptors by glucocorticoids: Functional and pathological significance. *Trends Pharmacol Sci* **19:**317–321.
Wientjes FB, Hsuan JJ, Totty NF and Segal AW (1993) p40^{phox}, a third cytosolic component of the activation complex of the NADPH oxidase to contain *src* homology 3 domains. *Biochem J* **296:**557–561.
Wilde CG, Snable JL, Scott RW, Loegering DA and Gleich GJ (1992) Identification of eosinophil-derived neurotoxin as the major protein constituent of human basophil granules (Abstract). *FASEB J* **6:**A1722.
Williams CMM, Smith L, Flanagan BF, Clegg LS and Coleman JW (1997) Tumour necrosis factor-α expression and cell recruitment in Sephadex particle-induced lung inflammation: Effects of dexamethasone and cyclosporin A. *Br J Pharmacol* **122:**1127–1134.
Williams DM, Linder JE, Hill MW and Gillett R (1978) Ultrastructural localization of alkaline phosphatase in rat eosinophil leucocytes. *J Histochem Cytochem* **26:** 862–864.
Williams GT, Smith GA, Spooncer E, Dexter TM and Taylor TR (1990) Haemopoietic colony stimulating factors promote cell survival by suppressing apoptosis. *Nature (Lond)* **343:**76–79.
Wilson C, Wilson S, Piercy V, Sennitt MV and Arch JRS (1984) The rat lipolytic β-adrenoceptor: Studies using novel β-adrenoceptor agonists. *Eur J Pharmacol* **100:**309–319.
Wilson DJ, Fortner KA, Lynch DH, Mattingly RR, Macara IG, Posada JA and Budd RC (1996) JNK, but not MAPK, activation is associated with Fas-mediated apoptosis in human T-cells. *Eur J Immunol* **26:**989–994.
Winqvist I, Olofsson T and Olsson I (1984) Mechanisms for eosinophil degranulation; release of the eosinophil cationic protein. *Immunology* **51:**1–8.
Winqvist I, Olofsson T, Olsson I, Persson AM and Hallberg T (1982) Altered density, metabolism and surface receptors of eosinophils in eosinophilia. *Immunology* **47:**531–539.
Wise H and Jones RL (1994) Characterization of the prostanoid receptors on rat neutrophils. *Br J Pharmacol* **113:**581–587.
Wong DT, Donoff RB, Yang J, Song BZ, Matossian K, Nagura N, Elovic A, McBride J, Gallagher G, Todd R, Chiang T, Chou LS, Yung C, Galli SJ and Weller PF (1993) Sequential expression of transforming growth factors α and β_1 by eosinophils during cutaneous wound healing in the hamster. *Am J Pathol* **143:**130–142.
Wong DT, Elovic A, Matossian K, Nagura N, McBride J, Chou MY, Gordon JR, Rand TH, Galli SJ and Weller PF (1991) Eosinophils from patients with blood eosinophilia express transforming growth factor β_1. *Blood* **78:**2702–2707.
Wong DT, Weller PF, Galli SJ, Elovic A, Rand TH, Gallagher GT, Chiang T, Chou MY, Matossian K, McBride J and Todd R (1990) Human eosinophils express transforming growth factor α. *J Exp Med* **172:**673–681.
Woods JW, Coffey MJ, Brock TJ, Singer II and Peters-Golden M (1995) 5-Lipoxygenase is located in the euchromatin of the nucleus in resting human alveolar macrophages and translocates to the nuclear envelope upon cell activation. *J Clin Invest* **95:**2035–2046.
Woods JW, Evans JF, Ethier D, Scott S, Vickers PJ, Hearn L, Heibein JA, Charleson S and Singer II (1994) 5-Lipoxygenase and 5-lipoxygenase-activating protein are localized in the nuclear envelope of activated human leukocytes. *J Exp Med* **178:**1935–1946.
Woodward DF, Krauss AH, Nieves AL and Spada CS (1991) Studies on leukotriene D_4 as an eosinophil chemoattractant. *Drugs Exp Clin Res* **17:**543–548.
Woodward DF, Spada CS, Hawley SB and Nieves AL (1985) Histamine H_1- and H_2-receptor involvement in eosinophil infiltration and the microvascular changes associated with cutaneous anaphylaxis. *Agents Actions* **17:**121–125.
Woodward DF, Spada CS, Hawley SB and Nieves AL (1986) Conjunctival eosinophil infiltration evoked by histamine and immediate hypersensitivity. Modification by H_1- and H_2-receptor blockade. *Invest Ophthalmol Vis Sci* **27:**1495–1503.
