Cellular Mechanisms of Leukocyte Adhesion*

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CONTENTS

Introduction

Endothellial cells and leukocytes express adhesion promoting receptors that mediate cell-cell interactions and in some cases cell-matrix interactions. These interactions regulate the trafficking and migration of leukocytes across the vascular endothelial barrier and into tissues where they are involved in immune and inflammatory responses. Several adhesion receptors with highly regulated and specialized functions have been characterized.

The first family of adhesion receptors, termed "selectins," are characterized by a common lectin domain which binds to carbohydrate residues (e.g., sialyl Lewis X-antigen) present on leukocytes and on endothelial cells. The selectin, LECCAM-1, a human homologue of the murine homing receptor Mel-14, is expressed on leukocytes. Endothelial adhesion molecule-1 (ELAM-1) and the granule membrane protein (GMP-140) are expressed on the endothelial cell plasmalemmal membrane; GMP-140 is also present on platelets (in α-granules).

Second, the leukocyte integrin family comprises three α/β heterodimer membrane glycoproteins sharing a common β-subunit, CD18, 3 different α-subunits which comprise the three members of the CD18 integrin family. These are referred to as the

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α-subunits of each of three members, lymphocyte function associated antigen-1 (LFA-1), macrophage antigen-1 (Mac-1), and p-150,95 or CD11a/CD18, CD11b/CD18, and CD11c/CD18, respectively. These adhesion molecules bind to the "counter-receptor" intercellular adhesion molecule-1 (ICAM-1) on endothelial cells. The cytoadhesions, the second integrin sub-family, are receptors on platelets (e.g., gpIIb/IIIa) and endothelial cells which bind to extracellular matrix proteins. Another integrin family found on leukocytes is termed the "very late activation antigens" (VLA group). VLA-1 and VLA-2 appear late in T-cell activation responses. Members of this family function to bind leukocytes to extracellular matrix adhesion receptors and in some cases to molecules expressed on the endothelial cell surface (e.g., VLA-4_α binds to VCAM-1 expressed in response to cytokines). This set of integrins plays an important role in cellular functions including tissue organization, monocyte adhesion and migration, lymphocyte recirculation, and T-cell immune responses.

The third family of adhesion receptors is the immunoglobulin superfamily, some members of which are ICAM-1 and ICAM-2. ICAM-1 is particularly important for leukocyte adhesion and migration since it binds to the CD18 integrins.

This review will focus on the biology of these adhesion molecules and on the recent studies that have defined their role in the pathogenesis of inflammatory disease. Particular emphasis will be paid to studies dealing with lung inflammation in which leukocytes play an important role in the pathogenesis of acute lung injury which leads to the derangement of lung gas exchange function.

Endothelial Leukocyte Adhesion Molecule-1 (ELAM-1)

Structure-Activity Relationship of ELAM-1

ELAM-1 is a member of the selectin family which includes the granule membrane protein-140 (GMP-140) and LECCAM-1, the selectin found on leukocytes (which is analogous to the Mel-14 antigen on murine leukocytes). ELAM-1 molecules on endothelial cells interact with lymphocytes, monocytes, and granulocytes (Pilarski et al. 1991). A panel of monoclonal antibodies to activated endothelial cells have defined the structural components of ELAM-1 that mediate leukocyte adhesion (Pigott et al. 1991). These antibodies recognized epitopes within the lectin and complement regulatory protein domains. Antibodies directed against the lectin domain prevented adhesion, whereas antibodies to complement regulatory protein domain were ineffective (Pigott et al. 1991). Also, the EGF-like domain (which is adjacent to the lectin domain) was important in the adhesion response since it maintained the conformation of the neighboring lectin domain. Leukocytes did not adhere to cells expressing ELAM-1 mutant lacking either the lectin binding or the EGF-like domains (Pigott et al. 1991), indicating that the lectin site is essential for leukocyte adhesion to ELAM-1.

Cloning of ELAM-1

Bevilacqua et al. (1989) have cloned the ELAM-1 molecule. A full-length complementary DNA-ELAM-1 was isolated by transient expression in COS cells. Cells transfected with the ELAM-1 clone expressed a molecule which was recognized by anti-ELAM-1 monoclonal antibodies and induced adhesion of isolated neutrophils and HL60 cells. Expression of ELAM-1 transcripts in cultured endothelial cells was induced by cytokines, reaching maximum at 2–4 h and decaying by 24 h (Bevilacqua et al. 1989). The sequence of ELAM-1 consisted of N-terminal lectin-like domain, an EGF domain, and 6 repetitive motifs (containing approximately 60 amino acids, each related to those found in complement regulatory proteins).

GMP-140 has also been cloned in a similar fashion. The cDNA-derived primary structure of GMP-140 indicated similar lectin and EGF-like domains, but with 9 tandem repetitive motifs instead of 6 in ELAM-1 (Johnston et al. 1989).

ELAM-1 Gene Expression

The factors regulating ELAM-1 gene expression are the subject of much interest since they are likely to be important in the inflammatory response. It is known that various cytokines, including TNF_α and IL-1, as well as LPS can directly induce ELAM-1 by gene transcription. Whelan et al. (1991) isolated a ELAM-1 genomic clone containing nucleotide sequences upstream of the transcription start sites. A series of ELAM-1 deletion mutants linked to reporter gene were constructed and analyzed for their expression. A fragment of 233 base pairs upstream of the transcription start site was responsible for induction of ELAM-1 expression (Whelan et al. 1991). The two elements involved in this region mediating cytokine inducibility of the ELAM-1 gene were between base pairs 233–117 and between base pairs 94–85 (which is the NF kappa B

consensus binding site). Therefore, cytokine induction of ELAM-1 gene transcription may be the result of action of cytokines on the promoter region of the gene with one factor being the NF kappa B-like transcription factor. The ELAM-1 promoter also contains an inverted CCAAT box and AP-1-binding site (Collins et al. 1991); the role of these regulatory components has not been examined.

The intracellular mechanisms mediating induction of ELAM-1 gene are also poorly understood. Evidence indicates that protein kinase c (PKC) is likely not involved since inhibition of PKC with staurosporine failed to prevent the TNF_{α}-induced expression of endothelial adhesivity (Ritchie et al. 1991). The role of other key second messengers (e.g., cytosolic Ca^{2+}) has not been examined.

Soluble ELAM-1

Lobb et al. (1991) have obtained a soluble form of ELAM-1 that may be important in studying how ELAM-1 modulates neutrophil adhesion. A truncated cDNA for ELAM-1 was constructed and stably expressed in CHO cells (Lobb et al. 1991). The secreted recombinant form of ELAM-1 was a functional adhesion molecule as it selectively bound to cells (e.g., HL60 cells, neutrophils, monocytes, NK cells and T cells) that are known to attach to ELAM-1 (Lobb et al. 1991). It is unknown whether soluble ELAM-1 is present in the microcirculation, but if it is, it may serve to modulate leukocyte attachment to endothelial cells.

