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Perspective

Leukotrienes: A Major Step in the Understanding of Immediate Hypersensitivity Reactions

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Kellaway and Trethewie[1] in 1940, described a myotropic substance which was released from the lung during anaphylaxis and could possibly account for some of its symptoms. Because of its slow contracting effect on the guinea pig jejunum, this entity was called "slow reacting smooth-muscle-stimulating substance" (SRS).[2] Brocklehurst[3a] coined the term SRS-A (for slow-reacting substance of anaphylaxis) to differentiate the substance produced by lungs upon immunological challenge by specific antigens from those generated upon nonimmunological stimulation (SRS). During the 1950's, growing amounts of information supported important functions for SRS-A in hypersensitivity reactions. Indeed, crude preparations of SRS-A were found to induce strong and long-lasting constrictions of human bronchioles, and the substance was produced in significant amount by the lungs of sensitized animal or from asthmatic lungs.[3b] In the following years, considerable efforts were put into the chemistry and pharmacology of SRS-A; much progress was made, but the substance resisted identification.[4-7]

In the last decade, our knowledge of the biochemistry of arachidonic acid has progressed considerably with the successive findings of the prostaglandin (PG) endoperoxides, thromboxanes (TX), and prostacyclin (PGI_2) (Figure 1; see ref 8 for a review). The latest addition to the list of oxygenated metabolites of arachidonic acid in mammalian cells are the leukotrienes (Figures 2 and 3). This new family of compounds, first described by Borgeat and Samuelsson,[9-12] was named leukotrienes[13] because of their origin from leukocytes and a structural characteristic, the conjugated triene. Recently, the interest in these new compounds was tremendously emphasized when a slow-reacting substance (SRS) was recognized as a leukotriene.[14,15]

In the present paper, we summarize the basic studies on the matabolism of arachidonic acid which led to the structure determination of the leukotrienes and to the elucidation of their mechanism of formation. This paper also dicusses the biological significance of the leukotrienes, i.e., their role in immediate hypersensitivity reactions and their possible involvement in nonimmunological inflammatory processes. Some possible consequences of the discovery of leukotrienes are presented, with particular emphasis on the biomedical impact of this finding on the development of new asthma treatments.

Leukotrienes: A Novel Family of Metabolites of Arachidonic Acid

The discovery of leukotrienes, as well as the elucidation of their mechanism of formation, resulted from a com-

(1) C. H. Kellaway and E. R. Trethewie, *Q. J. Exp. Physiol.*, **30**, 121–145 (1940).
(2) Abbreviations used are: SRS, slow-reacting substance; SRS-A, slow-reacting substance of anaphylaxis; PG, prostaglandin; TX, thromboxane; PMNL, polymorphonuclear leukocytes; LC, liquid chromatography; RBL, rat basophil leukemia cells; PMA, phorbol myristate acetate; cAMP, 3′,5′-cyclic adenosine monophosphate; cGMP, 3′,5′-cyclic guanosine monophosphate.
(3) (a) W. E. Brocklehurst, *J. Physiol.*, **120**, 16P (1953). (b) W. E. Brocklehurst, *Prog. Allergy*, **6**, 539–558 (1962).
(4) W. E. Brocklehurst, in "Clinical Aspects of Immunology", P. F. H. Gell and R. R. A. Coombs, Eds., Blackwell Scientific Publications, Oxford, 1969, pp 611–632.
(5) R. P. Orange and P. L. Austen, *Adv. Immunol.*, **10**, 105–144 (1969).
(6) R. P. Orange, D. J. Stechshulte, and K. F. Austen, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **28**, 1710–1715 (1969).
(7) R. P. Orange, R. C. Murphy, M. L. Karnovsky, and K. F. Austen, *J. Immunol.*, **110**, 760–770 (1973).
(8) B. Samuelsson, S. Hammarström, and P. Borgeat, *Adv. Inflammation Res.*, 1, 405–412 (1979).
(9) P. Borgeat and B. Samuelsson, *J. Biol. Chem.*, **254**, 2643–2646 (1979).
(10) P. Borgeat and B. Samuelsson, *J. Biol. Chem.*, **254**, 7865–7869 (1979).
(11) P. Borgeat and B. Samuelsson, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 3213–3217 (1979).
(12) P. Borgeat and B. Samuelsson, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 2148–2152 (1979).
(13) B. Samuelsson, P. Borgeat, S. Hammarström, and R. C. Murphy, *Prostaglandins*, **17**, 785–787 (1979).
(14) B. Samuelsson, P. Borgeat, S. Hammarström, and R. C. Murphy, *Adv. Prostaglandin Thromboxane Res.*, **6**, 1–18 (1980).
(15) R. C. Murphy, S. Hammarström, and B. Samuelsson, *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 4275–4279 (1979).

