review
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LEUKOCYTE CD11/CD18 INTEGRINS: BIOLOGICAL AND CLINICAL RELEVANCE

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ABSTRACT

The integrin family consists of a series of related $\alpha\beta$ heterodimers (subunits of 95,000-200,000 Mw) involved in a variety of cell-matrix and cell-cell adhesion functions. Leukocyte adhesion has biological importance in numerous processes involving host defense. The CD11/CD18 integrins are differentiated antigens which play a critical role in this mechanism. CD11a/CD18 are apparent on early progenitors of all myeloid and erythroid cells. CD11b/CD18 and CD11c/CD18 are more restricted antigens normally expressed on monocytes, macrophages, PMN and natural killer cells. Activated granulocytes and monocytes express far more CD11b/CD18 than the other two antigens: 6 to 8×10^5 CD11b/CD18 molecules appear on maximally activated granulocytes. These integrins and in particular the β_2 subunit are lacking in a genetic disease. On the other hand, they are fundamental in numerous physiological processes and in various hematological and cardiovascular diseases. The biochemical characterization and behavior of the CD11/CD18 complex in various clinical conditions are the subject of this review.

Key words: CD11/CD18, integrins, leukocytes

Cell adhesion molecules play an important role in leukocyte-endothelial cell interactions.[1-3] In the immune system these molecules encourage leukocyte-endothelial cell interactions and orchestrate many other types of cell interactions through a variety of adhesion receptors belonging to evolutionarily distant gene superfamilies, including integrins, selectins, immunoglobulin-like molecules and cadherins.[4-10] Adhesion molecules coordinate the various phases of leukocyte adherence to resting or inflamed endothelium in a stepwise fashion through a regulated mechanism of ligand binding. This typically includes qualitative changes in receptor avidity,[11] quantitative increases in receptor surface expression following inflammatory challenges, and transient activation of reversible pathways of leukocyte-endothelium interaction.[12-14] The integrins have been organized into eight distinct subfamilies based on β subunit associations (Table 1). Members of the β_1 subfamily (also called VLA proteins) each contain the β_1 subunit in association with one of at least nine different α subunits. In the β_2 subfamily, there are three distinct α subunits which associate with β_2 (CD11/CD18). The other groups associated with the β_3-β_8 subfamilies have various roles and functions.[13-15] This review will focus on recent studies concerning the structure and function of leukocyte CD11/CD18 receptors and their clinical significance.

CD11/CD18 structure

Various studies and reviews[17-25] have determined the structure of leukocyte CD11/CD18 adhesion molecules: a family of cell-surface glycoproteins, consisting of 3 heterodimers sharing a common β subunit (CD18) with a distinct α subunit (CD11a, CD11b, CD11c), called *inte-*

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Table 1. The integrin families.

Leukocytes CD11/CD18							
β1	*β2*	*β3*	*β4*	*β5*	*β6*	*β7*	*β8*
α1-α9 αV	αL αM αX	αIIb αV αLRI	α6	αV	αV	α4 αIEL	αV

grins. The three α chains of the CD11/CD18 family have molecular weights of 177 kD (CD11a, LFA-1), 165 kD (CD11b, CR3), and 150 kD (CD11c, p150, 95), and are the products of three separate genes.[24] Linking of the α and β_2 subunit precursors occurs in the Golgi apparatus, and the assembled receptors are then transported to the cell surface or to intracellular stores.[25]