ELAM-1-Induced Leukocyte Adhesion

Basophils, eosinophils and neutrophils adhere to IL-1-activated vascular endothelial cells by expression of the endothelial cell adhesion molecules, ELAM-1, ICAM-1, and VCAM-I may also be involved (Bochner et al. 1991). Activation of endothelial cell monolayers with IL-1 for 4 h resulted in adhesion of each cell type (Bochner et al. 1991). Treatment of endothelial cells with either anti-ICAM-1 or anti-ELAM-1 monoclonal antibodies inhibited the IL-1-induced adhesion of each cell type; the effect of each antibody was additive. However, a treatment with anti-VCAM-1 antibody inhibited only basophil and eosinophil adhesion. Thus, there are distinct roles of ICAM-1 and ELAM-1 versus VCAM-1 in mediating basophil, eosinophil, and neutrophil adhesion. Basophil and eosinophil adhesion depends to a significant extent on VCAM-1 expression and interaction of VCAM-1 with VLA-4α (the "counterreceptor" for VCAM-1) and also on ELAM-1 and ICAM-1 expression. However, neutrophil adhesion to activated endothelial cells depends only on ELAM-1 and ICAM-1 expression.

Neutrophil adhesion to cytokine-activated endothelial cells involves both CD18-dependent and ELAM-1-dependent mechanisms. The relative contributions to these mechanisms is time-dependent in that ELAM-1 activity was important at 4 h after stimulation of endothelium with cytokines, whereas ELAM-1 activity became dominant at 24 h (Luscinskas et al. 1989). There was very little ELAM-1 activity at 24 h, whereas some ICAM-1 activity was present at 4 h, indicating that both ELAM-1 and ICAM-1 are important adhesion molecules at earlier time points.

Monocytes, like basophils and eosinophils, bind to the ELAM-1 as well as the VCAM-1 induced by cytokines (Carlos et al. 1991). Monocytes adhered to CHO cells transfected with cDNA of ELAM-1 or VCAM-1. Binding to ELAM-1 was inhibited by monoclonal antibodies to ELAM-1 and binding to VCAM-1 was inhibited by antibodies to VCAM-1 or VLA-4_{α} (CD49d). Both ELAM-1 and VCAM-1 participated in the monocyte adhesion response since the effect of both antibodies was additive (Carlos et al. 1991).

ELAM-1-Mediated Leukocyte Migration

Both ELAM-1 and ICAM-1 are involved in neutrophil transmigration, although ICAM-1 appears to be of less importance. Extravasation of blood-borne tumor cells may also require expression of ELAM-1 (Rice and Bevilacqua 1989).

Treatment of endothelial cells with recombinant IL-1β caused neutrophil adhesion and migration (Luscinskas et al. 1991). Maximum enhancement of adhesion and migration was observed at 4 h after rIL-1β treatment when expression of ELAM-1 had peaked and ICAM-1 was modestly increased. Antibodies to ELAM-1 and ICAM-1 inhibited more than 90% of the neutrophil transmigration. In contrast, at the 24- or 48-h time-points, ELAM-1 expression had decreased, whereas ICAM-1 expression had increased over the 4-h level; however, neutrophil adhesion remained elevated (at about half of the 4-h value) and the migration of the neutrophils returned to the value of normal unactivated endothelial cells. These results indicate that

both ELAM-1 and ICAM-1 are involved in the early neutrophil migration, but that ICAM-1 expression per se is not a prerequisite for the response whereas ELAM-1 appears to be a strict requirement. The data fit in with the generally held view that neutrophil migration occurs rapidly and is not a chronic and persistant phenomenon.

ELAM-1-Induced Upregulation of CR3

Studies have indicated that ELAM-1 stimulates upregulation of the leukocyte integrin CR3 (CD11b/CD18) on neutrophils (Lo et al. 1991); ELAM-1 may thereby augment leukocyte sequestration in microvessels. In these studies, ELAM-1 binding to carbohydrate residues (presumably the sialyl Lewis x-antigen) on neutrophils resulted in expression of the integrin CR3 (Lo et al. 1991). Inhibition of ELAM-1 with monoclonal antibodies prevented activation of CR3 by the cytokine-treated endothelial cells (Lo et al. 1991). This study raises the interesting possibility that ELAM-1 serves as a "tethered chemoattractant" (Lo et al. 1991); hence, ELAM-1 may initiate leukocyte aggregation in microvessels. This study also provides the first evidence of a linkage between expression of ELAM-1 and upregulation of another essential adhesion molecule, CR3 (CD11b/CD18), which then binds to ICAM-1. The mechanism by which ELAM-1 upregulates CR3 is unknown, but may involve activation of second messenger Ca^{2+} and protein kinase c pathways in leukocytes.

ELAM-1 Expression in Inflammatory Diseases

ELAM-1 expression has been observed in inflammatory diseases. ELAM-1 expression of vascular endothelium was found in skin biopsies taken from humans with erythema and delayed hypersensitivity (Leung et al. 1991). ELAM-1 induction and polymorphonuclear leukocyte infiltration were first noted at 6 h and were maximum by 24 h in both skin inflammatory lesions. ELAM-1 expression also occurred in endothelial cells of skin in the late phase reaction between 20 min and 24 h after the intradermal allergen challenge (Leung et al. 1991). Activation of endothelial cells in allergic skin reactions may play a role in the migration of inflammatory cells.

In psoriasis, both ELAM-1 expression and neutrophil margination were observed (Groves et al. 1991) suggesting a functional link. ELAM-1 was widely induced in cutaneous inflammation with a more sustained time course than observed in vitro (Groves et al. 1991) perhaps reflecting a sustained ability to activate ELAM-1 gene expression in chronic inflammation. The long-lived in vivo expression as compared to response in cultured endothelial cells indicates that ELAM-1 can retain its ability to recruit neutrophils to inflammatory sites from the circulation for extended periods.

ELAM-1 expression was also observed in venules and capillaries in rheumatoid and osteoarthritic synovial tissues (Koch et al. 1991). In addition, VCAM-1 and ICAM-1 expression was noted, indicating that all three adhesion molecules may be involved in mediating leukocyte migration into synovium of patients with rheumatoid arthritis and osteoarthritis.

ELAM-1 may also be expressed in the vasculature of allografted tissue which may contribute to acute rejection of transplanted organs (Sedmack and Orosz 1991). Endothelial cells, which are important for normal inflammatory responses, can be pathogenic in allograft rejection following expression of adhesion molecules and migration of lymphocytes. It is known that ELAM-1 is of primary importance in attachment of T-cells in the inflamed endothelium in vivo (Shimizu et al. 1991; Picker et al. 1991) which might be critical cells contributing to the rejection process.

Studies have attempted to quantify recruitment of neutrophils in the local endotoxin response in primates. Injection of endotoxin (500 μg of *E. coli*-derived lipopolysaccharide) resulted in a marked expression of ELAM-1 within 2 h after the challenge and this was associated with adhesion and extravasation of neutrophils (Munro et al. 1991). ELAM-1 expression subsequently decreased and was not evident by 9 h. Interestingly, ICAM-1 activity did not change in response to endotoxin challenge (Munro et al. 1991), pointing to the essential role of ELAM-1 in mediating neutrophil adhesion and migration in sepsis.