Woolley MJ, Wattie J, Ellis R, Lane CG, Stevens WH, Woolley KL, Dahlback M and O'Byrne PM (1994a) Effect of an inhaled corticosteroid on airway eosinophils and allergen-induced airway hyperresponsiveness in dogs. *J Appl Physiol* **77:**1303–1308.
Woolley MJ, Woolley KL, Otis J, Conlon PD, O'Byrne P and Jordana (1994b) Inhibitory effect of IL-10 on allergen-induced airway inflammation and airway responses in Brown Norway rats (Abstract). *Am J Respir Crit Care Med* **149:**A760.
Wright CD, Kuipers PJ, Kobylarz-Singer D, Devall LJ, Klinkefus BA, Weishaar RE (1990) Differential inhibition of human neutrophil functions. Role of cyclic AMP-specific, cyclic GMP-insensitive phosphodiesterase. *Biochem Pharmacol* **40:**699–707.
Wright MD and Tomlinson MG (1994) The ins and outs of the transmembrane 4 superfamily. *Immunol Today* **15:**588–594.

Wu D, Kuang Y, Wu Y and Jiang H (1995) Selective coupling of β_2-adrenergic receptor to hematopoietic-specific G proteins. *J Biol Chem* **270:**16008–16010.
Wymann MP, Kernen P, Von Tscharner V, Tai PC, Spry CJ and Baggiolini M (1995) Activation of the respiratory burst in eosinophil leucocytes—A transduction sequence decoupled from cytosolic Ca^{2+} rise. *Eur J Clin Invest* **25:**25–31.
Wynn TA, Jankovic D, Hieny S, Zioncheck K, Jardieu P, Cheever AW and Sher A (1995) IL-12 exacerbates rather than suppresses T helper 2-dependent pathology in the absence of endogenous IFNγ. *J Immunol* **154:**3999–4009.
Xiao RP, Ji X and Lakatta EG (1995) Functional coupling of the β_2-adrenoceptor to a pertussis toxin-sensitive G protein in cardiac myocytes. *Mol Pharmacol* **47:**322–329.
Xu Q, Leiva MC, Fischkoff SA, Handschumacher RE and Lyttle CR (1992) Leukocyte chemotactic activity of cyclophilin. *J Biol Chem* **267:**11968–11971.
Yagisawa M, Yuo A, Yonemaru M, Imajoh Ohmi S, Kanegasaki S, Yazaki Y and Takaku F (1996) Superoxide release and NADPH oxidase components in mature human phagocytes: Correlation between functional capacity and amount of functional proteins. *Biochem Biophys Res Commun* **228:**510–516.
Yamaguchi Y, Ackerman SJ, Minegishi N, Takiguchi M, Yamamoto M and Suda T (1998) Mechanisms of transcription in eosinophils: GATA-1, but not GATA-2, transactivates the promoter of the eosinophil granule major basic protein gene. *Blood* **91:**3447–3458.
Yamaguchi Y, Hayashi Y, Sugama Y, Miura Y, Kasahara T, Kitamura S, Torisu M, Mita S, Tominaga A and Takatsu K (1988a) Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs *in vitro* survival. IL-5 as an eosinophil chemotactic factor. *J Exp Med* **167:**1737–1742.
Yamaguchi Y, Suda T, Ohta S, Tominaga K, Miura Y and Kasahara T (1991) Analysis of the survival of mature human eosinophils: Interleukin-5 prevents apoptosis in mature human eosinophils. *Blood* **78:**2542–2547.
Yamaguchi Y, Suda T, Suda J, Eguchi M, Miura Y, Harada N, Tominaga A and Takatsu K (1988b) Purified interleukin-5 supports the terminal differentiation and proliferation of murine eosinophilic precursors. *J Exp Med* **167:**43–56.
Yamaguchi Y, Tenen DG and Ackerman SJ (1994a) Transcriptional regulation of the human eosinophil peroxidase genes: Characterization of a peroxidase promoter. *Int Arch Allergy Immunol* **104 (Suppl 1):**30–31.
Yamaguchi Y, Zhang DE, Sun Z, Albee EA, Nagata S, Tenen DG and Ackerman SJ (1994b) Functional characterization of the promoter for the gene encoding human eosinophil peroxidase. *J Biol Chem* **269:**19410–19419.
Yamashita T, Someya A and Hara E (1985) Response of superoxide anion production by guinea pig eosinophils to various soluble stimuli: comparison to neutrophils. *Arch Biochem Biophys* **241:**447–452.