The cDNA sequences encoding the α and β_2 subunits have been cloned.[22,25,26] The α subunit has a long extracellular domain, a transmembrane domain and a short cytoplasmic domain.[27-32] The β_2 subunit has a highly conserved cysteine-rich region which gives it a rigid tertiary structure.[33-36] The divalent cations Ca^{2+} and Mg^{2+} are essential in the stabilization and function of the αβ complex.[33] The gene encoding the β_2 subunit has been mapped on chromosome 21 band 21q22, which is a breakpoint in chromosomal translocation t(3;21)(q26;q22) associated with the blast phase of chronic myeloid leukemia.[32] All three α subunits have been localized to bands p11-p13.1 on chromosome 16. Inversions in translocations involving this region have been reported in patients with acute myelomonocytic leukemia (AMMoL).[31-34] Whether the α and β_2 subunit genes are actually involved in these chromosomal rearrangements remains to be shown. A comparison of the primary structure of the α and β_2 subunits of leukocyte adhesion receptors with extracellular matrix receptors demonstrates strong homologies between the two groups of receptors. All have a heterodimeric α-β_2 structure and recognize the RGD (arginine-glycine-aspartic acid) sequence in the adhesive proteins.[24,35,36] In neutrophils CD11b/CD18 integrins are found in a large intracellular pool, in myeloperoxidase negative granules, and in other granules which are mobilized to the cell surface by inflammatory mediators.[1,2,15,36-43] In resting conditions, each granulocyte in a normal subject bears 6-7,000 CD11/CD18 molecules; after stimulation this number can increase many times, probably as a result of mobilization of reserve molecules stored in the granules.[3] Whereas CD11a/CD18 are constitutively expressed on the plasma membrane and are not up-regulated, CD11b/CD18 can be up-regulated several fold from intracellular granules by chemotactic factors such as C5a, interleukin-8, platelet activating factor, f-MLP and others chemoattractants,[15] causing a marked increase in adhesion that is completely inhibited by monoclonal antibodies against either the αM or the β_2 subunit of CD11b/CD18 receptors.

Distribution of CD11/CD18 in immune cells

CD11a/CD18 were first described as receptors that encourage the adhesion of cytotoxic T cells to their targets.[1,5,13] In T and B lymphocytes all CD11/CD18-dependent functions such as mitogen, antigen and alloantigen induced proliferation, T-cell-mediated cytotoxicity, B-cell aggregation, and Ig production are inhibited by anti-CD11a/CD18 MoAbs, supporting the fact that CD11a/CD18 are the only heterodimers normally apparent on these cells.[3] Moreover, CD11a/CD18 have recently been shown to play a role in strengthening the adhesion of T lymphocytes to dendritic cells. Effective adhesion with a dendritic cell appears to be a requirement for the stimulation of resting T cells.[5] Both T cells and dendritic cells express CD11a/CD18; CD11c also mediates some lymphocyte adhesive functions, e.g. it contributes to the conjugated formation of some cytotoxic T-cell clones and their targets. Neutrophil-mediated inflammatory responses depend on adherence to endothelium, migration into an inflammatory site, and the release of toxic products by neutrophils.[1,3,8]

Unstimulated neutrophils demonstrate a baseline level of spontaneous adherence to cultured endothelial monolayers *in vitro*. In contrast to unstimulated adherence, enhanced adherence in response to neutrophil mediators is almost entirely leukocyte integrin-dependent, mainly

CD11b, with a variable contribution from CD11a.[9] Under conditions of endothelial activation, however, neutrophil adhesion is only partly dependent on the leukocyte integrin family; this time CD11a plays a major part but all three members have a cooperative effect.[44] Transendothelial migration of neutrophils appears to be heavily dependent on leukocyte integrins, even at high flow rates, and requires the synergistic action of CD11a and CD11b.[8]

CD11c/CD18 are expressed on monocytes, macrophages, and on a subpopulation of cytotoxic cells, and appear to be important in mediating several types of adhesive interactions for monocytes. In fact, antibodies to CD11c also interfere with several functions of human monocytes, including random and directed migration, and adhesion to endothelial monolayers.[1,5,8,9,13,15]

Ligands recognized by members of the CD11/CD18 family

ICAM-1 (CD54), a major ligand for CD11a, was initially identified by raising monoclonal antibodies to cells from patients with a congenital deficiency of leukocyte integrin expression (see Table 2). Another ligand for CD11a, ICAM-2 was cloned by screening a cDNA library from endothelial cells.[15,44-51] The regulation of ICAM-1 (CD54) expression on a wide range of cells, and its up-regulation during cell activation and inflammation point to an important role in leukocyte immune responses, lymphocyte traffic and tissue localization. ICAM-1 also functions as a receptor for rhinoviruses. ICAM-2 is also present in endothelial cells, the U937 monocytic cell line and B and T lymphoblastoid lines, but unlike ICAM-1, its basal expression is high and not affected by cytokines.[52-58] There is some evidence to suggest that under certain conditions ICAM-1 can function as a ligand for CD11/CD18.[59,60] This is also true for CD11b/CD18, which binds with a region of complement protein C3bi containing RGD. A study testing the ability of CD11b/CD18 to bind with RGD-containing ligands found that macrophages are able to bind erythrocytes coated with a 21-amino-acid peptide from C3bi that contained this sequence. Although CD11b/CD18 were originally described as a receptor for C3bi, more recent work has identified several additional ligands for these receptors.[61] CD11b/CD18 bind with protein gp63 on the surface of leishmania and promote the internalization of this intracellular parasite.[62] Surfaces coated with the protein fibrinogen are also recognized by CD11b/CD18, and this receptor may therefore promote the adhesion of neutrophils to clots and the subsequent digestion of fibrin. A recent