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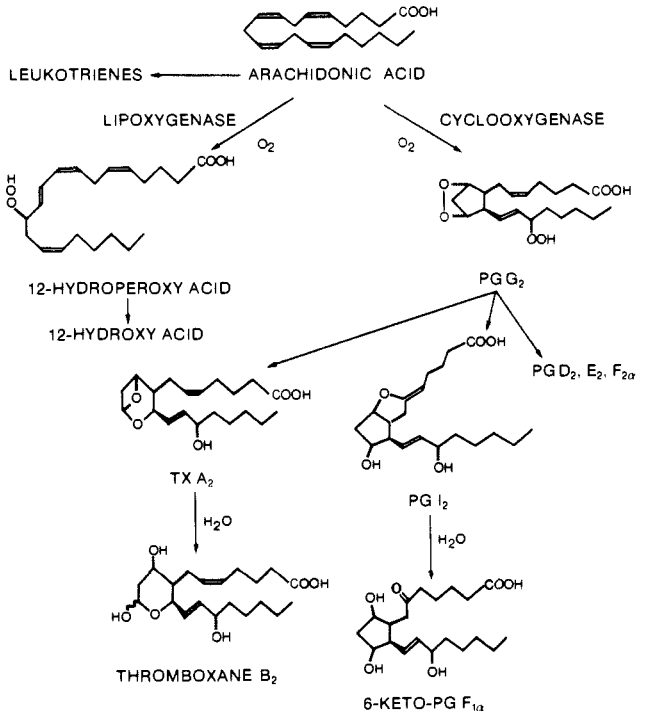

Figure 1. General scheme of the metabolism of arachidonic acid.

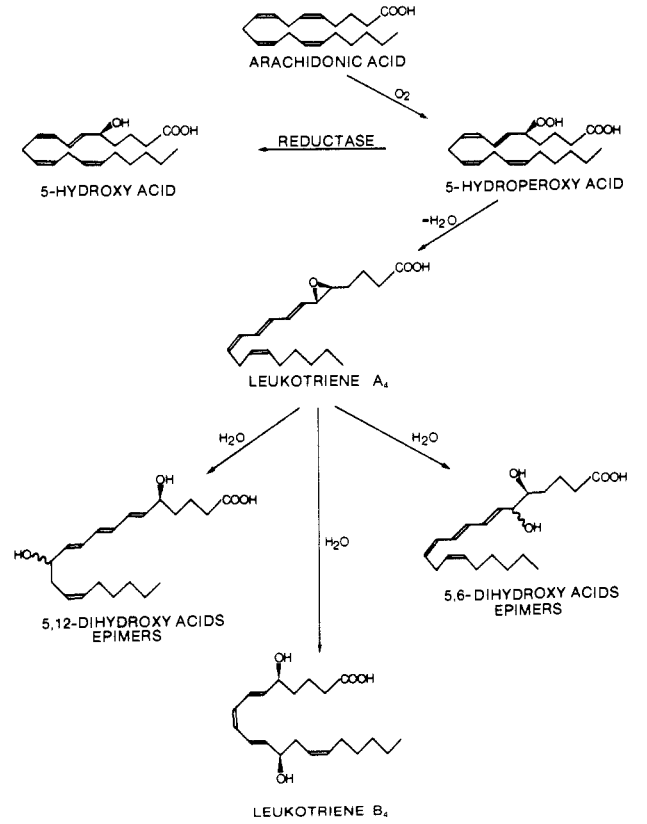

Figure 2. Transformation of arachidonic acid through the leukotriene pathway (partial scheme, see Figure 3). The geometry of the double bonds in leukotriene B_4 has not been fully resolved.[9]

prehensive study on the metabolism of arachidonic acid in rabbit peritoneal polymorphonuclear leukocytes (PMNL). Since PGs and other metabolites of arachidonic acid were found to have proinflammatory activities in various systems,[16] it seemed worthwhile to investigate the