Yang L, Cohn L, Zhang DH, Homer R, Ray A and Ray P (1998) Essential role of nuclear factor κB in the induction of eosinophilia in allergic airway inflammation. *J Exp Med* **188:**1739–1750.
Yang J, Xia M, Goetzl EJ and An S (1994) Cloning and expression of the EP_3-subtype of human receptors for prostaglandin E_2. *Biochem Biophys Res Commun* **198:**999–1006.
Yarden Y, Escobedo JA, Kuang WJ, Yang-Feng TL, Daniel TO, Tremble PM, Chen EY, Ando ME, Harkins RN, Francke U, Ullrich A and Schlessenger J (1986) Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. *Nature (Lond)* **323:**226–232.
Yasruel Z, Humbert M, Kotsimbos TC, Ploysongsang Y, Minshall E, Durham SR, Pfister R, Menz G, Tavernier J, Kay AB and Hamid Q (1997) Membrane-bound and soluble αIL-5 receptor mRNA in the bronchial mucosa of atopic and nonatopic asthmatics. *Am J Respir Crit Care Med* **155:**1413–1418.
Yazdanbakhsh M, Eckmann CM, De Boer M and Roos D (1987a) Purification of eosinophils from normal human blood, preparation of eosinoplasts and characterization of their functional response to various stimuli. *Immunology* **60:**123–129.
Yazdanbakhsh M, Eckmann CM, Koenderman L, Verhoeven AJ and Roos D (1987b) Eosinophils do respond to fMLP. *Blood* **70:**379–383.
Yeadon M, Dougan FL, Petrovic A, Beesley JE and Payne AN (1993) Effect of BW B70C, a novel inhibitor of arachidonic acid 5-lipoxygenase, on allergen-induced bronchoconstriction and late-phase lung eosinophil accumulation in sensitised guinea-pigs. *Agents Actions* **38:**8–18.
Ying S, Meng Q, Barata LT, Robinson DS, Durham SR and Kay AB (1997) Associations between IL-13 and IL-4 (mRNA and protein), vascular cell adhesion molecule-1 expression, and the infiltration of eosinophils, macrophages, and T cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Immunol* **158:**5050–5057.
Ying S, Meng Q, Taborda Barata L, Corrigan CJ, Barkans J, Assoufi B, Moqbel R, Durham SR and Kay AB (1996) Human eosinophils express messenger RNA encoding RANTES and store and release biologically active RANTES protein. *Eur J Immunol* **26:**70–76.
Yokomizo T, Izumi T, Chang K and Shimizu T (1997) A G-protein-coupled receptor for leukotriene B_4 that mediates chemotaxis. *Nature (Lond)* **387:**620–624.
Yokota A, Kikutani H, Tanaka T, Sato R, Barsumian EL, Suemura M and Kishimoto T (1988) Two species of human Fcϵ receptor II (FcϵRII/CD23): Tissue-specific and IL-4-specific regulation of gene expression. *Cell* **55:**611–618.
Yokota S, Deimann W, Hashimoto T and Fahimi HD (1983) Immunocytochemical localization of two peroxisomal enzymes of lipid β-oxidation in specific granules of rat eosinophils. *Histochemistry* **78:**425–433.
Yokota S, Tsuji H and Kato K (1984) Localization of lysosomal and peroxisomal enzymes in the specific granules of rat intestinal eosinophil leukocytes revealed by immunoelectron microscopic techniques. *J Histochem Cytochem* **32:**267–274.
Yonish-Rouach E, Resnitsky D, Lotem J, Sachs L, Kimchi A and Oren M (1991) Wild type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature (Lond)* **352:**345–347.
Yoshida K, Suko M, Matsuzaki G, Sugiyama H, Okudaira H and Ito K (1995) Effect of fibronectin on the production of leukotriene C_4 by eosinophils. *Int Arch Allergy Immunol* **108 (Suppl 1):**50–51.
Yoshie O, Majima T and Saito H (1989) Membrane oxidative metabolism of human eosinophilic cell line EoL-1 in response to phorbol diester and formyl peptide: Synergistic augmentation by interferon-γ and tumor necrosis factor. *J Leukoc Biol* **45:**10–20.
Young JD, Peterson CG, Venge P and Cohn ZA (1986) Mechanism of membrane damage mediated by human eosinophil cationic protein. *Nature (Lond)* **321:**613–616.
Yousefi S, Blaser K and Simon HU (1997) Activation of signaling pathways and prevention of apoptosis by cytokines in eosinophils. *Int Arch Allergy Immunol* **112:**9–12.