In an important observation, Matis et al. 1990 have shown that substance P induces expression of ELAM-1 in microvascular endothelial cells. Exposure of endothelial cells for 6 h with substance P showed evidence of ELAM-1 expression as documented histochemically using H4/18 anti-ELAM-1 antibody (Matis et al. 1991). This observation may be important in neurogenic modulation of the immune response.

Binding of ELAM-1 to Carbohydrate Moieties on Leukocytes

The leukocyte receptors for ELAM-1 have been recently identified. All of the identified ELAM-1 receptors are carbohydrate-binding proteins (Tiemeyer et al. 1991). Tiemeyer et al. (1991) used radiolabelled COS cells transfected with plasmids containing cDNA for ELAM-1 to screen for glycolipids extracted from human leukocytes. The results indicated that ELAM-1 binds to terminally sialylated lactosylceramides with a variable number of *N*-acetyllactosamine repeats and one fucosylated *N*-acetylglucosamine residue. Adhesion to these glycolipids required Ca^{2+}, but was not inhibited by heparin, chondroitin sulfate, or keratin sulfate (Tiemeyer et al. 1991).

A fucosylated carbohydrate structure appears to be an essential component of the ELAM-1 receptor. Studies have shown that the transfection of cells with the cDNA of $\alpha(1,3)$ fucosyltransferase resulted in binding of these cells to ELAM-1 (Goelz et al. 1990). The $\alpha(1,3)$ fucosyltransferase activity may regulate cell adhesion to the ELAM-1 by modulating cell surface expression of one or more $\alpha(2,3)$ sialylated, $\alpha(1,3)$ fucosylated lactosaminoglycans represented by the sialyl-Lewis X carbohydrate determinant (Lowe et al. 1990). The $\alpha(1,3)$ fucosyltransferase activity was specifically expressed in the cell types that bind to ELAM-1 (Goelz et al. 1990), indicating that this enzyme is an important regulator of expression of fucosylated carbohydrates on leukocytes.

Sialyl-Lewis X antigen is the primary determinant of ELAM-1 binding to leukocytes (Walz et al. 1990; Phillips et al. 1990). Sialyl-Lewis X is a terminal structure found on cell surface glycoprotein and glycolipid carbohydrate groups of leukocytes. ELAM-1 specifically recognized sialyl-Lewis X molecule

[NeuAcα2–3 Galβ1–4(Fucα1–3)GlcNAc-]

(Walz et al. 1990; Philips et al. 1990). This carbohydrate is also found on the surface of some tumor cell lines. Binding of myeloid cells to soluble ELAM-1 was inhibited by monoclonal antibodies recognizing sialyl-Lewis X or by proteins bearing sialyl-Lewis X such as orosomucoid (Walz et al. 1990).

Sialyl-Lewis X antigen is well known to be expressed on mucin-like glycoproteins in breast and colon-rectal cancer metastases (Matsushita et al. 1990). Monoclonal antibodies used by these investigators included SH-1 specific for Lewis X antigen; FH4, specific for dimeric Lewis X antigen; and FH6, specific for sialylated dimeric Lewis X antigen. The availability of these antibodies may be useful in identifying ELAM-1-leukocyte interactions and addressing the pathophysiological consequences of this interaction.

Athero-ELAM (or VCAM-1)

A monocyte-specific adhesion molecule on endothelial cells may be involved in mediating monocyte adhesion to the vessel wall during atherogenesis. An inducible endothelial adhesion molecule on the surface of cultured rabbit endothelial cells (athero-ELAM) was found to be homologous human VCAM-1 (Cybulsky and Gimbrone 1991). Dietary hypercholesterolemic rabbits as well as Watanabe heritable hyperlipidemic rabbit models of atherosclerosis showed expression of this adhesion molecule in the aortic endothelial cells (Cybulsky and Gimbrone 1991). Endothelial VCAM-1 expression may be important for mononuclear leukocyte recruitment in atherogenesis and may provide a molecular marker for early atherosclerosis.

Granule Membrane Protein (GMP-140)

GMP-140 [also known as platelet activation-dependent granulocyte external membrane (PAD-GEM)] is a membrane glycoprotein expressed on platelet and endothelial cell surfaces following degranulation (McEver 1990). GMP-140 plays an important role in platelet interactions with other blood cells as well as endothelial interactions with leukocytes. GMP-140 is a part of the selectin family along with ELAM-1 and LECCAM-1.

LECCAM-1, GMP140, and ELAM-1 constitute a gene cluster on mouse and human chromosome 1 (Watson et al. 1990). Gene linkage analysis in the mouse indicates that these genes and serum coagulation factor V all map to a region of the mouse chromosome that is analogous with human chromosome 1 (Watson et al. 1990). Therefore, LECCAM-1, ELAM-1, and GMP-140 comprise an adhesion protein family (the selectins) that likely arose by multiple gene duplication events before divergence of mouse and human. The complement receptor-related gene is also positioned on the same chromosome of both species (Watson et al. 1990) indicating a close evolutionary relationship between the region encoding the selectins and encoding the complement receptor genes.

Chemical Characteristics and Structure of GMP-140

GMP-140 has a molecular weight of 140 kD and is located in secretory granules of human platelets and endothelial cells (McEver 1990). The molecule is cysteine-rich and heavily glycosylated. Stimulation of these cells causes rapid distribution of GMP-140 to the plasmalemmal membrane. The cDNA-derived amino acid sequence analysis indicated that GMP-140 contains a number of domains that fold independently. The N-terminus has the lectin domain followed by EGF domain and nine tandem consensus repeats similar to the complement binding protein, then a transmembrane domain, and cytoplasmic tail (McEver 1990). There is also a soluble form of GMP-140 lacking a transmembrane domain, which may be important in modulating neutrophil adhesion.

Distribution of GMP-140 in Endothelial Cells and Platelets

Distribution of GMP-140 within cultured human umbilical vein endothelial cells coincides with distribution of Von Willebrand factor (Hattori et al. 1989), indicating that GMP-140 is located in membranes of the Von Willebrand factor-containing storage granules. Stimulation of Von Willebrand factor secretion resulted in an increase in GMP-140 on the surface as detected by increased binding of monoclonal antibody which recognized the extracytoplasmic domain of GMP-140 (Hattori et al. 1989). Histamine, thrombin, phorbol-12 myristate 13-acetate, and the calcium ionophore, A23187, caused redistribution of GMP-140 to the endothelial cell surface. The translocation of GMP-140 can occur rapidly and reach a plateau by 10 min (Hattori et al. 1989), indicating that this molecule is rapidly mobilized and can rapidly mediate adhesion of leukocytes.