Table 2. Families of adhesion receptors and their counter receptors.

Leukocyte integrins.

Subunits	*Names*	*Ligands*
$\alpha L\beta 2$	LFA-1 (CD11a/CD18)	ICAM-1, ICAM-2, ICAM-3
$\alpha M\beta 2$	Mac-1 (CD11b/CD18)	ICAM-1, iC3b, Factor X, LPS
$\alpha X\beta 2$	p150, 95 (CD11c/CD18)	iC3b, Fibrinogen

Other functions of CD11/CD18 integrins

Receptor	*Ligand*	*Intracellular*	*Cellular*
CD11a/CD18	ICAM-1-2	PI turnover increase (Ca^{2+})	proliferation fibronectin-binding CD11b/CD18 up-regulation T-cell differentiation
CD11b/CD18 (Mac-1)	ICAM-1 C3bi fibrinogen LPS	increase (Ca^{2+}) actin polymerization granule exocytosis in neutrophils	transendothelial migration

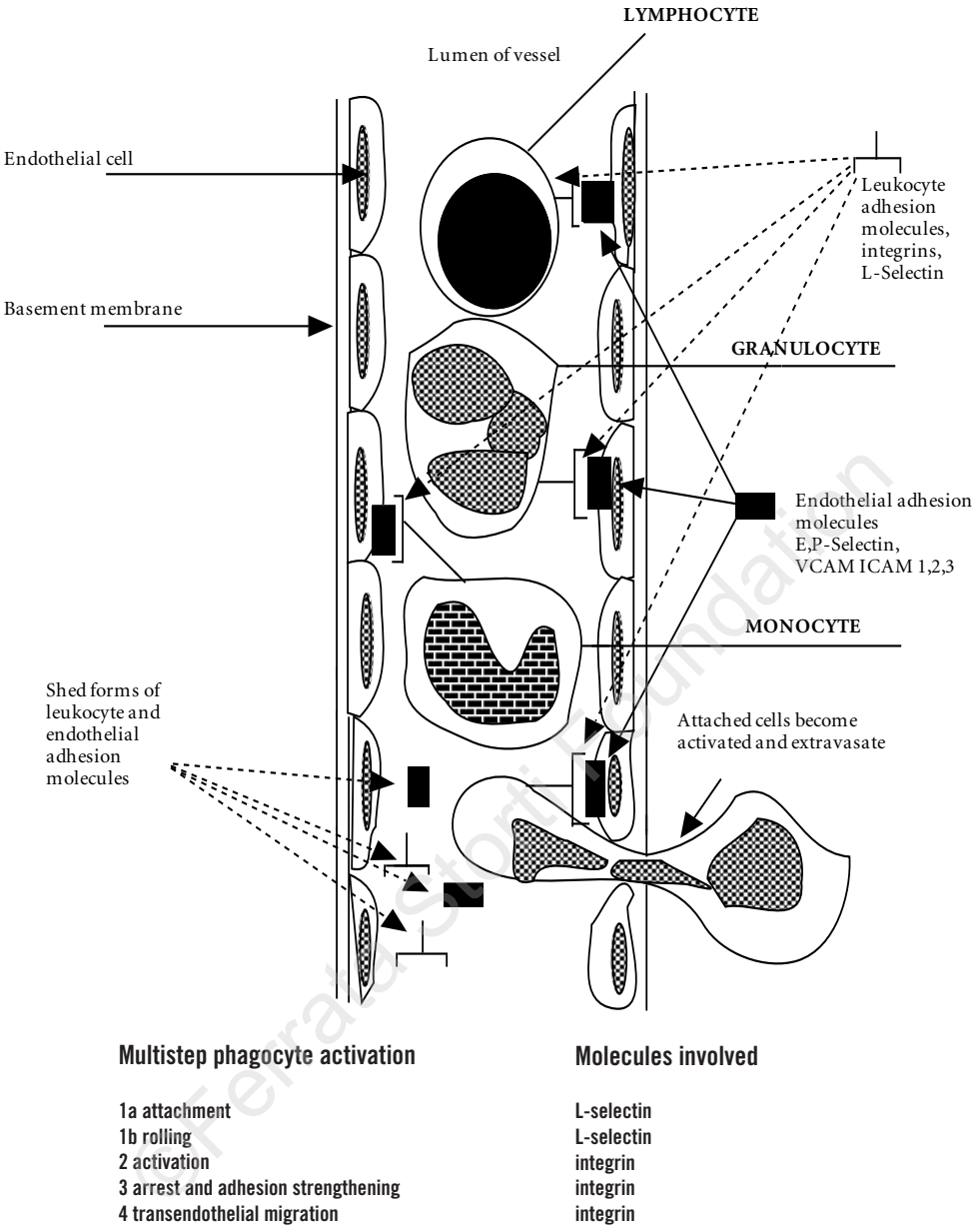

Figure 1. A schematic picture of interactions between white blood cells and endothelial cells mediated by adhesion molecules.

report[15] also describes the binding of soluble clotting factor X to CD11b/CD18. Fibrinogen and factor X effectively compete with each other and with C3bi for binding to CD11b/CD18, suggesting spatial proximity or identity in the binding sites involved.[63,64] The physiological significance of factor X and fibrinogen binding with activated CD11b/CD18 *in vivo* is unclear. Recently, reports[65,66] have shown that fibrinogen or normal plasma enhances the adhesion of myeloid cells to endothelium 2-5 times. This mechanism is mediated by fibrinogen binding to endothelial cells (ICAM-1) and leukocytes (CD11b/CD18).

In accordance with this hypothesis, the same authors have proposed a model in which fibrinogen binding to a variety of vascular cell