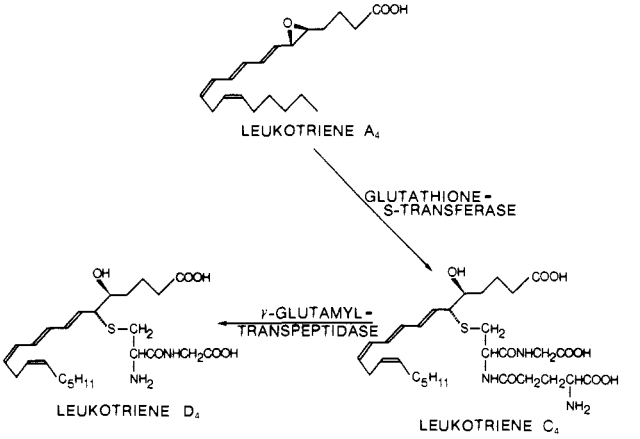

Figure 3. Hypothetical scheme of the formation of leukotriene D_4 (slow-reacting substance of anaphylaxis). The stereochemistry of leukotriene D_4 is temptative.[43]

metabolism of the fatty acid in the PMNL, an important cell type in inflammatory reactions.

Hydroxy Acids, Leukotriene A_4 and Leukotriene B_4. In a first report, Borgeat et al.[17] described the biosynthesis of the 5(*S*)-hydroxy-6,8,11,14-eicosatetraenoic acid (Figure 2) and 8(*S*)-hydroxy-9,11,14-eicosatrienoic acid in rabbit peritoneal PMNL (glycogen-induced) from arachidonic acid and 8,11,14-eicosatrienoic acid, respectively; the two hydroxy acids were the major metabolites of the C_{20}-unsaturated fatty acids. These findings revealed the occurrence of novel lipoxygenase-type reactions in leukocytes. Indeed, the positional specificity of the reactions at C-5 and C-8 was yet unreported.

Later, the same workers reported the presence of another metabolite of arachidonic acid in PMNL,[9] the 5-(*S*),12(*R*)-dihydroxy-6,8,10,14-eicosatetraenoic acid, recently named leukotriene B_4[13] (Figure 2). The compound was unique in that it contained a conjugated triene structure. The stereochemical purity of leukotriene B_4 indicated that it was formed enzymatically. In another paper, Borgeat and Samuelsson[10] described the structures of four minor metabolites of arachidonic acid in PMNL; the compounds were 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acids (epimers at C-6) and two geometric isomers of leukotriene B_4 (epimers at C-12; see Figure 2). Although these four compounds are probably devoid of biological activity, their isolation and identification proved to be of major importance in the subsequent studies on the mechanism of formation of leukotrienes. Indeed, the structural similarities between the five dihydroxy acids isolated so far from PMNL, i.e., the presence of a conjugated triene and of an alcohol at C-5 with the "*S*" configuration (see Figure 2), left little doubt about a common mechanism of formation for these compounds. Studies on the incorporation of isotopic oxygen indicated that the hydroxyl group at C-5 in all metabolites (including the 5-hydroxy acid) was derived from molecular oxygen and consequently indicated that the hydroxyl groups at C-6 or C-12 in the dihydroxy acids were derived from water. These data strongly supported a common pathway of formation for these compounds, involving the participation of an unstable intermediate that could undergo reaction with water.

Trapping experiments with alcohols were performed and proved unambiguously that an unstable compound which could easily react with weak nucleophiles (water, methanol,

(16) J. R. Vane, *Adv. Prostaglandin Thromboxane Res.*, **2**, 791–801 (1976).

(17) P. Borgeat, M. Hamberg, and B. Samuelsson, *J. Biol. Chem.*, **251**, 7816–7820 (1976).

ethanol, etc.) was generated during incubation of PMNL with arachidonic acid. Further studies have shown that the intermediate was very labile, particularly at acidic pH and more stable in alkaline medium. Other experiments allowed to establish that leukotriene B_4 was formed upon enzymatic hydrolysis of the unstable intermediate, whereas the four other dihydroxy acids were generated by nonenzymatic hydrolysis.[11] Taking into consideration the data available on the structure of the various products of hydrolysis of the intermediate (Figure 2), the results on the incorporation of isotopic oxygen in these compounds, and the experiments on the reactivity of the molecule, it was proposed that the unstable intermediate was 5(6)-oxido-7,9,11,14-eicosatetraenoic acid[11] or leukotriene A_4[13] (Figure 2).