Expression of GMP-140 on Endothelial Cell Surface

Histamine, thrombin, and oxidants (e.g., H_2O_2) have been shown to stimulate GMP-140 expression on the endothelial cell surface and cause binding to neutrophils (McEver 1990). In a recent study (Patel et al. 1991), treatment of endothelial cells with H_2O_2, *t*-butylhydroperoxide, or menadione resulted in adhesion of neutrophils to endothelial cells between 1 h and 4 h after the oxidant exposure even though the exposure period with these oxidants at the initiation of the assay was brief. The response was not dependent on ELAM-1, which requires de novo protein synthesis. The endothelial cells exposed to oxidants expressed GMP-140 on their surface and antibodies against GMP-140 or soluble GMP-140 inhibited neutrophil adhesion to the oxidant-challenged cells (Patel et al. 1991). The response was also inhibited with antioxidants and intracellular iron chelators (Patel et al. 1991).

GMP-140 Binding to Leukocytes

GMP-140-mediated leukocyte adhesion is dependent on sialic acid residues on leukocytes (Corral et al. 1990). This conclusion is based on the observation that treatment of neutrophils and HL-60 cells with broad spectrum sialidases prevented binding of neutrophils or HL60 cells to activated platelets (Corral et al. 1990).

GMP-140 binding kinetics to neutrophils have also been examined (Moore et al. 1991). Unstimulated neutrophils rapidly bound ^{125}I-GMP-140 at 4 °C, reaching equilibrium in 10–15 min with the binding being Ca^{2+}-dependent, reversible, and saturated at 3–6 nmol free GMP-140. Receptor density and affinity were not altered with treatment of neutrophils with PMA, whereas treatment of neutrophils with sialidase (neuraminidase) from vibrio cholerae (which cleaves α2–3-, α2–6-and α2–8-linked sialic acid) diminished the binding. The α-2–6 linkage of sialic acid may be critical for leukocyte binding (Moore et al. 1991; Corral et al. 1990). However, the precise nature of GMP-140 binding molecule on leukocytes has not been characterized.

GMP-140 binding to leukocytes was not inhibited by monoclonal antibodies to the abundant myeloid oligosaccharide Lewis X (CD15) or sialyl Lewis X (Moore et al. 1991). These findings indicate that sialic acid residues are essential for GMP-140 interaction with neutrophils and support the view that GMP-140 interacts with leukocytes by a lectin-like mechanism via sialic acid residues.

GMP-140-Mediated Leukocyte Adhesion

GMP-140 mediates adhesion of neutrophils to endothelial cells occurring within minutes after stimulation of endothelial cells with phorbol esters, thrombin, or histamine (Geng et al. 1990). This re-

sponse was inhibited by antibodies to GMP-140. Neutrophils and HL 60 cells also bound specifically to COS cells transfected with GMP-140. GMP-140 mediates platelet-neutrophil adhesion as well as neutrophil-endothelial adhesion (Hamburger and McEver 1990), and thus may also be involved in accumulation of platelets and neutrophils at sites of inflammation and tissue injury.

Soluble GMP-140 can have anti-adhesive functions as it strongly inhibited CD18-dependent adhesion of TNF_{α}-activated neutrophils to endothelial cells (Skinner et al. 1991). Binding of GMP-140 to neutrophils and HL-60 cells was also strongly inhibited by heparin, fucoidin, and dextran sulfate 500000, but was partially inhibited by dextran sulphate 5000. This indicates that molecules interacting with sugar molecules on the GMP-140 receptor modify the binding of leukocytes to the endothelial GMP-140.

While the GMP-140 causes a rapid adhesion of leukocytes, there may be another form of rapid adhesion which also does not require de novo protein synthesis. This may be the result of clustering or conformational change of constitutive ICAM-1 molecules on endothelial plasmalemmal membranes. Studies indicate that α-thrombin can cause a rapid induction of ICAM-1, which can bind to CD18 integrin on leukocytes (Bizios et al. 1988; Fisher et al. 1991). These mechanisms may operate independently of the thrombin-induced upregulation of GMP-140 (Geng et al. 1990).

CD11/CD18 Integrins on Leukocytes

Interaction of CD11/CD18 Integrins with ICAM-1 and ICAM-2

ICAM-1, but not ICAM-2, is a counter-receptor for CD11b/CD18, whereas CD11a/CD18 recognizes both (Staunton et al. 1989).

The binding site for CD11b is also different from that of CD11a in that CD11a/CD18 binds to the first NH_2 terminal immunoglobulin-like domain of ICAM-1, whereas CD11b/CD18 binds to a third NH_2 terminal immunoglobulin-like domain (Diamond et al. 1991). This finding provides a function for the tandem duplication for the immunoglobulin-like domains in ICAM-1 and provides support for the interaction of CD11b with ICAM-2, which has fewer such transmembrane immunoglobulin domains (Staunton et al. 1989). Glycosylation of these domains on ICAM-1 influenced the binding of either CD11a/CD18 or CD11b/CD18 (Diamond et al. 1991), indicating that the leukocyte adhesion can be subtly regulated by this mechanism.

Intracellular Activation of CD11/CD18

The maximum neutrophil adhesion response mediated by CD11/CD18 in response to C5a, TNF_{α}, or phorbol dibutyrate occurred within 5–15 min (Lo et al. 1989c); thus, the CD18-dependent adhesion response is a transient one and regulated by changes in the nature of existing CD18 molecules on the neutrophil surface (i.e., clustering or conformational changes in cell membrane-bound CD18 followed by down-regulation of the signal).

The activation of CR3 receptors is mediated by phosphorylation of the β-chain (CD18) which converts the molecule to an activated state (Roubey et al. 1991). Unstimulated neutrophils have a constitutive level of phosphorylation of CD11 subunits, whereas the basal phosphorylation of CD18 subunits is minimal (Chatila et al. 1989). Phosphorylation of CD18 only occurred following neutrophil activation (Chatila et al. 1989), indicating that conformation changes of CD18 (common β-subunit) of the integrin is required for the adhesion response.

LFA-1 binding to ICAM-1 was found to be regulated by the cytoplasmic domain of the β-subunit (Hibbs et al. 1991). This conclusion is based on the observation that truncation of the cytoplasmic domain of β- but not the α-subunit of LFA-1 eliminated binding to ICAM-1 and the sensitivity to phorbol esters. Therefore, phosphorylation of the β-subunit is a critical signal for activation of CD11/CD18 integrins. These results may explain why antibodies to the β-subunit are more effective in inhibiting adhesion than antibodies to α-subunits.

It appears that upregulation of CD18 differs according to whether the activation is induced by protein kinase c activators or chemoattractants. Direct protein kinase c activation with PMA or diacylglycerol caused prolonged neutrophil aggregation associated with intense phosphorylation of the β-subunit, whereas a more transient aggregation as induced by fMLP was associated with minimal β-subunit phosphorylation (Merrill et al. 1990). Phosphorylation induced by diacylglycerol analogues occurred via a staurosporine-sensitive pathway (dependent on protein kinase c activation), whereas fMLP-induced activation occurred by a distinct mechanism which may not depend on

phosphorylation of the β-chain (Merrill et al. 1990).

