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Vascular Endothelial Adhesion Molecules and Tissue Inflammation[a]

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I. Introduction

Adhesion molecules expressed on vascular endothelial cells mediate cell-cell and cell-matrix adhesive interactions that are essential for the regulation of trafficking of leukocytes across the vascular endothelial barrier and have been shown to be critically involved in the inflammatory response. Several endothelial adhesion molecules with highly specialized functions have been characterized thus far (see table 1). A family of endothelial "selectins" consisting of (*a*) E-selectin [i.e., the endothelial leukocyte adhesion molecule-1 (ELAM-1)] is expressed exclusively on endothelial cells and (*b*) P-selectin is expressed on endothelial and platelet plasmalemmal membranes. The common lectin domain on E- and P-selectins is involved in the binding to specific carbohydrate residues (e.g., sialyl-Lewis X) present on leukocytes. Another set of adhesion receptors, the intercellular adhesion molecules (ICAMs), ICAM-1, ICAM-2 and ICAM-3, are members of

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TABLE 1
Cell adhesion molecules expressed on endothelial cells

Adhesion Molecules	Family	Recognized Ligands	Inducers
E-selectin	Selectin	sialyl-CD15 and related carbohydrate structures	cytokines (TNF, IL-1), LPS
P-selectin	Selectin	sialyl glycoproteins	thrombin, histamine, phorbol esters
VCAM-1	Immunoglobulin	VLA-4	cytokines (TNF, IL-1), LPS
ICAM-1	Immunoglobulin	CD11a/CD18, CD11b/CD18	cytokines (TNF, IL-1), LPS, oxygen radicals
ICAM-2	Immunoglobulin	CD11a/CD18	cytokines, (TNF, IL-1), LPS

the immunoglobulin superfamily. The CD11/CD18 family of integrins are the counter-receptors for ICAM-1 and ICAM-2 (Springer, 1990). The vascular cellular adhesion molecule (VCAM-1), another member of immunoglobulin superfamily, binds to the integrin "very late activation antigens-4" (VLA_4 group) found on leukocytes (Elices et al., 1990). There are numerous excellent reviews elaborating the biological significance of VCAM-1 and other potentially important vascular adhesion molecules such as CD31 (PECAM-1) (Gimbrone et al., 1995; Newman, 1994; De Lisser et al., 1994). However, in this review, we will focus on the structure and biology of ICAMs and E-/P-selectins and their respective ligands and discuss their involvement in the pathophysiology of inflammatory diseases.

II. Intercellular Adhesion Molecule-1 (ICAM-1; CD54)

A. Structure and Cloning of ICAM-1

ICAM-1 is a 90-kD inducible cell surface glycoprotein that promotes leukocyte adhesion in inflammatory reactions (Rothlein et al., 1986). ICAM-1 was first identified with a monoclonal antibody (Rothlein et al., 1986), and the human ICAM-1 gene was subsequently cloned (Staunton et al., 1988). Analysis of ICAM-1 complementary deoxyribonucleic acid (cDNA) sequence revealed it to be a member of the immunoglobulin gene superfamily (Staunton et al., 1989). Horley et al. (1989) reported that the nucleotide sequence of mouse ICAM-1 bears a striking homology to human ICAM-1, which mapped to chromosome 19 whereas the mouse ICAM-1 gene was located on chromosome 9 (Ballantyne et al., 1991).

Human ICAM-1 is encoded by an inducible 3.3-kb messenger ribonucleic acid (mRNA) (Staunton et al., 1988). The amino acid sequence revealed a transmembrane protein with an extracellular domain of 453 residues containing 5 immunoglobulin-like domains. ICAM-1 is homologous to the neural crest adhesion molecules and myelin-associated glycoprotein (Staunton et al., 1989; Simmons et al., 1988).

ICAM-1 is the known ligand for CD11a/CD18 as well as CD11b/CD18 (Diamond et al., 1991). However, in contrast to other integrin ligands, ICAM-1 does not contain the Arg-Gly-Asp (RGD) sequence on integrins (Staunton et al., 1988), indicating that CD11/CD18 integrins bind to ICAM-1 via RGD-independent sites. This does not rule out the possibility that peptides containing a "critical" ICAM-1 sequence is responsible for binding to CD11/CD18 and that such peptides (if discovered) can inhibit leukocyte adhesion.

B. Induction of ICAM-1 Expression

ICAM-1 is constitutively present on the endothelial cell surface. Its expression increases progressively over a 24-hr period after TNFα, interleukin 1β (IL-1β) or lipopolysaccharide (LPS) challenge and can remain elevated for 72 hrs (Pober et al., 1986). Although the evidence is circumstantial, it is believed that ICAM-1 expression is regulated by activation of second-messenger pathways. Staurosporine or the isoquinosulphonamide derivative, H7, both of which prevent protein kinase C (PKC) activation, inhibited the cytokine-mediated ICAM-1 expression and neutrophil adhesion to endothelial cells (Lane et al., 1990; Renkonen et al., 1990). Other studies showed that TNFα-induced activation of ICAM-1 occurred independently of PKC activation (Ritchie et al., 1991); therefore, the involvement of PKC in the response remains far from clear. The synthesis of ICAM-1 may also be regulated by 3-deazaadenosine (Jurgensen et al., 1990), a nucleoside analog that selectively decreased steady-state ICAM-1 activity, suggesting a possible mechanism by which adenosine mediates its immunomodulatory function.

Unlike the effects of cytokines, both thrombin-induced and hydrogen peroxide (H_2O_2)-induced neutrophil adhesion to the endothelium involves the expression of ICAM-1 that occurrs within a 30 to 60 min period (Suguma and Malik, 1992; Lo et al., 1993). The rapidity of the response induced by thrombin (which paralleled the expression of endothelial adhesivity) (Bizios et al., 1988), together with the finding that ICAM-1 expression was not inhibited by cycloheximide (Suguma and Malik, 1992) suggests that ICAM-1 expression can also occur independently of protein synthesis. The increase in ICAM-1 functional activity, similar to the CD11b/CD18 activation on neutrophils (Detmers et al., 1987; Lo et al., 1989) and GPIIb/IIIa activation on platelets (Coller, 1985; Shattil et al., 1985), may involve conformational or allosteric modification of the constitutive cell surface ICAM-1. This "qualitative" increase in ICAM-1 activity may regulate the rapid phase of transendothelial leukocyte trafficking.

The basis of H_2O_2-induced ICAM-1 expression is not clear; however, in contrast to thrombin's effect, it requires increased mRNA activity (Lo et al., 1993). Oxidant-induced activation of transcription factors such as activator protein (AP)-1 or AP-2 and the oxidant-activated early response genes (c-*fos* and c-*jun*) are both capable of increasing ICAM-1 transcriptional activity (Malik et al., unpublished observation).

C. Binding of CD11/CD18 Integrins to ICAM-1, ICAM-2, and ICAM-3

ICAM-1 is the primary ligand for CD11a/CD18 (Marlin and Springer, 1987), although there may exists more than one ligand for CD11a/CD18 (Fawcett et al., 1992). This evidence is derived from observations that monoclonal antibodies to CD11a/CD18 but not to ICAM-1 fully inhibited the homotypic aggregation of lymphocytes (Rothlein et al., 1986). Staunton et al. (1989) and Fawcett et al. (1992) have identified and cloned the second and third putative CD11a/CD18 ligands, namely ICAM-2 and ICAM-3.

ICAM-2 is an integral membrane protein (MW 55 kD) with two immunoglobulin-like domains, whereas ICAM-1 has five such domains (Staunton et al., 1988, 1989). The two immunoglobulin-like domains on ICAM-2 are related to the two NH_2-terminal domains of ICAM-1. ICAM-2 is likely responsible for CD11a/CD18-dependent and ICAM-1-independent pathways of leukocyte adhesion to endothelial cells. The contribution of ICAM-2 in tissue inflammatory responses has yet to be examined, but it is likely to be different than that of ICAM-1, because ICAM-2 is found in relatively high concentrations on unstimulated endothelial cells and is unaltered by cytokines or LPS stimulation (Staunton et al., 1989).

Recent characterization (De Fougerolles and Springer, 1992) and molecular cloning (Fawcett et al., 1992; Vazeux et al., 1992) of ICAM-3, a third ligand for CD11a/CD18, indicated that ICAM-3 is related to ICAM-1 (48% homology), but consists of 5 immunologlobulin domains. The binding sites for CD11a/CD18 reside on the first two NH_2-terminal domains of ICAM-3. Unlike ICAM-1 and ICAM-2 , ICAM-3 is absent on vascular endothelial cells (Fawcett et al., 1992); ICAM-3 is not likely to play a role in leukocyte infiltration given its distinct cellular distribution only on resting leukocytes (Fawcett et al., 1992).