Yousefi S, Green DR, Blaser K and Simon HU (1994) Protein-tyrosine phosphorylation regulates apoptosis in human eosinophils and neutrophils. *Proc Natl Acad Sci USA* **91:**10868–10872.
Yousefi S, Hemmann S, Weber M, Holzer C, Hartung K, Blaser K and Simon HU (1995) IL-8 is expressed by human peripheral blood eosinophils. Evidence for increased secretion in asthma. *J Immunol* **154:**5481–5490.
Yousefi S, Hoessli DC, Blaser K, Mills GB and Simon HU (1996) Requirement of *lyn* and *syk* tyrosine kinases for the prevention of apoptosis by cytokines in human eosinophils. *J Exp Med* **183:**1407–1414.
Yu W, Bozza PT, Tzizik DM, Gray JP, Cassara J, Dvorak AM and Weller PF (1998) Co-compartmentalization of MAP kinases and cytosolic phospholipase A_2 at cytoplasmic arachidonate-rich lipid bodies. *Am J Pathol* **152:**759–769.
Yuan Q, Austen KF, Friend DS, Heidtman M and Boyce JA (1997) Human peripheral blood eosinophils express a functional *c-kit* receptor for stem cell factor that stimulates very late antigen 4 (VLA-4)-mediated cell adhesion to fibronectin and vascular cell adhesion molecule 1 (VCAM-1). *J Exp Med* **186:**313–323.
Yukawa T, Kroegel C, Chanez P, Dent G, Ukena D, Chung KF and Barnes PJ (1989a) Effect of theophylline and adenosine on eosinophil function. *Am Rev Respir Dis* **140:**327–333.
Yukawa T, Kroegel C, Evans P, Fukuda T, Chung KF and Barnes PJ (1989b) Density heterogeneity of eosinophil leucocytes: Induction of hypodense eosinophils by platelet-activating factor. *Immunology* **68:**140–143.
Yukawa T, Terashi K, Terashi Y, Arima M, Sagara H, Motojima S, Fukuda T and Makino S (1992) Sensitization primes platelet-activating factor (PAF)-induced accumulation of eosinophils in mouse skin lesions: contribution of cytokines to the response. *J Lipid Mediat* **5:**121–126.
Yukawa T, Ukena D, Kroegel C, Chanez P, Dent G, Chung KF and Barnes PJ (1990) Beta$_2$-adrenergic receptors on eosinophils. Binding and functional studies. *Am Rev Respir Dis* **141:**1446–1452.
Zabucchi G, Menegazzi R, Soranzo MR and Patriarca P (1986) Uptake of human eosinophil peroxidase by human neutrophils. *Am J Pathol* **124:**510–518.
Zabucchi G, Soranzo MR, Menegazzi R, Cattin L, Vecchio M, Lanza F and Patriarca P (1991) Eosinophilic granuloma of the bone in Hand-Schuller-Christian disease: Extensive *in vivo* eosinophil degranulation and subsequent binding of released eosinophil peroxidase (EPO) to other inflammatory cells. *J Pathol* **163:**225–231.
Zanardo RCO, Costa E, Ferreira HHA, Antunes E, Martins AR, Murad F and De Nucci G (1997) Pharmacological and immunohistochemical evidence for a functional nitric oxide synthase system in rat peritoneal eosinophils. *Proc Natl Acad Sci USA* **94:**14111–14114.
Zanders ED (1994) Interleukin-5 receptor α chain mRNA is down-regulated by transforming growth factor β_1. *Eur Cytokine Netw* **5:**35–42.
Zeck Kapp G, Czech W and Kapp A (1994) TNFα-induced activation of eosinophil oxidative metabolism and morphology—Comparison with IL-5. *Exp Dermatol* **3:**176–188.
Zeck Kapp G and Kapp A (1995) Subcellular mechanisms of eosinophil degranulation: The role of RANTES, interleukin-5 and tumor necrosis factor-α. *Int Arch Allergy Immunol* **107:**345.
Zeck Kapp G, Kroegel C, Riede UN and Kapp A (1995) Mechanisms of human eosinophil activation by complement protein C5a and platelet-activating factor: Similar functional responses are accompanied by different morphologic alterations. *Allergy* **50:**34–47.
Zeiger RS and Colten HR (1977) Histaminase release from human eosinophils. *J Immunol* **118:**540–543.