Altieri et al. (1988) have suggested that cytosolic Ca^{2+} is another signal for upregulation of CD18. Increase in cytosolic Ca^{2+} may mediate the quantitative mobilization of subcellular storage pools of CD11/CD18 to the plasma membrane. These studies also indicated that calcium channel blockers inhibited CD11/CD18 upregulation in a dose-dependent manner (Altieri et al. 1990b), supporting a role of Ca^{2+}-influx in signalling CD18 expression.

In an interesting study, Pryzwanski et al. (1991) characterized the kinetics of phorbol ester-induced translocation of CR3 to neutrophil cell membranes as examined in whole-mount preparations of adherent neutrophils by stereo high-voltage immunoelectronmicroscopy. In the absence of PMA, immunogold-labelled CR3 (C3bi receptor; CD11b/CD18) was uniformly distributed in the plasmalemmal membrane. However, within 5–15 min of incubation with PMA, the average density of CR3 in the membrane doubled and the increased amounts of CR3 co-localized with the secreted lactoferrin, a specific neutrophil granule marker (Pryzanski et al. 1991). With high concentrations of PMA, lactoferrin staining became rare and high density areas of CR3 were no longer present, suggesting that PMA caused "shedding" of CR3 from the cell surface along with lactoferrin. The decrease of CR3 on the cell surface may reduce neutrophil adhesion as the CR3 is dissipated into the circulation. This could be responsible for the transient nature of CD18-dependent adhesion (Lo et al. 1989a).

Studies have attempted to determine whether upregulation of CD18 occurs via a receptor-linked mechanism. Nourshargh et al. (1989) investigated the effects of in vitro pretreatment of rabbit neutrophils with pertussis toxin on their in vivo response and their accumulation in the microcirculation. Pertussis toxin prevented the fMLP- and C5a- but not PMA-induced increases in CD18 expression and the neutrophil adhesion and degranulation response in vitro. Pertussis toxin pretreatment also prevented accumulation of radiolabelled neutrophils in vivo in response to intradermally injected fMLP, C5a, LTB_4, IL-8, or zymosan. These results suggest that accumulation of neutrophils induced by chemoattractants depends on a pertussis toxin-sensitive receptor on neutrophils. This study further supports the role of activation of second messengers in mediating CD11/CD18 upregulation.

Role of CD18 Integrins in Leukostasis and Neutrophil Migration

CD18 appears to be an important mechanism of neutrophil uptake in vivo. Exogenous mediators, fMLP, $C5a_{desArg}$, LTB_4, or IL-1 were injected intradermally and the effects of inhibition of CD18 using antibodies were examined (Nourshargh et al. 1989). Treatment with anti-CD18 antibody inhibited the accumulation of neutrophils in all tissues (Nourshargh et al. 1989), demonstrating a common mechanism of leukostasis in these inflammatory reactions. Interestingly, the CD18 antibody-treated neutrophils responded normally to fMLP and C5a with respect to granular enzyme release, indicating that degranulation is mediated by a CD18-independent mechanism.

Neutrophils from a patient with leukocyte adhesion deficiency (LAD) of a moderate phenotype demonstrated a normal adhesion to endothelial cells, but an impaired migration response. At autopsy, there was no evidence of neutrophils in the lung interstitium and alveolar spaces, indicating that CD11/CD18 molecules have an important role in facilitating neutrophil emigration from blood vessels to sites of inflammation (Davies et al. 1991). However, there are phenotypic variations in that neutrophils in patients from severe LAD demonstrated reduced adhesion as well as migration.

Neutrophil migration across endothelial barrier occurs by both CD18-dependent and CD18-independent mechanisms. The dominant mechanism appears to depend on the particular stimulus that was used to elicit migration. Neutrophil migration into peritoneum in which *E. coli* or *S. pneumonia* was instilled was used to examine the role of CD18 integrin in the response (Mileski et al. 1990). Addition of macrophages into the peritoneum enhanced neutrophil migration by a CD18-dependent mechanism (Mileski et al. 1990). Therefore, humoral mediators originating from macrophages (e.g., TNF_α and IL-1) are capable of inducing CD18 upregulation. This mechanism may also be operative in lungs subsequent to activation of airspace and interstitial macrophages and the release of cytokines. Therefore, whether neutrophils migrate by a CD18-dependent mechanism requires that humoral mediators cause upregulation of CD18.

An important humoral signal for CD18 upregulation critical for transendothelial leukocyte migration is IL-8, a cytokine produced by a variety of cell types (e.g., monocytes, endothelial cells, fibroblasts) in response to inflammatory stimuli. IL-8 induces neutrophil adhesion and migration by

upregulation of CD18 (Detmers et al. 1990). The migration response was observed in the absence of a quantitative increase in CD18 receptor expression; there was, however, a qualitative increase in CD18 activity (Detmers et al. 1990; Schleiffenbaum et al. 1989).

Pretreatment of rabbits with anti-CD18 and anti-ICAM-1 (but not anti-CD11a) monoclonal antibodies inhibited by more than 60% the neutrophil migration into PMA-induced inflamed rabbit lungs (Barton et al. 1989). Therefore, ICAM-1 functions to induce transendothelial migration of neutrophils at inflammatory sites because binding of CD18 to ICAM-1 is required for the CD18-dependent migration response.

Oxidant-Mediated Leukocyte Adhesion and Modulation of Adhesion by Nitric Oxide

Suzuki et al. (1989) have shown that superoxide generated during anoxia followed by reoxygenation of endothelial cells mediates neutrophil-endothelial cell adhesion and that this response is CD18-dependent. This study points to a critical role of oxidant generation in mediation of neutrophil uptake. Other studies have elaborated on the important effect of oxidants in inducing leukocyte adhesion. H_2O_2-caused monoblastoid U937 adhesion to plastic with a half-time of 6 min; the response was optimally simulated by 100 μM H_2O_2 with an ED_{50} of 50 μM which closely resembled the adhesive response of U937 cells to phorbol esters (Skoglund et al. 1988). The response was dependent on CD11b and CD18 since antibodies to both of these integrins inhibited the response. Phorbol ester treatment of U937 cells stimulated the phosphorylation of three endogenous substrates: PP28, PP34, and PP43, which were also phosphorylated by H_2O_2 (Skoglund et al. 1988), suggesting that H_2O_2 can mediate its effect by activation of protein kinase c.

Nitric oxide (the putative endothelial-derived relaxing factor) appears to be an important regulator of CD18 upregulation. Inhibition of nitric oxide production by NG-monomethyl-L-arginine resulted in upregulation of CD11/CD18 as assessed by flow cytometry and enhanced leukocyte emigration (Kubes et al. 1991). Therefore, an impairment of nitric oxide production (such as with endothelial injury) may cause a pattern of leukocyte adhesion and migration that is typical of the acute inflammatory response.