Although many studies indicate that CD11a/CD18 is the key receptor for ICAM-1, other studies showed that the CD11b/CD18 integrin under some conditions also binds to ICAM-1, albeit with lower affinities (Diamond et al., 1990). Endothelial cells expressing high density of ICAM-1 were shown to bind to immuno-affinity purified CD11b/CD18 adsorbed to an artificial surface in a manner inhibitable by monoclonal antibodies to ICAM-1 as well as to CD11b/CD18 (Diamond et al., 1990). Moreover, transfected cells expressing human ICAM-1 bound to purified CD11b/CD18 in a specific manner (Diamond et al., 1990). Of interest is the finding that only a small population of CD11b/CD18 (~10%) on the activated leukocyte is capable of mediating ICAM-1 binding (Diamond and Springer, 1993). In summary, these results provide conclusive evidence that ICAM-1 is the "counter-receptor" for CD11b/CD18 in addition to the known ability of ICAM-1 to bind to CD11a/CD18.

The binding sites on ICAM-1 for CD11a/CD18 and CD11b/CD18 are different; CD11a/CD18 binds to the first and CD11b/CD18 binds to the third NH_2-terminal immunoglobulin-like domains of the molecule (Diamond et al., 1991). Differential binding provides a function for the tandem duplications of immunoglobulin-like domains on ICAM-1; moreover, the result provides support for the interaction of CD11a/CD18 with only ICAM-2, which has fewer such domains (Staunton et al., 1989). Glycosylation of the immunologlobulin domains can influence the binding of CD11b/CD18 (Diamond et al., 1991), suggesting that the leukocyte adhesion to endothelial cells is critically regulated by this process.

D. ICAM-1-Induced Leukocyte Adhesion and Migration

The interaction of CD11/CD18 and ICAM-1 is an important event in both neutrophil adhesion and migration events. Monoclonal antibodies (mAbs) against CD11/CD18 (β_2) integrins were used to study the relative contribution of CD11a/CD18 and CD11b/CD18 in mediating neutrophil adhesion (Lo et al., 1989). Each mAb inhibited approximately 50% of the adhesion induced by phorbol dibutyrate, whereas both mAbs together fully inhibited the response. Anti-ICAM-1 mAbs also produced a 50% inhibition of the adhesion. In neutrophils activated with chemotactic agents, combination of anti-CD11a and anti-CD11b mAbs fully inhibited neutrophil adhesion and diapedesis (Smith et al., 1989). These results support the hypothesis that a cooperative interaction of CD11a/CD18 and CD11b/CD18 with ICAM-1 is required for the neutrophil adhesion and transendothelial migration events. The availability of increased sites on both CD11a/CD18 and CD11b/CD18 for binding to ICAM-1 strengthens the neutrophil adhesion to the en-

Abbreviations: AP-1, activator protein-1; CD, Cluster of Differentiation Nomenclature; cDNA, complementary deoxyribonuncleic acid; CFR, cysteine-rich fibroblast growth factor receptor; CR3, complement receptor type 3; CRE/ATF, cyclic adenosine monophosphate response element/activation transcriptional factor; dsLe^x, dimeric sialyl-Lewis X, dimeric sialyl-CD15; EGF, epidermal growth factor; E-selectin (ELAM-1, CD62E); ESL-1, E-selectin ligand-1; ICAM-1, intercellular adhesion molecule-1 (CD54); ICAM-2, intercellular adhesion molecule-2; ICAM-3, intercellular adhesion molecule-3; I$\kappa\beta$, inhibitory factor κ β; IL-1β, interleukin-1β; LFA-1, lymphocyte function-associated antigen-1; Le^x, Lewis X (CD15); L-selectin, LAM-1, TQ-1, CD62L; LPS, lipopolysaccharide; mAbs, monoclonal antibodies; NF$\kappa\beta$, nuclear factor κ β; PAF, platelet-activating factor; PECAM-1, platelet endothelial cell adhesion molecule-1 (CD31); PKC, protein kinase C; P-selectin, PADGEM, granule member protein-140 (CD62P); SLe^x (sialyl-Lewis X, sialyl-CD15); TNFα, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4 (CD49d/CD29); NIF, neutrophil inhibitory factor.

dothelium and thus may facilitate the migration process.

In contrast to the involvement of both CD11a/CD18 and CD11b/CD18 in mediating adhesion of activated neutrophils to unstimulated endothelial cells, CD11a/CD18 appears to be the primary integrin responsible for the adhesion of unstimulated neutrophils to stimulated endothelial cells. Pretreatment of endothelial cells with anti-ICAM-1 mAbs reduced the adhesion of neutrophils to IL-1β- or LPS-stimulated endothelial cells by 50%, whereas the reduction in migration across the stimulated endothelial cells was greater than 85% (Smith et al., 1988). Anti-CD11a and anti-ICAM-1 mAbs produced the same decreases in adhesion and migration, and their combined effects were not additive, indicating that neutrophil adhesion to "activated" endothelial cells and neutrophil migration across these cells are regulated by separate mechanisms. Thus, the adhesion of neutrophils to LPS- and cytokine-stimulated endothelial cells had a significant (~50%) CD11a/CD18- and ICAM-1-independent component, whereas migration across the stimulated endothelial monolayers was largely the result of interaction of CD11a/CD18 with ICAM-1.

E. Sequential Neutrophil Adhesion Response

Endothelial cells treated with inflammatory cytokines expressed E-selectin and by ICAM-1 (Pober et al., 1986; Bevilacqua et al., 1987). Both adhesion molecules were involved in neutrophil adhesion with the initial response being more E-selectin-dependent, with the later response being more ICAM-1 dependent (Luscinskas et al., 1989; Luscinskas et al., 1991). Neutrophils did not migrate across endothelial cells when endothelial cells had been treated with cytokines for 24 hrs, whereas significant ICAM-1 and adhesivity activities were expressed at this time (Luscinskas et al., 1991), suggesting that the adhesion and transendothelial migration events are separate events regulated by different mechanisms.

Neutrophil migration across the endothelium may depend on the sequential series of interactions between the activated endothelial cells and neutrophils (Lo et al., 1991). Circulating neutrophils express minimal CD11/CD18 integrin activity and have low binding toward the unstimulated endothelium. However, the expression of E-selectin after endothelial activation by cytokines or LPS and the engagement of E-selectin to siayl Lewis X on neutrophils may induce the activation of CD11/CD18 integrins (Lo et al., 1991). The enhanced CD11/CD18 activity promotes ICAM-1-dependent adhesiveness between neutrophils and endothelial cells (fig. 1). This sequence of adhesion events is capable of delivering cellular signals to the neutrophil to induce high avidity neutrophil adhesion that can withstand the shear stress forces encountered in microvessels (Lawrence et al., 1987; Worthen et al., 1987; Perry and Granger, 1991). Once the neutrophils are stably adherent, they are then capable of migrating across the endothelial barrier.

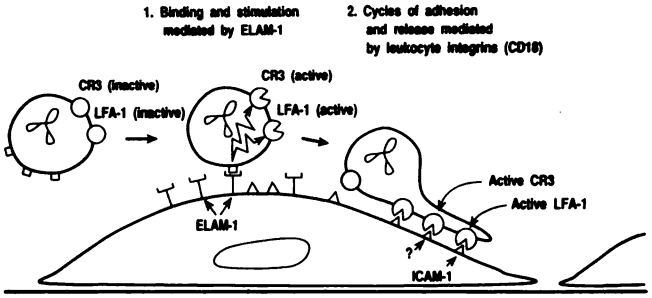

FIG. 1. Proposed model of sequential activation of E-selectin and CD11/CD18 integrins on neutrophils. Engagement of carbohydrate structures related to sialyl-Lewis X on neutrophils with the E-selectin on endothelial cells signals neutrophils to increase their CD11/CD18 functional activity. This sequential activation ensures tightness of adhesion and enables neutrophil recruitment at inflammatory sites (from Lo et al., 1991, used with permission of the authors and from The Rockefeller University Press).

Hence, according to this model, the selectin family, which mediates leukocyte rolling (Lawrence and Springer, 1991; Ley et al., 1991), and the CD11/CD18 integrins, which strengthen leukocyte attachment to the endothelium (Springer et al., 1990; Smith et al., 1989; Lo et al., 1991), act in concert to promote transendothelial migration of leukocytes.

An analogous combinatorial process is hypothesized to occur in thrombin-challenged endothelial cells that express both P-selectin and platelet-activating factor (PAF) (Zimmerman et al., 1990). Whereas P-selectin functions to strengthen the adhesion response, PAF serves as a "tethering" and "signaling" molecule for neutrophil activation (Zimmerman et al., 1990).