The geometry of the double bonds and the configuration of the epoxide were not known at that time. Professor Corey, who was already involved in the studies on leukotriene A_4, performed the synthesis of the (±)-5,6-oxido-7,9-*trans*,11,14-*cis*-eicosatetraenoic acid.[18] The compound was found to be very unstable, a finding which was in agreement with the reactivity reported for the biosynthetic material (see above). The degradation products generated by solvolysis (water and methanol) of the synthetic epoxide and of the natural material were identical. These data indicated that the unstable intermediate in the formation of dihydroxy acids in neutrophils does have the covalent structure and the double bond geometry of the synthetic epoxy acid described above. Shortly later, Corey et al.[19] reported the total stereo-specific synthesis of 5(*S*)-*trans*-5,6-oxido-7,9-*trans*,11,14-*cis*-eicosatetraenoic acid from D(−)-ribose. The compound was shown to be transformed by neutrophils into a product undistinguishable from leukotriene B_4.[20] This enzymatic conversion suggested that the configuration of the epoxide, as well as the geometry of the double bonds of the synthetic compound, was likely similar to that of the natural substance. The detailed structure of this synthetic epoxy acid was thus assigned to leukotriene A_4.

The data obtained so far by various investigators still support the first hypothetical scheme presented for the mechanism of formation of leukotrienes A_4 and B_4.[11] The first reaction clearly seems to be of the lipoxygenase type, as indicated by the typical structure of the immediate product of the reaction, the 5(*S*)-hydroperoxy-6,8,11,14-eicosatetraenoic acid (Figure 2). The 5-hydroxy acid is very likely the product of the action of reductases on the 5-hydroperoxy acid and is thus considered as a byproduct in this reaction scheme.

Corey et al.[21] have reported a method for the chemical synthesis of the 5(*S*)-hydroperoxyeicosatetraenoic acid, as well as an enzymatic procedure for the preparation of this compound from incubations of arachidonic acid with potato lipoxygenase.

The formation of leukotriene A_4 from the 5-hydroperoxy acid is believed to occur through a mechanism involving abstraction of a proton at C-10 of the hydroperoxy acid in analogy with the mechanism of synthesis of PGG_2 where a proton abstraction at C-13 has been demonstrated.[22]

The presence in neutrophils of an hydrolase, converting leukotriene A_4 into leukotriene B_4, has been suggested from the very first studies[11] and has recently been confirmed.[20] However, none of the enzymatic component of the leukotriene pathway has been isolated or characterized so far. The availability of leukotriene A_4 and of the 5(*S*)-hydroperoxy acid from chemical synthesis[19,21] will be of major importance in studies of the enzymology of this metabolic pathway of arachidonic acid.

Leukotriene C_4 and Leukotriene D_4. The discovery of leukotrienes C_4 and D_4 was essentially linked to the efforts for unraveling the structure of SRS-A. Indeed, in parallel to the first studies on the leukotriene pathway by Borgeat and Samuelsson,[9-12] several groups involved in studies on SRS-A made considerable progress in the purification of the substance, mostly because of the application of high-pressure liquid chromatography (LC) to the field. In fact, Morris et al.[23] purified a sample of SRS-A (guinea pig anaphylactic lung) to a high degree of purity and obtained an ultraviolet spectrum which showed the characteristic absorption bands of leukotrienes. At about the same time, some experimental data suggested a precursor role of arachidonic acid in the synthesis of SRS-A,[24-27] and Jakschik et al.[24] and Sirois et al.[26] suggested that SRS were unidentified metabolites of this fatty acid formed through an unknown metabolic pathway. Previous studies had already shown that the ionophore A23187 stimulated the release of SRS from leukocytes[6,24,28] and of SRS-A from perfused lungs.[29] It was also known that, under certain conditions, the cyclooxygenase inhibitors indomethacin and aspirin could stimulate the release of SRS-A.[30-36] These data were taken as good indications of a relationship between SRS-A and leukotrienes in view of the following observations: (a) leukotrienes are metabolites of arachidonic acid in leukocytes, (b) the synthesis of the 5-hydroxy acid (a product of the leukotriene pathway) is not inhibited by indomethacin,[17] (c) the ionophore A23187 is a powerful stimulus of the transformation of arachidonic acid into leukotriene B_4 in human PMNL,[12] and (d) the conjugated triene is a structural characteristic of leukotrienes.