CD18-Mediated Phagocytosis versus Adhesion

The phagocytic function mediated by CD11b/CD18 is different from that mediating adhesion of this integrin to its receptor on endothelial cells (Graham et al. 1989). Binding of neutrophils to anti-CD11b- and anti-CD18-coated surfaces did not interfere with the phagocytosis of complement- or IgG-coated red blood cells (Graham et al. 1989), indicating separate mechanisms of CD18-dependent phagocytosis and adhesion. There may be functionally distinct populations of CD11b/CD18 on monocytes and neutrophils: one involved in C3bi rosetting and cell-cell interaction and the other involved in phagocytosis. It is believed that the cell binding component of CD11b/CD18 is mobile whereas the region mediating phagocytosis is immobile (Graham et al. 1989).

Inhibition of protein kinase c (PKC) activation with staurosporine increased the binding of neutrophils to C3bi-opsonized sheep red blood cells (Rubey et al. 1991), an event caused by increased surface expression of CR3 after exocytosis of specific granular contents. Staurosporine alone did not stimulate phagocytosis of these cells; however, it markedly inhibited PMA-induced phagocytosis of C3bi-opsonized sheep red blood cells (Rubey et al. 1991). Therefore, phagocytosis mediated by CR3 requires activation of CR3 via the PKC-dependent pathway; but the binding to CR3 to C3bi does not require the PKC pathway, indicating that the binding and phagocytosis induced by CR3 are distinct processes. Kaslovsky et al. (1990) and recently Lum et al. (1991 a) have shown that anti-CD18 monoclonal antibody IB4 did not reduce phagocytosis of opsonized particles, whereas it effectively inhibited neutrophil adhesion to endothelial cells, further supporting the claim that adhesion and phagocytosis are separate and independent processes.

CD18-Dependent and -Independent Binding of Leukocytes to Subendothelial Matrix Proteins

Neutrophils can bind to subendothelial matrix proteins after migration across the endothelial barrier. The process is influenced by CD18-dependent and -independent mechanisms. The kinetics of adhesion and spreading on thrombospondin (TSP) were similar to that on vitronectin, laminin, and fibronectin (Suchard et al. 1991). Activation of PMN with the Ca^{2+} ionophore, A23187, or the chemotactic peptide, fMLP, increased neutrophil adhesion to laminin and fibronectin, but not to TSP and

vitronectin, indicating that PMN activation may differentially regulate expression of TSP and vitronectin receptors as compared to laminin or fibronectin receptors. Since TSP contains the Arg-Gly-Asp sequence (similar to the cell recognition site of fibronectin and vitronectin), it is possible that RDGS peptides would inhibit neutrophil adhesion. The results indicated that RDGS did not inhibit neutrophil adhesion to TSP, vitronectin, or laminin, whereas PMN adhesion to fibronectin was reduced (Suchard et al. 1991). Neutrophils from leukocyte adhesion deficient (LAD) patients exhibited normal adhesion to TSP and the adhesion to vitronectin, laminin and fibronectin was reduced by 95%. These results indicate that neutrophil adhesion to TSP is not mediated by β subunit (CD18) of the integrin receptor whereas adhesion to vitronectin, laminin, or fibronectin is dependent on binding of CD18 to the Arg-Gly-Asp sequence.

Monocyte cell line, U937 cells, have also been shown to adhere to fibronectin (Cavendar et al. 1991); however, these cells adhere only when activated with agents such as phorbol ester or fMLP. The pentapeptide containing the amino acid sequence Arg-Gly-Asp inhibited the phorbol ester-induced adhesion of U937 to fibronectin, whereas this peptide had no effect on the binding of these cells to endothelial cells (Cavendar et al. 1991). This study further documents that adhesion of neutrophils and monocytes to extracellular matrix proteins is dependent on CD18 integrin and its binding to the Arg-Gly-Asp cell binding domain, but this is not the case with the interaction of CD18 and endothelial cells. However, the data do not rule out the possibility that a large peptide-containing the Arg-Arg-Asp region with the appropriate flanking sequences can prevent the binding of activated leukocytes to endothelial cells.

Interaction of fibrinogen with Mac-1 may be responsible for the fibrin-leukocyte interactions observed in inflammation (Cooper et al. 1988). Altieri et al. (1990a+b) found that a 30 kD molecular weight plasmic fragment of fibrinogen (D30) produced dose-dependent inhibition of interaction of intact fibrinogen with stimulated neutrophils and monocytes. The D30 fragment lacked COOH-terminal dodecapeptide of the γ-chain as well as the Arg-Gly-Asp sequence of the A-chain; therefore, fibrinogen may interact with the leukocyte integrin Mac-1 through a novel recognition site that is not shared with other known integrins.

Mechanisms of CD18-Dependent and -Independent Leukocyte Adhesion and Migration Under Flow Conditions

Smith et al. (1991) have examined the relative contributions of CD18, CD11a, and CD11b and the neutrophil lectin adhesion molecule, LECCAM-1 in neutrophil-endothelial interactions under conditions of shear stress. Under static conditions, CD18, CD11a, and LECCAM-1 antibodies inhibited neutrophil adhesion to cytokine-stimulated endothelial cell monolayers. Under flow conditions, CD18-dependent adhesion was minimal whereas the anti-LECCAM-1 antibody inhibited adhesion more than 50% (Smith et al. 1991). Only those neutrophils that attached to endothelial cell lost their surface LECCAM-1 and these were the neutrophils that migrated across the endothelial cell monolayer.

The mechanism of loss of LECCAM-1 from the cell surface is unclear. Smith et al. (1991) have suggested that it may involve soluble factor(s) released by the cytokine-stimulated endothelial cells. The migration response was inhibited by anti-CD18 antibody but was unaffected by anti-LECCAM-1 antibodies. These results point to the critical role of LECCAM-1 in mediating adhesion of unstimulated neutrophils to cytokine-stimulated endothelial cells under conditions of flow. The results also indicate that loss of LECCAM-1 from the neutrophil surface coincident with the engagement of the CD18-dependent binding which leads to transendothelial neutrophil migration. Therefore, according to their model, the initial step in adhesion is determined by LECCAM-1 binding to its ligand on endothelial cells, followed by the engagement of CD18 to ICAM-1, followed by "shedding" of LECCAM-1, and then the migration of the neutrophil. Aspects of this model have been validated in a study showing that CD18-dependent leukocyte adhesion occurred at low shear stresses (Perry and Granger 1990). ELAM-1 complicates this scheme since ELAM-1 binding to neutrophils contributes to the expression of CD18 (Lo et al. 1991), and hence this would contribute to the migration process.

Neutrophils may roll on the endothelial cell surface selectins under ambient flow conditions in postcapillary venules. Resting or unactivated neutrophils did not adhere to artificial lipid bilayers via the binding of LFA-1 and Mac-1 to ICAM-1 under physiological shear stresses (Lawrence and Springer 1991). Leukocytes rolled on the selectins but only the activation of LFA and Mac-1 served to

strengthen the adhesion, which resulted in arrest of neutrophils on the bilayer (Lawrence and Springer 1991).