F. Expression of ICAM-1 and its Role in Inflammation

Because ICAM-1 is present on many cell types, it is not surprising that it has been implicated in several inflammatory diseases (Springer et al., 1990; Dougherty et al., 1988). ICAM-1 was expressed in renal allografts and an mAb to ICAM-1 in primates was shown to prevent the acute rejection of renal transplants (Cosimi et al., 1990). ICAM-1 expression was also unregulated in the inflamed airway epithelium in a primate model of asthma in which it mediated eosinophil adherence to airway epithelial cells (Wegner et al., 1990). MAbs against ICAM-1 reduced airway inflammation and hyperreactivity in an experimental model of asthma (Wegner et al., 1990).

ICAM-1 was expressed on endothelial cells in autoimmune reactions, including lupus nephritis and Graves and Hashimoto's diseases (Wuthrich et al., 1990; Zheng et al., 1990). In a rabbit model of hemorrhagic vasculitis (i.e., Schwartzman-like reaction), Argenbright et al. (1992) demonstrated an increase of endothelial ICAM-1 expression. Intravenous administration of an anti-ICAM-1 mAb abrogated the hemorrhagic response (Argenbright et al., 1992) pointing to a crucial role of ICAM-1 in the pathogenesis of hemorrhagic vasculitis.

Endothelial ICAM-1 was also expressed in the central nervous system of guinea pigs during acute and allergic encephalomyelitis (Wilcox et al., 1990). Whether ICAM-1 is related to the pathogenesis of these neurological inflammatory disorders remains to be determined. ICAM-1 expression in metastatic melanoma may provide a useful marker of the disease and might also be pathogenic in this condition (Natali et al., 1990). Interestingly, a soluble form of ICAM-1 was found to dramatically inhibit rhinovirus infection (Marlin et al., 1990) by antagonizing the interaction of the virus with its putative receptor, ICAM-1.

A role for ICAM-1 in acute lung injury induced by phorbol myristate acetate has been shown by antibody blocking with an anti-ICAM-1 mAb (Barton et al., 1989). ICAM-1 plays an important role in neutrophil migration into inflammatory sites in lungs. Studies also showed that lung ischemia/reperfusion- and TNFα-induced lung injury was dependent in ICAM-1 expression on pulmonary vascular endothelial cells (Horgan et al., 1991; Lo et al., 1992). MAbs directed against ICAM-1 prevented the pulmonary vascular neutrophil sequestration and lung injury in both experimental models.

III. E-Selectin

A. Cloning of E-Selectin and Its Structure

E-selectin was first identified by the mAbs H18/7 and H4/18 in cultured endothelial cells challenged with TNFα, IL-1β, or LPS (Bevilacqua et al., 1987, , 1989). The expression of E-selectin was transient, reaching a maximum at 4 hr and decaying by 24 hr; the kinetics of E-selectin expression paralleled the adhesion of leukocyte to endothelial cells (Bevilacqua et al., 1987, 1989).

A full-length cDNA for E-selectin was isolated and cloned by transient expression in COS cells (Bevilaqua et al., 1989). E-selectin expressed on COS cells was recognized by anti-E-selectin mAbs, and the expressed E-selectin on COS cells was shown to support adhesion of neutrophils, monocytic cells, and a subset of lymphocytes (Bevilacqua et al., 1989). Cloning of E-selectin indicated a NH_2-terminal lectin-like domain, an epidermal growth factor (EGF) domain, and six repetitive motifs (containing ~60 amino acids related to those found in complement regulatory proteins) (Bevilacqua et al., 1989). E-selectin is a highly glycosylated protein (> 30%) consistent with 11 potential glycosylation sites (Bevilacqua et al., 1989). Using a direct cloning approach, a subsequent study confirmed the E-selectin sequence (Hession et al., 1990). The sequence of E-selectin shares a ~60% identity with the lectin and EGF domains of P-selectin (Bevilacqua et al., 1989; Johnston et al., 1989). The gene structures of E- and P-selectin also have similar intron and exon boundaries (Collins et al., 1991). All selectin gene family members have been mapped to the long arm of human chromosome 1 (Tedder et al., 1989; Watson et al., 1991; Whelan et al., 1991).

B. E-Selectin Gene Expression

The factors regulating E-selectin gene expression are likely to be important in the initial phases of the inflammatory response because selectins are expresed early after cytokine challenge of endothelial cells. TNFα, IL-1β, and LPS induced E-selectin by gene transcription; unlike ICAM-1, there is no preformed E-selectin. Analysis of nucleotide sequences upstream of the transcription start sites indicated that a fragment of 233 base pairs was responsible for E-selectin induction (Whelan et al., 1991). The two elements between base pairs 233–117 and base pairs 94–85 (the NFκB consensus binding sites) were involved in cytokine inducibility of E-selectin. The action of cytokines on the promoter region (probably involving the NFκB-like transcription factor) is likely responsible for activation of E-selectin gene transcription (Collins et al., 1991). The NFκB protein subunits (consisting of p50 and p65) bind to the E-selectin κB site. Cotransfection of IκB-α (one of the IκB isoforms) with E-selectin reporter gene predictably abolished the TNFα-mediated E-selectin gene induction (Read et al. 1994). The E-selectin promoter also contains an inverted CCAAT box and AP-1 binding sequences (Collins et al., 1991); their relative contributions and the possible synergy of the NFκB-IκB and AP-1 regulatory components to E-selectin expression have not been defined.

The second-messenger pathways responsible for E-selectin induction are poorly understood. Evidence suggests that PKC is not likely involved because inhibition of PKC activation failed to prevent TNFα-induced expression of endothelial adhesivity (Ritchie et al., 1991). The role of other second-messenger (e.g., Ca^{2+} mobilization or phosphorylation events) has not been examined. In bovine aortic endothelial cells, treatment with 1-methyl-3-isobutyl xanthine plus forskolin (to increase cAMP) decreased the TNFα inducibility of the E-selectin promoter, suggesting that a negative regulatory element exists to down-regulate the response. The cAMP-mediated inhibition mapped to the cyclic adenosine monophosphate response element (CRE)/activation transcriptional factor (ATF) elements (−153) on the E-selectin promoter (De Luca et al., 1994). Whether this is the mechanism of "turning off" the E-expression after cytokine stimulation of endothelial cells must be defined.

C. Structure-Function Relationships of E-Selectin

Studies using soluble E-selectin chimeras demonstrated that the binding epitope for anti-E-selectin blocking mAb H18/7 was on the lectin and EGF-like domains (Walz et al., 1990). Pigott et al. (1991) raised a panel of anti-E-selectin mAbs and defined the structural components of E-selectin responsible for leukocyte adhesion. MAbs directed against the lectin domain prevented the adhesion, whereas mAbs against complement regulatory protein domains were ineffective (Pigott et al., 1991). The EGF-like domain appeared to be critically

involved in the adhesion response, possibly by maintaining the correct conformation of the lectin domain. Because leukocytes did not adhere to cells expressing E-selectin mutants that lacked either lectin or EGF-like domains (Pigott et al., 1991), it is likely that both domains are equally important in mediating E-selectin-dependent leukocyte adhesion.

D. Soluble E-Selectin

The recombinant form of soluble E-selectin (lacking the transmembrane and cytoplasmic domains) is a functional adhesion molecule because it mediated the binding of neutrophils, monocytes, natural-killer cells, and a subpopulation of T cells known to attach to E-selectin (Lobb et al., 1991). Soluble E-selectin was also chemotactic for neutrophils (Lo et al., 1991). Immobilized soluble E-selectin mediated adhesion of neutrophils, and adherent neutrophils exhibited increased CD11b/CD18 activity (Lo et al., 1991). However, soluble E-selectin weakly inhibited the E-selectin-dependent leukocyte adhesion in vitro. Intravenous injection of soluble E-selectin also reduced LPS-mediated neutrophil emigration in a rat model of bronchoalveolar inflammation (Ulich et al., 1994). Circulating soluble E-selectin (if present) in sufficient concentrations in vivo may be capable of modulating leukocyte attachment to endothelial cells in the microcirculation.

E. E-Selectin-Induced Leukocyte Adhesion

Basophils, eosinophils, and neutrophils adhered to IL-1β-activated vascular endothelial cells by binding to E-selectin and ICAM-1 as well as to VCAM-1 (Bochner et al., 1991). Anti-ICAM-1 or anti-E-selectin mAbs inhibited IL-1β-induced adhesion of each cell type. The effect of each mAb was additive (Bochner et al., 1991), indicating that the endothelial cells interacted with a different set of molecules on leukocytes. Anti-VCAM-1 mAb inhibited only basophil and eosinophil adhesion, whereas anti-E-selectin and anti-ICAM-1 mAbs inhibited neutrophil adhesion. Basophil and eosinophil adhesion depends on VCAM-1 expression and the interaction of VCAM-1 with VLA-4 (CD49d/CD29, the "counter-receptor" for VCAM-1) and on expression of E-selectin and ICAM-1 (Bochner et al., 1991). Neutrophil adhesion to activated endothelial cells depends on E-selectin and ICAM-1 expression because VLA-4 is not present on neutrophils (Springer et al., 1990). Thus, these unique functions of ICAM-1, E-selectin, and VCAM-1 are responsible for orchestrating in a subtle manner basophil, eosinophil, and neutrophil adhesion to the endothelium.