Zhan S, Vazquez N, Wientjes FB, Budarf ML, Schrock E, Ried T, Green ED and Chanock SJ (1996) Genomic structure, chromosomal localization, start of transcription, and tissue expression of the human $p40^{phox}$, a new component of the nicotinamide adenine dinucleotide phosphate-oxidase complex. *Blood* **88:**2714–2721.
Zhang DH, Cohn L, Ray P, Bottomly K and Ray A (1997) Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. *J Biol Chem* **272:**21597–21603.
Zhang K, Gharaeekermani M, Mcgarry B, Remick D and Phan SH (1997) TNFα-mediated lung cytokine networking and eosinophil recruitment in pulmonary fibrosis. *J Immunol* **58:**954–959.
Zhang J, Kuvelkar R, Cheewatrakoolpong B, Williams S, Egan EW and Billah MM (1997) Evidence for multiple promoters of the IL-5 receptor α-subunit gene: a novel 6-base pair element determines cell-specific promotor function. *J Immunol* **159:**5412–5421.
Zhu X, Hamann KJ, Munoz NM, Rubio N, Mayer D, Hernrreiter A and Leff AR (1998) Intracellular expression of FcγRIII (CD16) and its mobilization by chemoattractants in human eosinophils. *J Immunol* **161:**2574–2579.
Zhu XD, Munoz NM, Rubio N, Hernreiter A, Mayer D, Douglas I and Leff AR (1996) Quantitation of the cytosolic phospholipase A_2 (type IV) in isolated human peripheral blood eosinophils by sandwich-ELISA. *J Immunol Methods* **199:**119–126.
Ziegler SF, Ramsdell F and Alderson MR (1994) The activation antigen CD69. *Stem Cells* **12:**456–465.
Ziltener HJ, Chavaillaz PA and Jorg A (1983) Leukotriene formation by eosinophil leukocytes. Analysis with ion-pair high pressure liquid chromatography and effect of the respiratory burst. *Hoppe-Seyler's Z Physiol Chem* **364:**1029–1037.
Zon LI, Yamaguchi Y, Yee K, Albee EA, Kimura A, Bennett JC, Orkin SH and Ackerman SJ (1993) Expression of mRNA for the GATA-binding proteins in human eosinophils and basophils: Potential role in gene transcription. *Blood* **81:**3234–3241.

Zora J, O'Connell EJ, Sachs MI and Hoffman AD (1984) Eosinophilic gastritis: A case report and review of the literature. *Ann Allergy* **53:**45–47.
Zoratti EM, Sedgwick JB, Bates ME, Vrtis RF, Geiger K and Busse WW (1992) Platelet-activating factor primes human eosinophil generation of superoxide. *Am J Respir Cell Mol Biol* **6:**100–106.
Zoratti EM, Sedgwick JB, Vrtis RR and Busse WW (1991) The effect of platelet-activating factor on the generation of superoxide anion in human eosinophils and neutrophils. *J Allergy Clin Immunol* **88:**749–758.
Zuany-Amorim C, Creminon C, Nevers MC, Nahori MA, Vargaftig BB and Pretolani M (1996) Modulation by IL-10 of antigen-induced IL-5 generation, and $CD4^+$ T lymphocyte and eosinophil infiltration into the mouse peritoneal cavity. *J Immunol* **157:**377–384.
Zuany-Amorim C, Haile S, Leduc D, Dumarey C, Huerre M, Vargaftig BB and Pretolani M (1995) Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. *J Clin Invest* **95:**2644–2651.
Zuany-Amorim C, Leduc D, Vargaftig BB and Pretolani M (1993) Characterization and pharmacological modulation of antigen-induced peritonitis in actively sensitized mice. *Br J Pharmacol* **110:**917–924.
Zucker Franklin D (1974) Eosinophil function and disorders. *Adv Intern Med* **19:**1–25.
Zucker Franklin D (1978) Eosinophil function related to cutaneous disorders. *J Invest Dermatol* **71:**100–105.
Zucker Franklin D (1980) Eosinophil structure and maturation, in *The Eosinophil in Health and Disease* (Mahmoud AAF and Austen KF eds) pp 43–60, Grune and Stratton, New York.
Zurawski SM and Zurawski G (1992) Receptor antagonist and selective agonist derivatives of mouse interleukin-2. *EMBO J* **11:**3905–3910.
Zwirner J, Gotze O, Moser A, Sieber A, Begemann G, Kapp A, Elsner J and Werfel T (1997) Blood- and skin-derived monocytes/macrophages resopnd to C3a but not to C3a(desArg) with a transient release of calcium via a pertussis toxin-sensitive signal transduction pathway. *Eur J Immunol* **27:**2317–2322.