Expression of CD18 Integrins in ARDS

Simms and D'Amico 1991 have shown the development of the adult respiratory distress syndrome (ARDS) in trauma patient coincides with the expression of leukocyte CD11b/CD18 and that neutrophil oxidative metabolism increases after the onset of CD11b/CD18 expression in neutrophils of these patients. Thus, there may be a relationship between CD11b/CD18 expression and development of ARDS in trauma patients. This has also been observed in experimental septic injury model where there was increased expression of neutrophil CD11/CD18 in a model of septicemia associated with acute lung injury (Walsh et al. 1991).

Role of CD18 in Models of Tissue Injury and Inflammation

Studies in rabbits have indicated that ischemia-reperfusion of the hind limb causes neutrophil CD18-dependent emigration (Goldman et al. 1991). Other studies have shown that neutrophil-mediated lung vascular injury following ischemia-reperfusion of lungs is dependent on CD18 as the response was inhibited by antibodies to CD18 (Atkins and Taylor 1990; Horgan et al. 1990). Horgan et al. (1990) demonstrated that CD18 integrin contributes to neutrophil uptake following ischemia-reperfusion of lungs and that both neutrophil uptake and lung injury occurring following reperfusion were inhibited by the monoclonal antibody to CD18 (IB4), indicating a specific role of the CD18 glycoprotein as a determinant of reperfusion-induced lung vascular injury. Polymorphonuclear leukocytes may also contribute to reperfusion injury in hearts (Lucchesi 1990).

Studies have shown that neutrophils are involved in generalized reperfusion injury associated with resuscitation from shock (Vedder et al. 1989). In this experiment, the anti-CD18 monoclonal antibody, 60.3, significantly reduced neutrophil adhesion even if administration was delayed until resuscitation. The treatment prolonged the survival time following shock and resuscitation. Anti-CD18 antibodies were also protective in reducing inflammation, tissue damage, and mortality in bacterial meningitis in rabbits (Tuomanen et al. 1989).

Neutrophil CD18 expression is clearly important in mediating neutrophil-dependent vascular endothelial injury (Kaslovsky et al. 1990). Studies in intact lungs showed that phagocytosis of opsonized zymosan particles was not inhibited by the anti-CD18 monoclonal antibody IB4, but IB4 prevented neutrophil adhesion which was responsible for inhibition of lung vascular injury. The results point to the importance of CD18-dependent neutrophil adhesion in mediating vascular endothelial injury induced by phagocytosing neutrophils.

Monoclonal antibodies to CD18 and to ICAM-1 inhibited in vivo leukocyte-endothelial interactions in rabbits (Argenbright et al. 1991). Intravital microscopy was used to visualize the microcirculation in rabbit mesentarium. Antibodies against CD18, CD11a, and ICAM-1 significantly inhibited leukocyte adhesion caused by C5a, whereas antibodies against CD11b produced a weaker effect (Argenbright et al. 1991). In some case antibodies were administered after C5a-induced neutrophil adhesion had begun. Both anti-CD18 and anti-CD11a antibody displaced the adherent neutrophils prevented further neutrophil accumulation, whereas the anti-CD11b and anti-ICAM-1 antibodies had no effect in displacing neutrophils or in inhibiting further neutrophil adhesion. These results indicate that CD18 and ICAM-1 are important in mediating neutrophil adhesion in vivo and, interestingly, they suggest the possibility of detaching adherent neutrophils by anti-CD18 antibodies.

The soluble functional form of human β_2 integrin CD11b/CD18 was shown to inhibit the binding of polymorphonuclear leukocytes to recombinant IL-1-activated endothelial cells (Dana et al. 1991). Soluble CD18 can be a potentially useful anti-inflammatory agent because it binds to expressed ICAM-1 ligand. The in vivo effects of soluble integrins in models of inflammation have not been examined.

Intracellular Adhesion Molecule-1 (ICAM-1)

Cloning of ICAM-1

ICAM-1 has been cloned in various species. Horley et al. (1989) reported a murine lymphocyte surface antigen which was the same as ICAM-1 (molecular weight of 95 kD). The nucleotide sequence as well as the deduced amino acid sequence of mouse ICAM-1 displayed homology to those reported by human ICAM-1 which included its homology with

neural crest molecule (NCAM), its internal repeat structure, and immunoglobulin-like structure.

The ICAM-1 gene is found on the mouse chromosome 9 (Ballantyne et al. 1991). ICAM-1 has a tissue distribution similar to that of the major histocompatibility complex class antigens. In humans, ICAM-1 maps to chromosome 19. Interestingly, murine ICAM-1 gene was found on the same region of chromosome 9 as the susceptibility genes for insulin-dependent diabetes mellitus (Ballantyne et al. 1991).

ICAM-1 Structure

The intracellular adhesion molecule ICAM-1 is a 90 kD inducible cell surface glycoprotein that promotes adhesion in immunological and inflammatory reactions. ICAM-1 is a ligand of LFA-1 and Mac-1, both members of the integrin family of cell-cell and cell-matrix receptors. ICAM-1 is coded by inducible 3.3 Kb mRNA (Staunton et al. 1988). The amino acid sequence indicates a transmembrane protein with an extracellular domain of 453 residues containing 5 immunoglobulin-like domains. ICAM-1 has homology with NCAM and myelin-associated glycoprotein (MAG) which also contain 5 immunoglobulin-like domains (Staunton et al. 1988). Unlike other integrin ligands, ICAM-1 does not contain the Arg-Gly-Asp (RGD) sequence indicating that the CD11/CD18 integrin binds to ICAM-1 by a RGD-independent mechanism.

Upregulation of ICAM-1 Expression

ICAM-1 biosynthesis in TNF_{α}-treated endothelial cells depends on de novo protein synthesis. The synthesis of ICAM-1 can be regulated by 3-deaza-adenosine (Jurgensen et al. 1990). Northern blot analysis indicated that this nucleoside analogue selectively decreased steady-state levels of ICAM-1 mRNA. This effect of adenosine may contribute to the drug's anti-inflammatory action.

Upregulation of ICAM-1 expression may be regulated by activation of second messenger pathways. Staurosporine or isoquinsulfonamide derivative H7, which prevented protein kinase c (PKC) activation, were able to inhibit the cytokine-mediated stimulation of ICAM-1 expression and neutrophil adhesion to endothelial cells (Lane et al. 1990). These results indicated that inflammatory mediators induce stimulation of PKC and thereby mediate ICAM-1 expression. Other studies have indicated that TNF_{α}-induced activation of ICAM-1 is independent of PKC activation (Ritchie et al. 1991). The role of PKC in the expression of ICAM-1, however, may be dependent on the stimulus used; for example, interferon γ caused ICAM-1 expression that was inhibited by the PKC inhibitor H7 (Renkonen et al. 1990). These investigators also showed a significant increase in Ca^{2+} flux into endothelial cells in response to interferon γ which was related to upregulation of ICAM-1 expression.