Although it is clear that neutrophil adhesion to cytokine-activated endothelial cells involves ICAM-1-dependent as well as E-selectin-dependent mechanisms, the relative contributions of each appears to be time-dependent in that E-selectin activity was important from 1 to 4 hr, whereas ICAM-1 activity (which interacts with CD11/CD18 on leukocytes) became the dominant adhesive molecule at the later times (Luscinskas et al., 1989). E-selectin activity was minimal at 24 hr, but both ICAM-1 and E-selectin activities were elevated at 4 hr (Luscinskas et al., 1989), indicating that both adhesion molecules are involved at the earlier time points.

Monocytes can bind to endothelium through ICAM-1, E-selectin as well as VCAM-1 (Carlos et al., 1991). Monocytes adhered to Chinese hamster ovary cells stably transfected with cDNAs of E-selectin or VCAM-1. Binding to E-selectin was inhibited by mAbs to E-selectin, and binding to VCAM-1 was inhibited by mAbs to VCAM-1 or VLA-4. E-selectin and VCAM-1 participated in the monocyte adhesion response because the effects of both mAbs were additive (Carlos et al., 1991).

F. E-Selectin-Mediated Leukocyte Migration

E-selectin and ICAM-1 are important in the mediation of transendothelial neutrophil migration. Maximum neutrophil adhesion and migration were evident at 4 hr after IL-1β treatment, when expression of E-selectin had peaked and ICAM-1 was also increased (Luscinskas et al., 1991). MAbs to E-selectin and ICAM-1 both inhibited more than 90% of the neutrophil migration at this time. In contrast, at the 24-hr or 48-hr points, E-selectin expression was decreased, whereas ICAM-I expression was significantly over the 4 hr; however, neutrophil adhesion remained elevated, whereas migration of neutrophils returned to baseline value evident in unstimulated endothelial cells (Luscinskas et al., 1991). These results indicate that migration of neutrophils is a rapid event and that both E-selectin and ICAM-1 mediate the the response at the early time points. Interestingly, the sustained ICAM-1 expression did not continue to promote emigration neutrophils, suggesting "factors" exist to prevent neutrophil migration from occurring despite the high ICAM-1 activities.

G. E-Selectin-Induced Up-Regulation of CD11b/CD18

E-selectin expression on the activated endothelium may provide a critical signal for leukocyte sequestration at inflammatory sites in light of the observations that E-selectin stimulated the functional activity of CD11b/CD18 integrin on neutrophils (Lo et al., 1991). Neutrophils binding to cytokine-stimulated endothelium and immobilized recombinant soluble E-selectin resulted in CD11b/CD18 activation. MAbs to E-selectin prevented the activation of CD11b/CD18 by cytokine-treated endothelial cells and soluble E-selectin. Because a truncated soluble E-selectin (lacking the transmembrane and cytoplasmic domains) induced chemotaxis and motility of neutrophils, this study raises the possibility that E-selectin serves as a "tethered chemoattractant" (Lo et al., 1991). The enhanced CD11b/CD18 activation of adherent neutrophil may also contribute to neutrophil aggregation in microvessels. These studies provide evidence of linkage between E-selectin expression and the up-regulation of another essential adhesion molecule

CD11b/CD18, which is thus capable of binding to ICAM-1 on the endothelium (fig. 1). Identification of the second-messengers by which E-selectin activates CD11b/CD18 would be an important area for future investigation.

H. E-selectin Engagement Leads to Monocyte Gene Activation

Adhesion of leukocytes is known to lead to an enhancement of functional activity of these cells. Kaplan et al. (1982) showed that adhesion of monocytes promoted differentiation, and Haskill et al. (1988) further established the c-*fos*/c-*jun* gene activation immediately after the adhesion step. Recent studies point to the specific engagement of leukocytes with selectins in mediating the adhesion-dependent leukocyte activation. It has been established that endothelial expression of PAF served as a possible costimulatory molecule in neutrophil migration. Monocyte tethering by P-selectin transduced the signals that increased the secretion of monocyte chemotactic protein-1 and TNFα when they were stimulated PAF (Weyrich et al., 1995). Thus, P-selectin is insufficient in itself to lead to activation of leukocyte functional activity; P-selectin signaling of leukocytes requires the juxtacrine co-expression of PAF.

We have recently obtained evidence that engagement of monocyte with E-selectin per se is sufficient to mediate monocyte gene activation. Thus, engagement of monocyte with E-selectin expressed on activated endothelial cells or transfectants during adhesion was shown to induce activation of multiple genes, including the tissue factor procoagulant, CD36 (a multifunctional glycoprotein that serves as a scavenger receptor for oxidized low density lipoprotein and adhesive receptor) and cytokines (IL-1β and TNFα) (Huh et al., 1995; Lo et al., 1995). Monocyte adhesion to cytokine-activated endothelial cells (expressing E-selectin) increased the expression of tissue factor in monocytes. The tissue factor induction was reduced by soluble CD15 (Lewis X, one of the E-selectin ligands) (Lo et al., 1995). Adhesion to cytokine-activated endothelial cells also induced an increase in CD36 expression and this effect could be reproduced by monocyte adhesion to E-selectin transfectants (but not ICAM-1 transfectants) (Huh et al., 1995). By immunologically cross-linking the monocyte surface E-selectin ligand (i.e., sialyl-CD15, CD15, and dimeric sialyl CD15), we showed that CD15 (but not sialyl-CD15 or dimeric sialyl CD15) mimicked monocyte activation similar to that induced by monocyte attachment to E-selectin. This study demonstrated that CD15-bearing cell surface structures are directly involved in E-selectin signaling. Quantitative reverse transcriptase-polymerase chain reaction has also shown an increase in the steady state TNFα and IL-1β mRNAs after cross-linking of CD15, without apparent change in the level of β-actin mRNA.

The induction of genes mediated by E-selectin ligand occurred at the level of mRNA transcription (Lo et al., 1995). Studies using tissue factor promoter coupled to luciferase reporter gene transfected to monocytic cells indicated that cross-linking CD15 was sufficient to induce gene activation. Promoter deletion studies revealed that a sequence (−224 to −194) on tissue factor promoter, which included two AP-1 sites, is required for E-selectin's effect (Lo et al., unpublished observation). Gel shift assay showed that CD15 selectively up-regulated AP-1 transcriptional factors (Lo et al., 1995), suggesting that engagement of E-selectin to its ligands is capable of initiating specific intranuclear signaling pathways. Because the P-selectin ligand induces NF$\kappa\beta$ nuclear translocation (Celi et al., 1994), all members of the selectin family were likely to mediate gene activation by binding to their counter-receptors.

I. E-Selectin Expression in Inflammatory Diseases

E-selectin was expressed in dermal postcapillary venule endothelial cells between 20 min and 24 hr after the interdermal allergen challenge (Leung et al., 1991). E-selectin was also found in skin biopsies of patients with erythema and delayed hypersensitivity (Leung et al., 1991; Gundel et al., 1991). E-selectin is involved in the pathogenesis of acute airway inflammation in primates. An anti-E selectin mAb prevented leukocyte recruitment into lungs after inhalation of antigen E-selectin and also prevented airway constriction (Gundel et al., 1991). In addition, E-selectin was expressed on the vascular endothelium in psoriasis and alopecia areata (Nickoloff et al., 1991; Groves et al., 1991). Induction of E-selectin paralleled leukocyte infiltration into tissues in that both were evident by 6 hr and were maximal by 24 hr (Munro et al., 1991). These results suggest that E-selectin expression on the activated vascular endothelium in allergic skin reactions contributed to transendothelial migration of inflammatory cells.

E-selectin is widely induced in cutaneous inflammation, with a more sustained time course than observed in vitro (Groves et al., 1991), reflecting a sustained ability to activate E-selectin gene expression in chronic inflammation in vivo. The duration of in vivo E-selectin expression as compared with the response in cultured endothelial cells has a potentially important implication in that E-selectin would be capable of recruitment of neutrophils at inflammatory sites for extended periods. E-selectin was also expressed in venules and capillaries in chronic inflammatory diseases, including rheumatoid and osteoarthritic synovial tissues along with VCAM-1 and ICAM-1 (Koch et al., 1991), suggesting that these adhesion molecules function in a complex manner to mediate leukocyte migration into the synovium in rheumatoid arthritis and osteoarthritis.