Many thiols and particularly cysteine were reported to stimulate the formation of SRS and SRS-A.[37-39] These

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(33) J. R. Boot, A. D. J. Brockwell, W. Dawson, and W. J. F. Sweatman, *Br. J. Pharmacol.*, **59**, 444–445P (1977).
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(35) J. F. Burka and P. Eyre, *Can. J. Physiol. Pharmacol.*, **52**, 1201–1204 (1974).
(36) D. M. Engineer, H. R. Morris, P. J. Piper, and P. Sirois, *Br. J. Pharmacol.*, **64**, 211–218 (1978).

observations suggested that SRS and SRS-A could be sulfur-containing compounds and, thus, possibly conjugates of cysteine (or related compounds) and of leukotriene A_4, which was expected to react easily with nucleophiles (e.g., thiols and alcohols). Working on this hypothesis, Murphy et al.[15] prepared some SRS from mouse mastocytoma cells upon incubation of the cells with arachidonic acid, L-cysteine, and the ionophore A23187. SRS was purified to near homogeneity by classical procedures and high-pressure LC. The pure and biologically active material showed the characteristic ultraviolet spectrum of leukotrienes. Labeling experiments showed that SRS incorporated cysteine and arachidonic acid. Further chemical analysis of the purified SRS clearly indicated that the compound was indeed a derivative of leukotriene A_4, i.e., a 5-hydroxy-7,9,11,14-eicosatetraenoic acid carrying a substituent at C-6 via a thioether linkage. The exact nature of the substituent at C-6 was yet unknown, although the experimental data suggested cysteine, a cysteine derivative, or a cysteine-containing peptide. These studies[14,15] established the relationship between SRS and the leukotriene family and thus constituted the first conclusive data on the exact nature of these substances.

In subsequent papers, Hammarström et al.[40,41] identified the substituent at C-6 in mouse mastocytoma cells SRS as glutathione and resolved the stereochemistry of the molecule by comparison of the biological, chemical, and physical properties of purified SRS with a totally synthetic product of known structure.[19] The mouse mastocytoma SRS was thus described as 5(*S*)-hydroxy-6(*R*)-*S*-glutathionyl-7,9-*trans*,11,14-*cis*-eicosatetraenoic acid and is now recognized as leukotriene C_4 (Figure 3).[41]

More recently, two groups reported the structure determination of an SRS from rat basophil leukemia cells (RBL-1). Morris et al.[42] purified some RBL-1 SRS by gel chromatography and high-pressure LC. The biologically active material showed the ultraviolet spectrum characteristic of the leukotrienes. The amino acid analysis revealed the presence of a cysteinylglycinyl moiety in the molecule. Interestingly, these workers were the first to obtain fruitful electron-impact mass spectrometric analysis of SRS. They reported the mass spectrum of intact SRS as the *N*-acetyl, methyl ester and trimethylsilyl ether derivative. Taken together, their data on the structure of RBL-1-SRS were fully consistent with a peptide conjugate of leukotriene A_4, a 5-hydroxy-6-*S*-cysteinylglycinyl-7,9,11,14-eicosatetraenoic acid. Örning et al.[43] also analyzed the RBL-1-SRS and obtained fully compatible results. These workers, on the basis of ultraviolet analysis, Raney nickel desulfurization experiments, and enzymatic conversion with soybean lipoxygenase, concluded that the RBL-1-SRS was a leukotriene with a fatty acid part identical with that of leukotriene C_4. Amino acid analysis further indicated that the RBL-1-SRS was a 5-hydroxy-6-*S*-cysteinylglycinyl-7,9,11,14-eicosatetraenoic acid, which was named leukotriene D_4. The same investigators also demonstrated that leukotriene D_4 could be generated by the action of γ-glutamyl transpeptidase on leukotriene C_4 (Figure 3). These data suggested that leukotrienes A_4 and C_4 were intermediates in the formation of leukotriene D_4 and that, consequently, the stereochemistry of leukotrienes D_4 and C_4 should be the same.[43]

Bach et al.[44] studied the major component of the SRS released by rat peritoneal cells upon stimulation with the ionophore A23187. Their data, based on high-pressure LC analysis, ultraviolet spectroscopy, amino acid analysis, and reaction with soybean lipoxygenase, indicated that this SRS was identical with leukotriene D_4.