receptors mediates a specific pathway for cell-to-cell adhesion by creating a bridge between leukocytes and endothelial cells. This may be important in the monocyte-associated initiation of the coagulation cascade, and/or in CD11b/CD18-dependent adhesion, and in the transmigration of phagocytic cells to and across endothelium.[15]

Several other ligands have since been identified and CD11b/CD18 have been implicated in a number of macrophage-microorganism interactions including binding with E. Coli, *Histoplasma capsulatum* and lipopolysaccharides.[67,68] A recent report described the direct binding of soluble IgG to CD11b/CD18 on monocytes and hypothesized that this activity could be mediated by an immobile subpopulation representing about 40% of all CD11b/CD18 integrins. This observation could explain the mild impairment in Fc-receptor-mediated phagocytosis in LeuCAM deficiency, and the ability of anti-CD11b MoAb to inhibit phagocytosis of IgG-coated particles.[69,70] The adhesion molecules are constitutively expressed at low level, and are up-regulated over a period of minutes or hours (Table 3).[48] Leukocyte adhesion molecules are regulated by changes in avidity as well as surface expression. Activation of neutrophils increases the surface expression and avidity of CD11b/CD18.[15] Lipopolysaccharide (LPS), interleukin-1 (IL-1), tumor necrosis factor (TNF) and interferon-γ (IFN-γ) play a key role in these modulations.[52,53,71] This observation suggests that the mechanisms used in controlling CD11/CD18 modulation are complex and in part unknown. Endothelial surface-bound interleukin-8 seems to enhance CD11/CD18 activation, change shape and shed L-selectin (LAM-1) only in those neutrophils that have already established initial adhesive contact with endothelial cells, as shown in Figure 1.[15,71] This would allow for effective control of the second step in neutrophil emigration.