E-selectin was present on the vascular endothelium of allografted tissue and hence may contribute to acute rejection of transplanted organs (Sedmak et al., 1991).

The expression of E-selectin and other adhesion molecules on endothelial cells may mediate to allograft rejection secondary to the recruitment of lymphocytes into transplanted tissues. E-selection is known to be of primary importance in the attachment of a subset of T-lymphocytes of inflamed endothelium in vivo (Shimizu et al., 1991; Picker et al., 1991), a critical effector cell contributing to organ rejection.

Studies quantifying neutrophil recruitment in primates after injection of endotoxin (500 μg of *Escherichia coli*-derived LPS) showed marked expression of E-selectin within 2 hr after LPS challenge (Munro et al., 1991). This was associated with neutrophil adhesion as well as neutrophil extravasation (Munro et al., 1991). E-selectin decreased with time and was not evident by 9 hr. In contrast, ICAM-1 activity did not change in response to endotoxin challenge during this period (Munro et al., 1991), pointing to E-selectin expression as the initiating event in the adhesion response during sepsis. Unlike septic shock that induces E-selectin on the vascular endothelium, traumatic or hypovolemic shock had no such effect (Redl et al., 1991).

Matis et al., (1991) showed that Substance P induced the expression of E-selectin in microvascular endothelial cells. Exposure of endothelial cells for 6 hr with Substance P showed histochemical evidence of E-selectin, indicating an important role of E-selectin in the neurogenic modulation of immune response, a phenomenon dependent on Substance P.

J. Binding of E-Selectin to Carbohydrate Moieties on Leukocytes

The presence of a homologous mammalian (C-type) lectin motif on the NH_2-terminal of E-selectin (Bevilacqua et al., 1989) suggests that it recognizes and interacts with carbohydrate moieties on leukocytes as the "counter-receptors." Recent studies have identified several E-selectin ligands on leukocytes as carbohydrates containing sialyl-lactosamines.

E-selectin-dependent adhesion of neutrophils and a variety of cell lines including HL-60 correlated with the cell expression of sialyl-Lewis X (sialyl-CD15, SLe^x). MAbs specific for SLe^x, fucosylated α_1-acid glycoprotein as well as liposomes containing difucosylated glycolipids with SLe^x moiety abrogated the HL-60 and Lec 11 CHO cells adhesion to E-selectin (Walz et al., 1990; Phillips et al., 1990). Enzymatic removal of sialic acid from SLe^x greatly reduced the ligand activity (Walz et al., 1990). Liposomes containing Lewis X (Le^X) structure lacking sialic acid had a partial inhibitory effect, suggesting that Le^x also interacts E-selectin, albeit with a lower affinity. These studies indicate that SLe^x structure is a predominant ligand for E-selectin and that Le^x may interact with E-selectin at low affinity. Interestingly, Le^x has been shown to be a low affinity ligand for P-selectin (CD62), indicating an overlapping in ligand recognition and specificity between these two selectin members.

Tiemeyer et al. (1991) used radiolabeled COS cells transfected with plasmids containing cDNA for E-selectin to screen for glycolipids extracted from human leukocytes. Mass spectrometry revealed that E-selectin bound to terminally sialylated lactosylceramides with a variable number of N-acetyllactosamine repeats and at least one fucosylated N-acetylglucosamine residue (Tiemeyer et al., 1991). Adhesion to these glycolipids required Ca^{2+} but was not inhibited by heparin, chondroitin sulfate, keratan sulphate, or yeast phosphomannan (Tiemeyer et al., 1991).

Fucosylation of lactosamine carbohydrate structures is an essential requirement of E-selectin ligands. Goelz et al., (1990) cloned out an E-selectin ligand fucosyltransferase and showed that transfection of cells with the α(1, 3) fucosyltransferase cDNA conferred binding activity of these cells to E-selectin. The α(1, 3) fucosyltransferase activity was expressed in cell lines that demonstrated binding to E-selectin (Goelz et al., 1990). The α(1, 3) fucosyltransferase activity may regulate cell adhesion to E-selectin by modulating the cell surface expression of one or more of the α(2, 3) sialylated, α(1, 3) fucosylated lactosaminoglycans of the SLe^x complex (Lowe et al., 1990). These studies point to α(1, 3) fucosyltransferase as the important regulator of fucosylation of carbohydrates on leukocytes, and hence in the regulation of on E-selectin-dependent cell adhesion.

Mouse pre-B cell line L1–2 transfected with E-selectin cDNA bound to SLe^x as well as SLe^a (Berg et al., 1991). SLe^a is composed of identical sugar residues as SLe^x, but differs in stereochemistry (Berg et al., 1991). The binding of both SLe^x and SLe^a to E-selectin is in accord with the hypothesis that E-selectin recognizes a spectrum of related carbohydrate structures. Whereas SLe^x is a terminal carbohydrate structure found on cell surface glycoproteins and glycolipids on leukocytes, SLe^a is not present on leukocytes. Therefore, the "counter-receptor" for E-selectin likely consists of a family of sialyated fucosylated lactosamines with SLe^x [NeuAcα2–3 Gal β1–4(Fucα1–3)G1cNAc-] as the central recognition site (Walz et al., 1990; Phillips et al., 1990).

SLe^x and Sle^a are both found on the surface of some tumor cells, including breast and colon-rectal cancer cells (Matsushita et al., 1990; Takara et al., 1991; Hoff et al., 1989; Giavazzi et al., 1990). As cell surface carbohydrate antigens—in particular, sialoglycoproteins—have been implicated in the metastatic potential of tumor cells (Hoff et al., 1989), it is reasonable to speculate that SLe^x and related structures play a role in tumor metastasis. In this regard, studies showed a correlation between the expression of dimeric SLe^x and increased metastatic potential in colon-rectal carcinoma (Giavazzi et al., 1990). Because cytokines are released in cancer patients, the resultant E-selectin expression on endothelial cells may increase the colonization of blood-borne tumor cells by facilitating tumor metastasis at sites of inflam-

mation. The role of E-selectin ligands in the tumor metastasis awaits further studies.

K. Glycoproteins on Leukocytes as E-selectin Ligands

Using the mouse E-selectin-immunoglobulin G fusion protein, Levinovitz et al. (1993) have recently identified a unique 150-kD transmembrane glycoprotein in detergent extracts of neutrophil progenitor cells as another ligand for murine E-selectin. Cloning of the glycoprotein indicates the E-selectin ligand (ESL-1) is a protein that is highly conserved (> 94% homology at the amino acid level between chicken and mouse) and is expressed on both neutrophils and monocytes (Steegmaier et al., 1995). Specific anti-ESL-1 antibodies inhibited attachment of leukocytes to TNFα-treated endothelial cells as well as to purified E-selectin. These data indicate that ESL-1 is an important counter-receptor for E-selectin on myeloid cells. The structural relationships between ESL-1 and the well described carbohydrate-based E-selectin ligands (such as sialyl-CD15 or CD15) are presently unknown. It is important to note that the E-selectin recognition required the expression of both the glycoprotein ligand and fucosyl transferase because cotransfection of ESL-1 cDNA along with the fucosyl transferase cDNA was necessary for full binding cells of the purified E-selectin. Cloning data indicated a high degree of structural homology of ESL-1 to the chicken cysteine-rich FGF receptor (CFR), a putative signaling receptor characterized by Burrus et al. (1989). It is possible that ESL-1 represents a splice variant of the murine CFR. The relationship between ESL-1 and CFR in mediating E-selectin binding, or possibly cell signaling, is an area that deserves study.

IV. P-Selectin

A. Structure of P-Selectin

P-selectin is an adhesive glycoprotein (MW 140 kD) translocated to the cell surface of platelets and endothelial cells after treatment with thrombin or phorbol esters (Hattori et al., 1989). Molecular cloning and cDNA sequence analysis revealed that P-selectin was structurally similar to E-selectin; the NH_2-terminus of P-selectin has a lectin domain followed by an EGF domain and nine tandem consensus repeats related to complement binding proteins, a transmembrane domain, and a short cytoplasmic tail (McEver, 1990). The P-selectin molecule is also cysteine-rich and heavily glycosylated. A soluble form of P-selectin lacking a transmembrane domain was effective in reducing neutrophil adhesion (Gamble et al., 1990).