The latest addition to this field came from Morris et al.;[45] using high-pressure LC, ultraviolet spectroscopy, amino acid analysis, and electron-impact mass spectrometry (of the *N*-acetyl, trimethylsilyl ether, methyl ester derivative), the authors showed that the SRS-A released upon immunological challenge of sensitized guinea pig lungs was identical with the SRS released by RBL-1 cells and, thus, to leukotriene D_4. This constituted the first conclusive report on the structure of an immunologically released SRS.

Distribution and Biological Significance of Leukotrienes

Leukotrienes as such have been conclusively identified in various leukocytes (including RBL-1 cells), mast cells, tumors, and lungs (see previous discussion), and the question is open whether or not these compounds have a more general distribution like prostaglandins or are limited to tissues and cells more directly involved with allergic reactions. Considering first that the SRS (or SRS-A) identified so far from four different sources were leukotrienes and second that "slow-reacting substances" with the characteristic biological, physical, and chemical properties are released by many tissues (other than those mentioned above), such as the human skin,[46] human nasal polyps,[47] blood vessels,[48] heart,[48] and cat paw[49] and in several species,[50] it is tempting to speculate that leukotrienes probably have a relatively broad distribution in the organism. The data accumulated so far do not allow to establish which cell type (tissue specific cells, tissue macrophages, mast cells) in the tissues (or organs) concerned would be the source of leukotrienes. However, that these substances are released upon immunological challenging strongly suggest that cells carrying receptors for immunoglobulins E (or G for the guinea pigs) are more likely involved.

There is at the present time little new to write on the biological activity of leukotrienes. The structural elucidation and the chemical synthesis of these compounds are very recent and most of the information is yet to come. In fact, the knowledge available today in this field is es-

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(50) P. Sirois, D. M. Engineer, P. J. Piper, and E. G. Moore, *Experimentia*, 35, 361–362 (1979).

sentially based on studies on SRS and SRS-A performed before the identification of these spasmogens as leukotrienes. These studies clearly pointed out the importance of SRS-A in immediate hypersensitivity reactions. It is not, however, the purpose of this paper to present these data, and the reader is referred to Brocklehurst[4] and Orange and Austen[5] for excellent reviews. The tremendous importance of immediate hypersensitivity reactions in medicine leave little doubt about the fact that in the near future the discussion on the biological importance of leukotrienes will be focused on the role of these compounds in allergy, anaphylaxis, asthma, etc. The availability of synthetic leukotrienes will permit to confirm and reevaluate the biological importance of leukotrienes in immediate hypersensitivity reactions (as established in the past years with partially purified preparations of SRS-A). Experiments on the effects of leukotrienes in various systems should also rapidly provide a broad picture of the biological properties of these new compounds.

Medical Consequences of Leukotrienes: Hopes for New Treatments of Asthma

As for many other diseases, the development of the actual asthma therapy has been empirical. The complexity of this disease, which is rather a series of symptoms resulting from the action of a number of incompletely understood mechanisms, hindered scientific approaches to treatment. Among others, the difficulties encountered over the last 40 years in the purification, assay, and structure elucidation of SRS-A has prevented the rational development of specific antagonists to this important mediator.

The recent discovery of the structure of leukotriene D_4 will certainly stimulate the development of new and more specific drugs. In fact, the knowledge of the chemical structure and of the pathway of biosynthesis of leukotriene D_4 suggests several possible ways of inhibiting the biological effects or of controlling the biosynthesis of this spasmogen. The methodologies already available for the chemical synthesis of leukotriene C_4[19] will permit the synthesis of analogues of leukotriene C_4 and D_4, the establishment of the structure–activity relationship of these compounds on various target organs, and thus the development of specific action-antagonists.

The biosynthesis of leukotriene D_4 is controlled by various enzymes. The lipoxygenase as the enzyme leading to the formation of the 5-hydroperoxy acid and leukotriene A_4 (Figure 2) could easily become the first target for pharmacological intervention. Interestingly, studies of the action of nonsteroid antiinflammatory drugs have also shown that the cyclooxygenase pathway could be blocked without inhibition of the lipoxygenase pathway (leukotrienes),[30-36] indicating the possibility of specific interventions. Thus, new drugs acting specifically on the lipoxygenase pathway could probably be developed. Another interesting approach to the control of leukotriene biosynthesis would be the use of "false-substrates" which could enter the lipoxygenase pathway and be transformed by the enzyme, but could not lead to the formation of active leukotrienes.