Interleukin-8 released from endothelial cells in the blood stimulates the release of L-selectin and the activation of CD11/CD18 on circulating neutrophils.[15,53,72] This prevents neutrophil emigration and, because of either homotypic aggregation of the neutrophils or a stiffening of their membrane, results in neutrophil sequestration in the lung microcirculation. The leukocyte-endothelial cell interaction necessary for host defense and repair may contribute to causing vascular and tissue injury in inflammatory and immune disorders.[1] Homotypic leukocyte adhesion within the vasculature may produce aggregation which occludes microcirculation and enhances ischemia. Adherent or diapedesing leukocytes may release mediators that cause endothelial barrier dysfunction, leading to local permeability of edema and thrombosis.

Finally, once they have emigrated, leukocytes may release toxic products (e.g. oxidants and proteases) which damage tissue.

Clinical situations characterized by decreased CD11/CD18 expression

Deficit in neonatal neutrophils

Neonatal neutrophils show well-documented defects in chemotaxis and adherence which are presumed to contribute to the increased susceptibility of neonates to bacterial infection. CD11b/CD18 on PMN play a pivotal role in the adhesion-related functions of PMN. In neonatal PMN, induced-chemoattractant increases in surface expression of CD11b and is deficient with respect to that of adult PMN, and this pre-

Table 3. Distribution and function of CD11/CD18 on leukocytes.

Leukocyte function	*Molecules involved*
Myeloid series	
Binding to C3bi	CD11b, c/CD18
Adhesion to endothelium	CD11a, b, c/CD18
Aggregation	CD11a, b/CD18
Random migration/chemotaxis	CD11a, b, c/CD18
Phagocytosis	CD11b/CD18
fMLP-induced oxidative burst	CD11b/CD18
ADCC	CD11a, b, c/CD18
Lymphoid series	
Antigen, mitogen, alloantigen-induced proliferation	CD11a/CD18
NK, K and CTL	CD11a, b, c/CD18
T and B cell aggregation	CD11a/CD18
Adhesion to endothelium	CD11a/CD18
Helper immunoglobulin production	CD11a/CD18

sumably contributes to defects described in neonatal PMN.[73] The basis for this deficiency in stimulated CD11b/CD18 surface expression has not been determined. Possibilities might include abnormalities in signal transduction, defects in the intracellular machinery responsible for CD11b/CD18 translocation, or a deficiency in the total cell content of CD11b/CD18. In a recent paper[73] it was reported that three different immunochemical methods determined that the total cell content of CD11b/CD18 in neonatal PMN is substantially less than that in adult PMN, thus providing a simple explanation for deficient CD11b/CD18 surface expression on stimulated neonatal PMN. Further studies are in progress to elucidate the developmental basis for the diminished total CD11b/CD18 content in neonatal PMN, and to determine the postnatal age at which PMN CD11b/CD18 content achieves adult levels.

Leu-CAM deficiency I and II

In the last ten years several congenital defects of chemotaxis and phagocytosis have been described in more than 100 patients worldwide who show a similar history of recurrent bacterial infections and an inherited deficiency of three related leukocyte membrane surface antigens called CD11/CD18.[74,75] Neutrophils have a reduced phagocytic response to bacteria and yeast, as well as a reduced ability to adhere to various substrates and to migrate to sites of infection.[76-80] *In vitro* functional studies of control neutrophils treated with monoclonal antibodies to the individual the α and β_2 chains of these antigens suggest that most of the clinical features of these patients may be caused by a CD11/CD18 neutrophil deficiency.[81-84] The incidence of these diseases has apparently increased lately but this is probably the result of newly available diagnostic techniques. The clinical manifestations due to the congenital lack of membrane glycoproteins have been classified[7,85-88] into two subgroups.

Partial deficiency. This group contains different intermediate clinical pictures varying from frequent and long-lasting infective episodes which respond poorly to antibiotics and take a long time to subside, to the presence of trivial skin infections.

Total deficiency. The disease results from a partial (type I) or total lack of surface membrane expression of CD11/CD18 and is often fatal in patients with type II deficiency. Those with type I have a milder course, often surviving into adulthood. Defects giving rise to type II are characterized by frequent infections and severe episodes of bacteremia; sepsis and severe infection of the umbilical stump,[74-88] *S. aureus* septicemias and high mortality occur in the infantile period. Recently, Etzioni et al.[89] described two unrelated children with an identical combination of anomalies and a defect in leukocyte adhesion molecules due to the absence of the Sialyl-Lewis X ligand of E-selectin. These authors[89] proposed designating the previously described defect leukocyte adhesion deficiency type I, and the disorder reported by the same authors leukocyte adhesion deficiency type II.