P-selectin gene was found in a cluster on mouse and human chromosome 1 (Watson et al., 1991). Gene linkage analysis indicated that the P-selectin gene mapped to a region of mouse chromosome 1 analogous to human chromosome 1 (Watson et al., 1991). Because all members of selectins share high sequence homology and domain organization, this gene family is likely produced by duplication and rearrangement of a central exon. This hypothesis gains further support from the finding that all three selectin genes are clustered on chromosome 1 in mice and humans (Watson et al., 1991). The selectin gene family likely arose by multiple gene duplications before divergence of mouse and human chromosomes. A close evolutionary relationship between complement receptor components and selectins is also evident by the finding that the complement receptor-related gene is located on the same chromosome of both species (Watson et al., 1991).

B. Cellular Distribution of P-Selectin

P-selectin is located in the secretory α-granules in platelets and Weibel-Palade bodies in endothelial cells (McEver et al., 1990). P-selectin redistribution to the surface of platelets and endothelial cells occurs as a result of exocytosis within minutes after stimulation with histamine, thrombin, H_2O_2 phorbol esters, or calcium ionophores (Hattori et al., 1989). P-selectin translocation in endothelial cells peaked by 10 min, and then decreased within 30 min (Hattori et al., 1989). The intracellular mechanisms leading to P-selectin exocytosis are not yet clear, but it is likely that they involve activation of second-messenger pathways.

In the intact microcirculation, P-selectin up-regulation persisted 1 to 2 hr after thrombin challenge (Anene et al., 1992). Oxidants also resulted in prolonged expression. H_2O_2, t-butylhydroperoxide, or menadione promoted adhesion of neutrophils to endothelial cells secondary to P-selectin expression (Patel et al., 1991). Antibodies against P-selectin inhibited neutrophil adhesion after oxidant challenge (Patel et al., 1991). Moreover, soluble P-selectin inhibited CD11/CD18-dependent adhesion (Gamble et al., 1990), whereas Lorant et al. (1991) reported that P-selectin did not affect CD18 activity. The question whether P-selectin interacts with CD11/CD18 is unresolved.

P-selectin was localized on pulmonary vascular endothelium in rats after systemic complement activation with cobra venom factor (Mulligan et al., 1991). The rapid kinetics of P-selectin expression in vivo paralleled the in vitro response, and an anti-P-selectin mAb reduced the neutrophil sequestration, permeability increase, and hemorrhage (Mulligan et al., 1991).

Agents up-regulating P-selectin also induced rapid synthesis and expression of PAF, a potent inducer of CD11/CD18 integrin (Zimmerman et al., 1990). It is believed that PAF serves as a "tethering" and "signaling" molecule and thus strengthens cell adhesive interactions. The cooperation of P-selectin and PAF is an illustration of the diverse pathways involved in leukocyte recruitment at inflammatory sites. The synergistic function of PAF and P-selectin in vivo has yet to be fully explored.

C. P-Selectin-Mediated Leukocyte Adhesion

P-selectin is responsible for rapid adhesion of leukocytes to endothelial cells (Geng et al., 1990). Neutrophils and promyelocytic HL-60 cells bound to COS cells transfected with cDNA encoding P-selectin (Geng et al., 1990). P-selectin also mediated platelet-neutrophil adhesion (Hamburger et al., 1990), and thus contributes to accumulation of platelets and neutrophils at the same sites.

Binding of P-selectin to neutrophils and HL-60 cells was strongly inhibited by heparin, fucoidin, and dextran sulphate (MW 500,000) and was partially inhibited by various dextran sulphates (MW 5000), but was unaffected by 4- and 6-chondroitin sulphates (Skinner et al., 1991). Thus, the interactions of these molecules with specific sugars on P-selectin can modify the binding of leukocytes to P-selectin on the endothelium, and may explain the known anti-inflammatory properties of carbohydrates such as dextrans.

D. P-Selectin Binding to Ligands on Leukocytes

P-selectin-mediated leukocyte adhesion is dependent on the sialic acid residues on leukocytes. This mechanism is based on the observation that treatment of neutrophils and HL-60 cells with broad spectrum sialidases prevented the binding of these cells to activated platelets (Corral et al., 1990). Unstimulated neutrophils rapidly bound ^{125}I-P-selectin at 40°C, reaching equilibrium in 10 to 15 min with the binding being Ca^{2+}-dependent, reversible, and saturated at 3 to 6 mol free P-selectin (Moore et al., 1991). Receptor density and affinity were not altered by treatment of neutrophils with phorbol esters, whereas treatment with sialidase from *Vibrio cholera* (which cleaves α2–6-and α2–8-linked sialic acid) reduced the binding (Corral et al., 1990). The α2–6 linkage of sialic acid is apparently critical for leukocyte binding to P-selectin (Moore et al., 1991; Corral et al., 1990). Sialic acid residues are required for P-selectin binding to neutrophils.

P-selectin binding to leukocytes was inhibited by mAbs to the myeloid oligosaccharide Le^x (CD15) present on leukocytes (Moore et al., 1991; Lavson et al., 1991). Purified soluble Le^x oligosaccharide inhibited the adhesive interaction of activated platelets with neutrophils and monocytes (Lavson et al., 1991). In an attempt to determine whether P-selectin and E-selectin bind to the same counter-receptor, SLe^x, Polley et al. (1991) demonstrated that P-selectin also recognized SLe^x. MAbs against SLe^x inhibited P-selectin-mediated binding of HL-60 cells to activated platelets. A soluble oligosaccharide containing SLe^x was effective in inhibiting neutrophil adhesion to activated platelets (Polley et al., 1991).

Experimental evidence form Steegmaier et al. (1995) showed that P-selectin also interacted with ESL-1, although probably with lower affinity than E-selectin. These data suggest an overlap in the ligand structures shared by E-selectin and P-selectin, even though P-selectin has a broader spectrum of ligand recognition than E-selectin and has different binding affinities to its oligosaccharide ligands.

V. Cell Adhesion Molecules in Experimental Models of Inflammation

Cell adhesion molecules on leukocytes and vascular endothelial cells are essential for recruitment of leukocytes at sites of inflammation and are thus critical in the pathogenesis of acute and chronic inflammatory diseases. The relevance of these adhesion molecules is underscored by the protective effects of mAbs or compounds targeted against the cell adhesion molecules in preventing the progression of inflammation. The protective effects of the agents are probably secondary to an interference of rolling, adhesion, migration response or ultimately of recruitment of leukocytes in numerous experimental models that include ischemic-reperfusion injury in lungs and heart, asthma, hemorrhagic/septic shock, bacterial meningitis, Schwartzman reaction, and cytokine-induced lung injury (table 2).

The current dogma is that leukocytes roll on endothelial cells expressing E- and P-selectins and that this selectin-mediated leukocyte rolling represents the initial event in the in vivo adhesive interaction. Interference with the rolling step prevented the leukocyte adhesion and migration, indicative of a crucial role of the selectins in these responses. In addition to the use of blocking antibodies, other approaches have included selectin chimeras and selectin ligand oligosaccharides, both of which have also been known to inhibit interaction of selections with their ligands. In a recent study using rat as a model, the immunolocalization of E-selectin in the vascular endothelium of lungs after immune complex challenge was associated with lung injury (Mulligan et al., 1991). MAbs against E-selectin prevented leukocyte sequestration and the lung injury. Administration of anti-E-selectin mAb in rats also reduced myocardial ischemia/reperfusion-induced tissue (Altavilla et al., 1994; Seekamp et al., 1994). In a primate model of asthma, E-selectin was present on vascular endothelium with time course of expression paralleling the neutrophil influx into the lungs (Gundel et al., 1991). Anti-E-selectin (but not anti-ICAM-1) prevented both neutrophil recruitment and late-phase airway obstruction.