The availability of synthetic leukotrienes will soon permit the development of sensitive, specific, and convenient methods of measurements which could be applied to the evaluation of blood or tissue concentrations in health or disease. The development of radioimmunoassays are particularly needed. Synthetic leukotrienes will also make possible studies of the mechanism of action of these compounds, including characterization of receptor molecules, effects on cyclic nucleotide systems, and effects on the release of other mediators of hypersensitivity reactions.

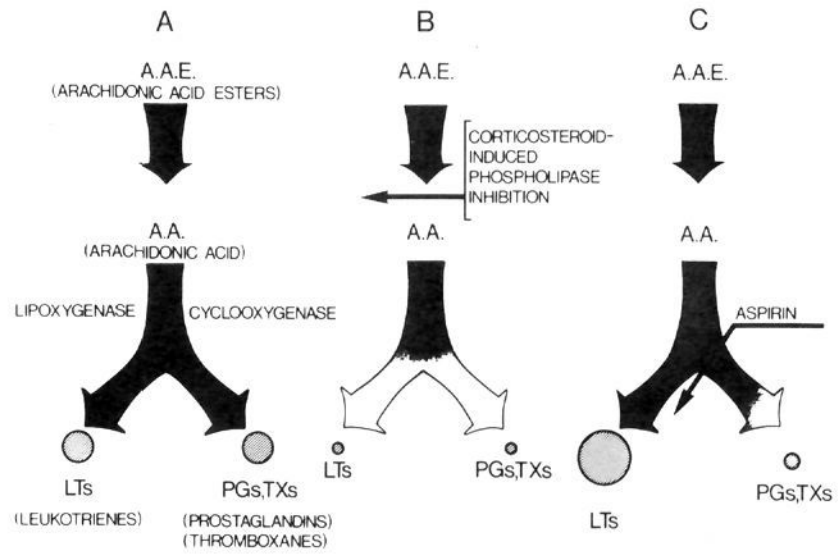

Figure 4. Illustration of the balance in the transformation of arachidonic acid through the leukotriene pathway (lipoxygenase) and the prostaglandin pathway (cyclooxygenase).

The discovery of leukotrienes has already brought some interesting explanations on the mechanism of action of widely used drugs. Corticosteroids have been used for a long time and with success in asthma therapy, but their proposed mechanisms of action could not fully explain their efficacy. Among the multiple actions of these drugs, they are known to block phospholipase A_2, an enzyme involved in the release of arachidonic acid from phospholipids.[51] However, until recently, this fatty acid was known to generate only PGs and TXs, which were not believed to play an important role in asthma.[52,53] When recent discoveries showed that leukotrienes were also synthesized from arachidonic acid via another pathway, it became apparent that at least some of the effects of corticosteroids in asthma were linked to the inhibition of phospholipase A_2, resulting in a decreased formation of leukotrienes (Figure 4). The fact that PGs and leukotrienes are generated from the same precursor brings also a possible explanation to the adverse action of aspirin-like drugs in asthmatics; indeed, there is likely a balance effect in the utilization of arachidonic acid by the cyclooxygenase and lipoxygenase pathways, and inhibition of the former leads to increased formation of leukotrienes (Figure 4).[30-36]

Leukotrienes and Inflammation, an Hypothesis

Since the discovery by Vane et al.[54] of the inhibitory effects of nonsteroidal antiinflammatory agents (aspirin, indomethacin, etc.) on PGs synthesis, the role of arachidonic acid metabolites has been extensively investigated in the various stages of the inflammatory process, and it was indeed shown that PGs do play important roles in some inflammatory reactions (see ref 55 for a review).