Hematological disorders

Myelodysplastic disorders are characterized by bone marrow dysmaturation with signs of pathological hematopoiesis in one to three cell lines, and are accompanied by various degrees of anemia and peripheral blood cytopenia (an acquired granulocyte anomaly has been found in association with both hematologic and non-hematologic diseases).[90] Dysplastic changes in neutrophilic granulocytes, i.e. nuclear abnormalities and cytoplasmic hypogranulation, which can be estimated by the percentage of pelgeroid polymorphs and the use of a granulation scoring system (G-score), respectively, were described in 90% of patients affected by myelodysplastic syndrome[91-94] and chronic myeloid leukemia.[43] Various studies have demonstrated decreases in the quantity of CD11b/CD18 expressed on the cell surface and present in the intracellular pool in patients suffering from myeloid disorders[96] and chronic myeloid leukemia.[43] These granulocytes showed cellular defects of locomotion *in vivo* and *in vitro*, aggregation and superoxide release. Flow cytometry and APAAP studies confirmed that the surface expression of CD11b/CD18 decreased. The data reported in two papers[95,96] are consistent with the hypothesis that qualita-

tive and quantitative changes in CD11b/CD18, or in the structures with which they interact, are responsible for the aggregation and locomotion defects in our patients. Several investigators, utilizing monoclonal antibodies which bind with integrin family antigens, have obtained data that are consistent with this hypothesis.[30] *In vivo* studies employing the skin-window technique confirmed the *in vitro* findings of a PMN chemotactic defect. Finally, patients exhibiting these anomalies were shown to express 10% of the normal amount of CD11b/CD18. This may explain the dysfunction of these cells.

Rosmarin[51] demonstrated that during the course of myeloid differentiation, CD11b/CD18 RNA levels present changes in pattern which are temporarily and quantitatively similar to the changes in their protein levels, suggesting that this RNA may regulate the expression of these proteins.

Studies by Hickstein et al.[30-32] with a cDNA clone for β-subunit mRNA indicate that surface expression closely parallels levels of mRNA expression in hematopoietic cells, and that in mRNA the levels (very low in dysplastic PMNs) appear to be an important determinant of CD11b/CD18 surface expression. The granulocyte anomalies characterized by a reduction in CD11b/CD18 binding are correlated with reduced granulocyte chemotaxis, aggregation, phagocytosis, and predisposition to infection.

Clinical situations characterized by increased CD11/CD18 expression

Increased expression of CD11/CD18 on circulating leukocytes occurs in several inflammatory disorders associated with neutrophil activation, e.g. in patients with burns, sepsis, hemodialysis, systemic lupus erythematosis,[97] and in diabetes mellitus.[98] In particular, strong clinical relevance was reported in diabetes[98] and in coronary artery disease.[99-103] Since excessive monocyte adhesion to blood vessel walls could produce endothelial cell injury, various papers have reported monocyte adhesiveness in insulin-dependent diabetics with vascular complications.[98] In these patients the extent of monocyte adhesion to fibronectin and autologous plasma-coated surfaces was significantly increased with respect to that observed in the control group. Monocyte adhesion to plasma-coated surfaces, but not to fibronectin-coated surfaces, could be inhibited in a dose-dependent manner by anti-CD11b/CD18 monoclonal antibodies. This study demonstrated a high level of adhesiveness on diabetic monocytes as a result of increased expression of fibronectin and CD11b/CD18 receptors. Because fibronectin is a major adhesive agent for monocytes and is produced by endothelial cells and fibroblasts, the increased expression of fibronectin and CD11b/CD18 receptors on diabetic monocytes could lead to increased monocyte adhesion to blood vessel walls.[66] Such an abnormality would be particularly enhanced after endothelial cells are injured, since they would express Fc, C3, and C3b receptors[104] and increase fibronectin production. If there was excessive monocyte adhesion, it could have consequences for the integrity of the vessel wall because it would lead to endothelial cell injury and alteration of vascular permeability.[15] Neutrophil and monocyte adhesion to the endothelial cells of the coronary arteries and subsequent leukocyte activation may be relevant in the progression and evolution of atherosclerotic coronary disease. Recent data also suggest a role for inflammation in the pathophysiology of unstable angina.[105] A study was undertaken to assess whether or not up-regulation of neutrophil and monocyte CD11b/CD18 adhesion receptors occurs during the passage of blood through the coronary tree of patients with coronary heart disease.[105] While neutrophils and monocytes adhere poorly to endothelial cells in the absence of stimulation of either cell type, stimulation by chemotactic factors produces a marked increase in adhesion that is demonstrable within minutes. Neutrophils may also adhere to each other, causing aggregates that can promote plugging of the microvascular bed.[106] This aggregation is inhibited by anti-CD18 or anti-CD11b monoclonal antibodies.[106,107] Chemotactic factors able to enhance the up-regulation of CD11b/CD18 adhesion receptors include complement factor C5a, cytokines like interleukin-8 and TNF-α, and lipid-mediated platelet activating factor.[52,53,108] Recent data suggest that all these