Similar to the effect of E-selectin, blockade of P-selectin is beneficial in inflammatory disorders. In anti-glomerular basement membrane antibody-induced glomerulonephritis in mice, acute glomerular injury after complement activation resulted in neutrophil accumulation and renal damage as manifested by proteinuria. Infusion of anti-P-selectin mAb inhibited glomerular neutrophil accumulation and prevented the proteinuria (Tipping et al., 1994). Weyrich et al. (1995) showed the P-selectin up-regulation in coronary arteries and veins after myocardial ischemia/reperfusion in a feline model

TABLE 2

Effects of monoclonal antibodies against cell adhesion molecules in experimental models of inflammatory diseases

Studies	MAbs	Disease Models	Outcomes
Arfors et al., 1986	60.3 (α CD18)	Inflammatory skin lesions in rabbits	Abolished neutrophil accumulation and plasma leakage
Vedder et al., 1988	60.3 (α CD18)	Hemorrhagic shock in rabbits	Ameliorated multiple organ failure and improved survival rate
Simpson et al., 1988	904 (α CD11b)	Ischemia-reperfusion in pig heart	Reduced infarct size
Barton et al., 1989	R3.3 (α CD18) R3.1 (α CD11a/CD18) R0.5 (α ICAM-1)	PMA-induced lung inflammation	Neutrophil migration reduced by α CD18 and α ICAM-1 mAbs (unaffected by α CD11a/CD18 mAb)
Tuomanen et al., 1989	IB4 (α CD18)	Bacterial meningitis in rabbits	Reduced tissue damage and mortality
Horgan et al., 1990	IB4 (α CD18)	Ischemia-reperfusion in rabbit lungs increase	Prevented pulmonary leukostasis and vascular permeability
Doerschuk et al., 1990	60.3 (α CD18)	Pulmonary and systemic inflammation in rabbits	Prevented neutrophil emigration by PMA; partial attenuation with endotoxin; no effect on S. pneumoniae or HCl-induced pneumonias
Mileski et al., 1990	60.3 (α CD18)	Hemorrhagic shock in primates	Reduced fluid requirement during resusitation
Kaslovsky et al., 1990	IB4 (α CD18)	Neutrophil-mediated lung injury in rabbits	Prevented lung vascular injury
Wegner et al., 1990	RR1/1(α ICAM-1) R15.7 (α CD18)	Asthma in primates	Attenuated airway eosinophilia and hyperresponsiveness
Gundel et al., 1991	CL-2 (α E-selectin)	Acute airway inflammation in primates	α-E-selectin mAb prevented neutrophil infiltration (no effect of α ICAM-1 mAb)
Mulligan et al., 1991	CL-3 (α E-selectin)	Immune complex-induced lung injury in rats	Reduced neutrophil recruitment and lung and skin vascular permeability increases
Horgan et al., 1991	RR1/1(α ICAM-1) IB4 (α CD18)	Ischemia-reperfusion in rabbit lungs	α CD18 and α ICAM-1 mAbs prevented lung vascular permeability increase to some degree
Argenbright et al., 1992	R6.5 (α ICAM-1) R15.7 (α CD18) R7.1 (α CD11a) LM2 (α CD11b)	Schwartzman reaction in rabbits	mAbs against ICAM-1, CD18, and CD11b inhibited skin hemorrhage (α CD11c mAb had no effect)
Ma et al., 1992	R15.7 (α CD18)	Reperfusion myocardial injury in cats	Reduced neutrophil-mediated myocardial injury and dysfunction during reperfusion
Lo et al., 1992	IB4 (α CD18) RR1/1(α ICAM-1)	TNF-induced lung injury in guinea pig	α CD18 and α ICAM-1 mAbs prevented lung vascular permeability increase
Winn et al., 1993	PB1.3 (α P-Selectin)	Reperfusion injury in rabbit ears	Reduced ischemic-reperfusion injury
Ma et al., 1993	(α L-Selectin)	Ischemic-reperfusion injury in cat myocardium	Attenuated neutrophil accumulation
Weyrich et al., 1993	PB1.3 (α P-Selectin)	Feline model of myocardial ischemia and reperfusion	Attenuated myocardial necrosis
Seekamp et al., 1994	(α E-/L-/P-Selectins)	Lung injury and rat hind limbs	Lung injury was E- and P-Selectins dependent and hind limb injury was dependent on L-Selectin
Altavilla et al., 1994	(α E-Selectin)	Myocardial ischemia-reperfusion in rats	Improved survival rate, reduced myocardial injury and decreased myeloperoxidase activity
Lo et al., 1994	(α E-Selectin Ligands)	TNF-induced lung injury in guinea pig	α sialyl-CD15 and α CD15 reduced lung permeability and neutrophil sequestration
Bernard et al., 1994	(Neutrophil Inhibitory Factor)	TNF-induced lung injury in guinea pig	Reduced neutrophil adherence and tissue injury
Bogen et al., 1994	(α CD31)	Peritonitis model	Block neutrophil emigration but not adhesion
Mulligan et al., 1994	(α L-Selectin)	Complement activation lung injury	Reduced lung permeability and prevented neutrophil adhesion
Lee et al., 1995	P-Selectin-Immunoglobulin G chimera	Rabbit ear model of ischemia-reperfusion	Attenuated reperfusion injury

and the cardioprotective effects of an P-selectin mAbs (Weyrich et al., 1993). Using a P-selectin immunoglobulin G chimera, Lee et al. (1995) demonstrated that the P-selectin-immunoglobulin G was protective in the rabbit ear model of ischemia-reperfusion.

The roles of E- and P-selectin ligands on leukocytes in tissue inflammation in vivo are less clear. Our evidence using challenged guinea pig lung indicated the participation of both ICAM-1 and CD11/CD18 in the response (fig. 2). Further studies in this animal model indicated

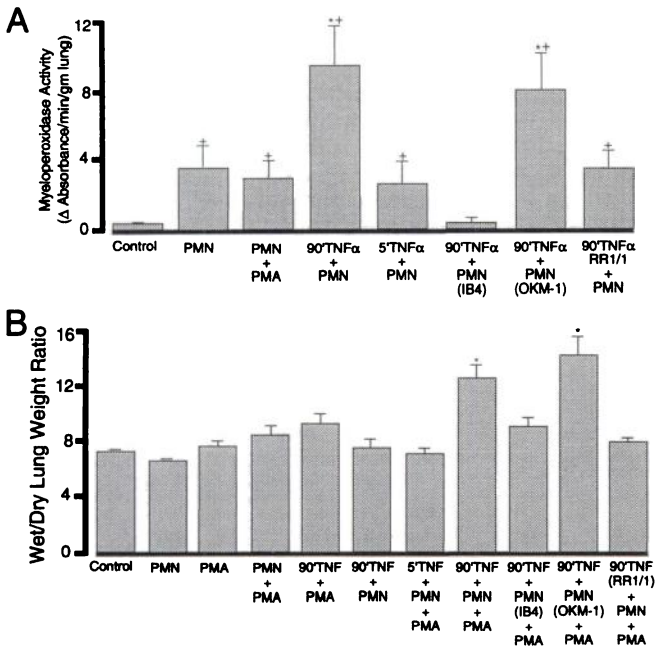

FIG. 2. Effects of anti-CD11/CD18 and anti-ICAM-1 mAbs (IB4 and RR1/1, respectively) on TNFα (1000 IU/ml) for various times before addition of human neutrophils (2×10^7 cells). Neutrophils or lungs were pretreated with mAbs (IB4, α CD18, OKM-1, α CD11b and RR1/1, α ICAM-1) at 10 μg/ml before addition of PMA (5×10^{-9} M). Both anti-CD18 and anti-ICAM-1 mAbs reduced the neutrophil sequestration in the pulmonary vascular bed (*A*) and prevented pulmonary edema formation (*B*) (from Lo et al., 1992, used with permission of the authors and from The Rockefeller University Press).

the role of the E-selectin counter-receptors (e.g., sialyl-CD15; CD15) on neutrophils. Both anti-sialyl CD15 and CD15 prevented neutrophil recruitment in the vasculature in a guinea pig acute lung injury model and reduced the lung edema and injury (Lo et al., 1994). Consistent with our finding is the observation that infusion of sialyl-CD15 oligosaccharide (a carbohydrate ligand for E-selectin and P-selectin) interfered with the adhesion between P-selectin and its ligand and reduced the lung injury accompanied by reduction of tissue accumulation of neutrophils (Mulligan et al., 1993).

Adhesion molecules E- and P-selectins that "tether" circulating leukocytes to endothelial cells is a prerequisite for activation-induced adhesion of leukocytes via the CD11/CD18-ICAM-1 interaction. Evidence indicates that the CD11/CD18 integrin family on neutrophils participated in the pathogenesis of tissue injury and inflammatory responses. Arfors et al. (1986) were the first to show that a mAb to CD11/CD18 (mAb 60.3) prevented the intradermal neutrophil infiltration and plasma leakage of proteins. Other experiments have documented the role of CD11/CD18 in hemorrhagic shock (Vedder et al., 1988; Mileski et al., 1988), lung edema (Kaslovsky et al., 1990), lung inflammation (Barton et al., 1989), meningitis (Tuomanen et al., 1989), asthma (Wegner et al., 1990), myocardial ischemia/reperfusion (Simpson et al., 1988; Ma et al., 1991), and skin allergic inflammation (Nourshargh et al., 1989; Nourshargh et al., 1990).