However, other experimental data, particularly those regarding the release of lysosomal enzymes and to some extent phagocytosis, are hardly reconcilable with a role of endogenous PGs, in view of the known effects of the compounds on these leukocyte functions in vitro. These data pertained to several studies where the divalent cation ionophore A23187 was reported to stimulate the synthesis of PGs and TXs in leukocytes (likely via the stimulation of Ca^{2+}-dependent phospholipase A_2)[56-59] and to induce

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an elevation of the intracellular concentration of 3',5'-cyclic guanosine monophosphate (cGMP) and the release of lysosomal enzymes.[60] Taking into consideration the principle now accepted that agents causing an increase of the 3',5'-cyclic adenosine monophosphate (cAMP) levels in leukocytes (e.g., PGE_1, PGE_2, isoproterenol) also inhibit the release of lysosomal enzymes, whereas those causing an increase in cGMP levels (e.g., acetylcholine) induce the release of enzymes,[61,62] the sequence of events observed upon addition of the ionophore A23187 to leukocytes suggested that the increased PG production and lysosomal enzyme release are unrelated phenomenons. Interestingly, it has been shown in similar studies that the ionophore A23187 strongly stimulates the synthesis of the 5-hydroxy acid and leukotriene B_4 (40-fold) in human peripheral blood PMNL.[12] These data raise the possibility that a product of the leukotriene pathway might be involved in the stimulation of cGMP accumulation and lysosomal enzyme release in leukocytes. Other experimental observations support this hypothesis; the tumor-promoting and proinflammatory agent phorbol myristate acetate (PMA), which was shown to cause the release of arachidonic acid in kidney cells[63] and to raise the levels of cGMP in various cell types,[62,64] was also found to stimulate lysozyme release from cytochalasin B treated human PMNL;[65] in similar experiments, PMA potentiated the release of lysozyme and β-glucuronidase from zymosan-stimulated cells. Several biochemical events have been observed during phagocytosis; for instance, it is known that phagocytosis is accompanied by a burst of oxygen consumption, by a marked decrease of arachidonic acid in the phospholipids of phagocytic vacuole membranes,[66] and by increased production of PGs.[67] Interestingly, phagocytosis is also accompanied by increased levels of cGMP and the release of lysosomal enzymes, whereas cAMP levels remain unchanged.[61] That the disappearance of arachidonic acid is associated with the synthesis of leukotrienes has not been shown yet, but the release of lysosomal enzymes and the changes of levels of cyclic nucleotides during phagocytosis do not favor a role of cyclooxygenase products. Recently, it was shown that the direct addition of arachidonic acid to rabbit peritoneal neutrophils (cytochalasin B treated) induces the release of lysosomal enzymes at doses below 10^{-6} M.[68] This effect was inhibited by the lipoxygenase inhibitor 5,8,11,14-eicosatetraynoic acid[69] and and was not produced by any other fatty acids used, including the PGs precursor 8,11,14-eicosatrienoic acid. Thus, among the fatty acids tested, only arachidonic acid, the leukotrienes precursor, was active in stimulating enzyme release. Again, these data suggest that leukotrienes might be involved in the release of lysosomal enzymes (and phagocytosis) possibly through a mediator role of cGMP.

This hypothesis is in agreement with the proposal of Sullivan and Parker[70] that some metabolites of arachidonic acid other than the cyclooxygenase products appear to be involved in the release of inflammatory mediators from mast cells. Such a role of leukotrienes at different steps of the inflammatory process would provide one possible explanation (see ref 62) of the action of steroidal antiinflammatory drugs. Indeed, as mentioned previously, corticosteroids, which have been shown to inhibit the release of arachidonic acid in a variety of tissues[51] and cells, including leukocytes,[71] and to block the formation of PGs, are expected to also block the biosynthesis of leukotrienes (Figure 4). Consequently, in view of the hypothesis presented here, these drugs might interfere with important functions of the leukocytes in inflammation and host defense, i.e., phagocytosis and the release of lysosomal enzymes.

The present discussion was to point out that some studies on the role of arachidonic acid metabolites in leukocyte physiology performed before the discovery of leukotrienes could now be interpreted to support a role of these compounds in some aspects of nonimmunological inflammation. Studies of the effects of leukotrienes on leukocyte functions and measurement of leukotrienes under various experimental conditions will be required to evaluate this hypothesis.

Conclusion

The question of the chemical nature of an important mediator of anaphylaxis has recently been answered. Furthermore, the biosynthetic pathway of the compounds have been partly elucidated, and the chemical sytnesis of leukotrienes C_4 and D_4, as well as their precursor leukotriene A_4, has been performed. This is without any doubt a major breakthrough in the research fields concerned. It is expected that these findings will lead to the development of better asthma treatments and to rapid progress in the understanding of the biochemical events and physiological process involved in immediate hypersensitivity reactions and possibly in nonimmunological inflammation.

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