agents are potentially released into the coronary circulation of patients with unstable angina. The presence of an acute thrombotic process leads to increased plasmin activity which can cause the activation of the complement system. Indeed, Yasuda et al.[108,109] found increased plasma levels of C3bi and C3d in 17 patients with unstable angina, suggesting complement activation in this clinical setting. Likewise, rupture or ulceration of coronary lesions may provoke the release of TNF-α, which is a constituent of coronary atherosclerotic plaques.[52,53] This cytokine increases the amount of endothelial cell adhesion receptors (E-selectin) that bind unstimulated neutrophils. Interaction of neutrophils with E-selectin-bearing endothelial cells causes neutrophils to show enhanced adhesive activity of the integrins CD11b/CD18.[15,110,111] Moreover, endothelial cells stimulated with thrombin or histamine-synthesized platelet-activating factor rapidly appear on the activated endothelial surface, where they can stimulate leukocytes bound to endothelial receptors.[111] The close proximity of neutrophils to the endothelial cell membrane may also induce subtle alterations in endothelial cell functions[112-115] like permeability changes and decreased production of prostaglandin I_2 and endothelial-derived relaxing factor (EDRF). In addition, the respiratory burst results in the formation of oxygen-derived free radicals which are able to alter microvascular permeability and influence vascular smooth muscle tone. Fibrinogen and factor X compete with each other for binding with the activated CD11b/CD18 complex.[63-66] The significance of this interaction is not clear but it could be important in monocyte-associated initiation of the coagulation cascade[13-15] during which activated leukocytes and platelets potentiate each other's effects, favoring coronary vasoconstriction and thrombosis.

CD11/CD18 as a target of new therapy

Several *in vivo* studies have demonstrated the anti-inflammatory effect of monoclonal antibodies directed against the leukocyte integrins or their ligands (Table 4). In rabbits, intravenous administration of anti-CD18 MoAb inhibited local neutrophil accumulation in response to intradermal injection of LTB4, C5a or FMLP, and neutrophils failed to migrate into endotoxin-impregnated sponges implanted subcutaneously.[114] The mechanism behind these results is suggested by studies using intravital microscopy on animals treated with the anti-CD18 monoclonal antibody.[115-117] Anti-CD18 did not inhibit the rolling of neutrophils along the surface of post-capillary venules, but it did inhibit their attachment in response to stimulation by LTB4 or zymosan-activated serum.[12,15] Neutrophils in animals treated with anti-CD18 antibodies[114] thus apparently fail to extravasate in response to inflammatory stimuli because they are unable to attach to the endothelium and diapedese. These studies suggest that CD11/C18 play a principal role in the relatively rapid inflammatory response to sterile stimuli at peripheral sites.

Ischemia reperfusion injury

The organ injury resulting from ischemia and reperfusion determines the outcome of many important clinical disorders including myocardial infarction, strokes, mesenteric and peripheral vascular diseases, organ transplantation and circulatory shock. A number of recent investigations into the mechanisms of ischemia-reperfusion injury have focused on oxygen free radicals and their production of microvascular injury.[107,116] A role for leukocytes in the pathogenesis of ischemia-reperfusion injury has recently been suggested by studies demonstrating significant injury reduction in neutrophil-depleted animals.[107] An association between leukocytes and ischemic organ injury has been known for many years.[118-120] There are several mechanisms by which neutrophils might cause tissue injury in the setting of ischemia-reperfusion.[121] Because they are larger and less deformable than erythrocytes, neutrophils may plug the small capillaries as perfusion pressure drops. When neutrophil adhesiveness increases, either in response to direct neutrophil stimulation or by endothelial-mediated mechanisms, this situation is worsened. Neutrophils may then actively adhere to the endothelium and to each other (aggregation), occluding the larger post-capillary venules and ultimately resulting in the

Table 4. Animal and human models of diseases in which anti-adhesion therapies have demonstrated efficacy.