Binding of CD11/CD18 Integrins to ICAM-1 and ICAM-2

Several lines of evidence have suggested the existence of second LFA-ligand. Homotypic adhesion of one cell line was inhibited by a monoclonal antibody to LFA-1, not by an antibody to ICAM-1 (Johnston et al. 1989). Staunton et al. (1989) have cloned a second ligand designated ICAM-2 which is an integral membrane protein with 2 immunoglobulin-like domains, whereas ICAM-1 has 5 such domains. ICAM-1 has a molecular weight of 90 kD and ICAM-2 has a molecular weight of 55 kD (Gahmberg et al. 1991). ICAM-2 is related to the two most end terminal domains of ICAM-1 (34% identity). ICAM-2 may be responsible for LFA-dependent and ICAM-1-independent pathway of adhesion of leukocytes to endothelial cells. The precise role of ICAM-2 in immune and inflammmatory responses has not been examined.

An important signal regulating expression of LFA-1 on leukocytes and the binding of leukocytes to ICAM-1 may be activation of CD14 by the lipopolysaccharide binding protein-lipopolysaccharide (LBP-LPS) complex (Lauener et al. 1990). In this study, activation of CD14 causes adhesion of leukocytes via the LFA-1-ICAM-1 interaction. In a recent study, Wright et al. (1991) have shown that activation of CD14 can upregulate on the monocyte CD18 integrin which indicates that LPS-LBP complex may mediate not only aggregation of leukocytes, but also leukocyte binding to endothelial cells.

Although most studies indicate that LFA-1 binds to ICAM-1, recent data have shown that Mac-1 can also bind to ICAM-1 (Diamond et al. 1990). Stimulated endothelial cells which express a high density of ICAM-1 were shown to bind to immunoaffinity purified Mac-1 absorbed to a artificial surface in a manner that was inhibited by monoclonal antibodies to ICAM-1 as well as

Mac-1. Moreover, transfected cells expressing human ICAM-1 were shown to bind to purified Mac-1 in a specific and dose-dependent manner. These results provide proof that ICAM-1 is also "counter-receptor" for Mac-1 and that this interaction is in past responsible for adhesion between stimulated neutrophils and unstimulated endothelial cells.

The binding site on ICAM-1 for LFA-1 was distinct from that for Mac-1 which binds to the third NH_2-terminal immunoglobulin-like domain (Diamond et al. 1990). In contrast, LFA-1 binds to the first NH_2-terminal immunoglobulin-like domain of ICAM-1. This observation provides a function for the tandem duplication of the immunoglobulin-like domains of ICAM-1.

ICAM-1-induced Leukocyte Adhesion and Migration

ICAM-1 is important in neutrophil adhesion and migration (Smith et al. 1988). Pretreatment of endothelial cells with ICAM-1 antibodies reduced by 50% the attachment of neutrophils to IL-1- or LPS-stimulated endothelial cells, whereas the reduction in migration was greater than 85% across the cytokine-stimulated endothelial cells. Both CD18 and ICAM-1 antibodies produced the same inhibition in adhesion and migration. Their combined effects were not additive. These results indicated that neutrophil adhesion and transendothelial migration in vitro depend on both CD18 on neutrophils and ICAM-1 on endothelial cells.

The relative contributions of CD11a/CD18 and CD11b/CD18 in mediating neutrophil adhesion were examined by using monoclonals to each integrin (Lo et al. 1989c). Each antibody inhibited about 50% of the adhesion induced by phorbol dibutyrate whereas the antibodies in combination fully inhibited adhesion. Anti-ICAM-1 antibodies also inhibited 50% of the adhesion, but combination of anti-CD11a and anti-ICAM-1 antibodies did not produce an additive inhibition. In contrast, anti-CD11b plus anti-ICAM-1 resulted in complete blockade of adhesion. These results suggest that the CD11a/CD18 recognizes ICAM-1 on endothelial cells, but that CD11b/CD18 recognizes additional and possibly different ligand(s) which could be ICAM-2.

LFA-1 and Mac-1 have cooperative interactions with ICAM-1 that facilitate adhesion and transendothelial migration of neutrophils in vitro (Smith et al. 1989). The adhesion of unstimulated neutrophils to IL-1 stimulated endothelial cells was inhibited by anti-CD11a but not anti-CD11b monoclonal antibodies. Anti-CD11a but not anti-CD11b antibodies also reduced adherence on unstimulated neutrophils to purified ICAM-1. The results indicate that the unstimulated neutrophils bind to ICAM-1 via CD11a integrin. However, when neutrophils were activated in this study by fMLP, both anti-CD11a and anti-CD11b antibodies were effective (Smith et al. 1989). This implies that chemotactic stimulation of neutrophils enhances the attachment of human neutrophils to ICAM-1 by a Mac-1-dependent process. Thus, the interaction between unstimulated neutrophil and activated endothelial cell is mediated by LFA-1, whereas the upregulation and activation of neutrophils also engages Mac-1 that can augment the adhesion response additional binding to ICAM-1.

Expression of ICAM-1 in Clinical Disorders

ICAM-1 has been implicated in clinical disorders since it is an ubiquitous adhesion molecule found on many cells. Studies indicated that ICAM-1 is expression is increased in renal allografts (Cosimi et al. 1990). A monoclonal antibody to ICAM-1 in non-human primates prevented acute rejection of renal transplants (Cosimi et al. 1990). A soluble form of ICAM-1 also inhibited rhinovirus infection (Marlin et al. 1990). These data indicate that soluble ICAM-1 antagonizes the virus and its receptor (which in ICAM-2), and thereby can prevent rhinovirus infection.

ICAM-1 upregulation also has been proposed as a mechanism of asthma in which airway eosinophils adherence to airway epithelial cells is a major contributing factor. ICAM-1 expression was upregulated in inflamed airway epithelium in primate model of asthma (Wegner et al. 1990). The results point to the possible protective effect of antagonism of ICAM-1 in reducing airway inflammation and hyperreactivity. ICAM-1 expression also occurred in metastatic melanoma which may be pathogenic in this condition and may provide a useful marker of the disease (Natali et al. 1990).

ICAM-1 expression is also upregulated in autoimmune reactions such as insulitis and Graves and Hashimoto's disease (Wutrich et al. 1990; Zheng et al. 1990) and may play a role in their pathogenesis (Simon et al. 1991). In addition, endothelial cells express ICAM-1 in the central nervous system of guinea pigs during acute and allergic encephalomyelitis (Wilcox et al. 1990).

Conclusion

Adhesion molecules on endothelial cells and leukocytes orchestrate a complex interaction between the endothelial cell and leukocyte. The primary role of this interaction is to facilitate migration of leukocytes across the endothelial barrier. The discovery of these molecules and their inhibitors may enable the precise control and modulation of the inflammatory response. Important questions remain to be addressed. These relate to the intracellular mechanisms of induction or expression of these adhesion molecules, the cooperation and the synergy that exists between these molecules, and their precise role in the host-defense response and in the mediation of inflammatory disorders.

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