Using a rabbit model of ischemia-reperfusion, we showed that the anti-CD18 mAb IB4 prevented lung edema (Horgan et al., 1990) (fig. 3). This protective effect was accompanied by inhibition of neutrophil recruitment in the lungs. The role of CD11/CD18 in TNFα-mediated lung injury was shown in guinea pig lungs (Lo et al., 1992). Inhibition of neutrophil sequestration induced by the anti-CD18 mAb IB4 prevented the lung vascular injury (Lo et al., 1992). Studies using neutrophil inhibitory factor (NIF), a 41-kD cloned protein from canine hookworm that binds to the I domain of CD11b, showed inhibition of CD11b/CD18-dependent neutrophil adhesion, confirming studies using the anti-CD11/CD18 mAbs (Bernard et al., 1995). NIF reduced neutrophil

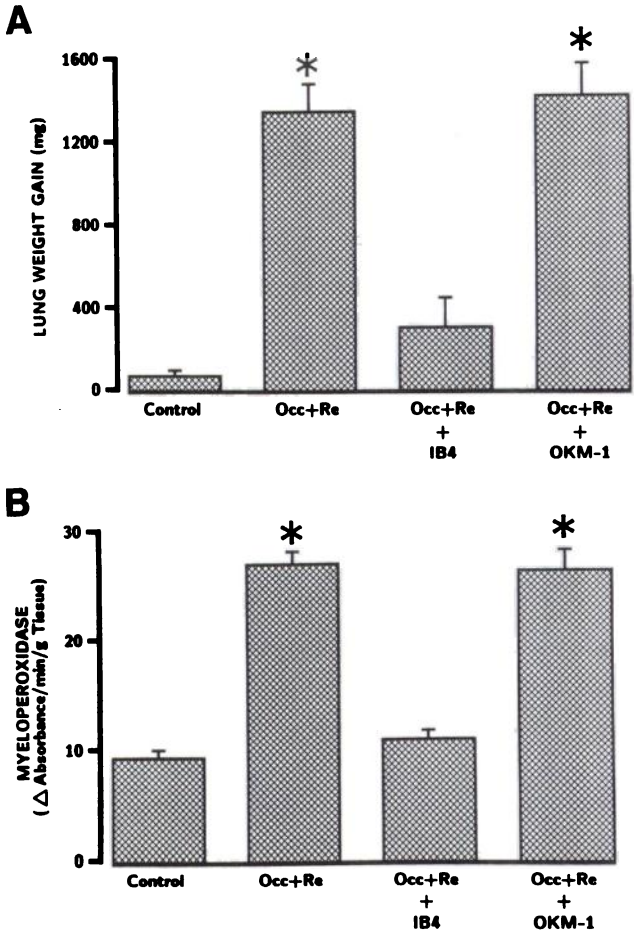

FIG. 3. Role of CD11/CD18 in the development of pulmonary edema after ischemia and reperfusion in the rabbit lung. The right pulmonary artery in rabbits was occluded for 24 hr, followed by reperfusion for 2 hr. Monoclonal antibodies against CD18 (IB4, 0.5 mg/kg) and CD11b (OKM-1, 0.5 mg/kg) were infused i.v.. Ischemia reperfusion caused lung injury and edema formation as indicated by the increase in lung wet weight (*A*). MAb prevented the pulmonary edema, whereas control mAb OKM-1 had no effect. MAb IB4, but not OKM-1, also prevented pulmonary leukostasis (*B*) (from Horgan et al., 1990, used with permission from the authors and from The American Physiological Society).

sequestration and also prevented the increase in vascular permeability in the TNFα-mediated lung injury (Bernard et al., 1995)

Of interest is the finding that the anti-CD18 mAb (60.3) failed to prevent neutrophil emigration into lung alveoli induced by *Streptococcus pneumoniae* (*S. pneumoniae*) (Doerschuk et al., 1990). The identity of adhesion molecules that mediate this CD18-independent response is not known.

Anti-ICAM-1 mAbs share identical protective effects to anti-CD11/CD18 mAbs in numerous experimental models of inflammation. This suggests that the CD11/CD18-ICAM-1 adhesive interaction is critical for neutrophil sequestration in vivo. Anti-ICAM-1 mAbs prevented lung inflammation (Barton et al., 1989), asthma (Wegner et al., 1990), ischemia/reperfusion (Nourshargh et al., 1990), and cytokine-induced lung injury (Lo et al., 1992).

The interesting work stemming from Bogen et al. (1994) indicated that CD31, an adhesion molecule constitutively expressed in the lateral borders between endothelial cells and leukocytes, plays a role in leukocyte recruitment during tissue inflammation. Thus, mAb against mouse CD31 prevented the emigration of leukocytes into peritoneal cavity induced by thioglycollate in a murine model of acute inflammation. Morphological examination of the mesenteric venules showed that leukocytes were present on the luminal surface of the blood vessels, but that they failed to emigrate. These observations support the hypothesis that CD31 also participates in the leukocyte migration response.

VI. Studies in Selectin and ICAM-1 "Knock-Out" Mice

In addition to the animal models using adhesion molecule-specific mAbs, another approach yielding much information has been the study of "knock-out" mice produced by homologous recombination techniques. In essence, experimental data from the knock-out mice have confirmed the crucial role of adhesion molecules in leukocyte migration in inflammatory diseases. Furthermore, it has revealed the complexity and redundancy of cell adhesion molecules in mediating these effects. Mice lacking P-selectin have a normal development, yet the ability of leukocytes to roll in mesenteric vessels was diminished, as was the capacity of these mice to mount the inflammatory response after the challenge of *S. pneumoniae* (Bullard et al., 1995). These mice had reduced peritoneal accumulation of inflammatory leukocytes. The data suggest that P-selectin is important for leukocyte rolling in peritoneal vessels at sites of inflammatory injury.

Mice deficient in E-selectin have a similar phenotype to that of P-selectin loss. Whereas immunological blockade of E-selectin in experimental animal models prevented inflammatory leukocyte infiltration, E-selectin-deficient mice appeared to have minimal, if any, defect in leukocyte accumulation (Labow et al., 1994). Thus, single "knock-out" of either P-selectin or E-selectin does not abrogate the leukocyte migration and inflammatory response. Mice that are deificient in one "selectin" system of the adhesion molecules can still mount the neutrophil emigratory response. In contrast, administration of anti-P-selectin mAb to E-selectin "knock-out" mice completely prevented neutrophil emigration (Labow et al., 1994). These data indicate a complementary relationship between P-selectin and E-selectin in leukocyte rolling and adhesion (Labow et al., 1994).

There also appears to be a great degree of functional overlap among adhesion molecules in mediating neutrophil emigration. Because neutrophil migration is dependent on ICAM-1 to produce firm attachment, it is no surprise that ICAM-1-deficient mice exhibited impaired neutrophil migration in response to bacterial challenge (Sligh et al., 1993). Furthermore, the neutrophil emigration was totally inhibited in P-selectin-/ICAM-1 double mutants (Bullard et al., 1995), supporting a co-operativity between selectins and ICAM-1. Interestingly, although P-/E-selectins and ICAM-1 are important in neutrophil rolling and emigration into the peritoneum during *S. pneumoniae*-induced peritonitis, the response appears to be organ-specific in that the neutrophil emigration into the parenchyma of lungs in response to the same stimulus was not altered in the P-selectin/ICAM-1 double mutant mice (Bullard et al., 1995). The explanation for the finding is the possibility that these are alternate adhesive mechanisms operating within the pulmonary capillaries.

The above studies (described in section V) all indicate the importance of cell adhesion molecules in leukocyte recruitment and in the pathogenesis of inflammatory diseases in vivo. A better understanding of the subtlety of molecular determinants of leukocyte-endothelial cell interactions and development of specific peptides and sugars that can simultaneously interfere with the multiple adhesive interactions will provide valuable interventions in acute and chronic inflammatory diseases.

VII. Conclusions

Adhesion molecules on endothelial cells orchestrate a complex series of responses between endothelial cells and leukocytes. The primary role of these adhesive interactions is to facilitate leukocyte migration across the endothelial barrier to defend the host during infection. The adhesion response serves as an important initial step in the host-defense response. However, inappropriate attachment of leukocytes to the "hyperadhesive" or "activated" endothelial cell surface can occur by the expression of one or more endothelial adhesion molecules. This may contribute to the pathogenesis of disorders such as the adult respiratory distress syndrome and ischemic-reperfusion tissue injury.

The finding that the adhesion molecules are essential for leukocyte adhesion and migration and that adhesion

and migration can be separately inhibited by mAbs directed against these molecules suggests that it is possible to control as well as reverse the progression of the inflammatory response. Important questions that remain to be addressed relate to the (*a*) mechanisms of induction of the endothelial adhesion molecules at the level of the gene and the involvement of second-messenger pathways, (*b*) cooperations and interactions between the leukocyte and endothelial adhesion molecules, (*c*) precise role of the adhesion molecules in the host-defense response and in the mediation of inflammatory disorders, (*d*) operation of these molecules in the intact microcirculation under varying shear stress and flow conditions, and (*e*) function of adhesion molecules in differentiated endothelial cells such as those of the blood-brain barrier.

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