Models of disease	*Adhesion protein*	*References*
1. Ischemia-reperfusion		
Intestine (cat)	CD18	114, 120
Hypovolemic shock (rabbit, primate)	CD18	122
Transected ear (rabbit)	CD18	123
Lung (rabbit)	CD18	49, 126
Frostbite (rabbit)	CD18	123
Myocardium (dog)	CD11b	121,133
2. Inflammation		
Skin edema (rabbit)	CD18	113, 124
Cerebral edema in bacterial meningitis (rabbit)	CD18	125,133
3. Immune reaction		
Autoimmune disease (mouse)	CD11b	70, 71
Bone marrow rejection (human)	CD11a	127,133

no-reflow phenomenon.[121] Once adhered to the endothelium, neutrophils may then release proteases, toxic oxygen metabolites and vasoactive substances. Together these may cause endothelial injury with subsequent loss of vascular integrity, edema, hemorrhage and tissue injury.[15,122] Studies in the intestine and myocardium have shown a dramatic increase in tissue leukocytes and microvascular plugging by leukocytes shortly after ischemia and reperfusion. These studies have also shown that neutrophil accumulation and tissue injury were markedly reduced by inhibitors of AA metabolism or by inhibitors of oxygen free radicals.[123] This suggests that the deleterious effects of AA metabolites and oxygen free radicals in ischemia-reperfusion injury may be due, at least in part, to their ability to generate or activate neutrophil chemoattractants. The finding that CD11/CD18 deficiencies in granulocytes and monocytes eliminate or markedly attenuate acute cellular inflammatory responses *in vivo* suggests that induction of these deficiencies by means of MoAbs may attenuate the tissue damage induced by these cells in several clinical situations.[124,125] Activated neutrophils have been implicated in some clinical states in which uncontrolled inflammation leads to autologous tissue damage, for instance in cardiac ischemia-reperfusion, and in the *adult respiratory distress syndrome* (ARDS).[126] A monoclonal antibody[127] to the common β subunit (CD18) decreases endothelial cell damage by PMA-activated neutrophils *in vitro*. In a dog model[121] of myocardial reperfusion injury, a monoclonal antibody to the alpha subunit of Mac-1 (CD11b) reduced infarct size by 40-50%, but only if administered well before reperfusion. In a similar rabbit model, monoclonal antibodies to the LFA-1 α subunit (CD11a), ICAM-1 and CD18 were comparably effective. The use of such therapies in combination with fibrinolytic agents could be of interest in the management of acute myocardial infarction. The CD18 antibody[107] was found to be similarly protective in ischemia-reperfusion injury in the cat intestine, the rabbit ear and in rabbits subjected to transient hypovolemic shock.[9]

Infectious diseases

Various studies have provided[124,125] clear evidence that leukocytes play a significant role in causing several types of intracranial disease severe enough to induce death following a challenge with both live bacteria and inflammatory bacterial surface components. Cerebral edema was absent in CD18 MoAb-treated animals, confirming previous evidence that activated leukocytes on endothelial cells decreased three additional parameters of blood brain barrier permeability in meningitis: these included the influx of serum proteins into CSF, the penetration of antibiotics from serum into CSF, and the onset of bacteremia resulting from intracisternal bacterial growth. In these rabbit models of meningitis, the CD18 MoAb reduced leukocyte accumulation, cerebral edema and mortality.

One of the apparent advantages of the isolated perfused rat lung-human neutrophil system is the ability to quantify neutrophil activation *in situ* by monitoring both the generation of oxidants and the release of specific granule constituents into the perfusate. In this rat lung model of ARDS, the CD11b antibody prevented lung injury caused by PMA-activated neutrophils as measured by pulmonary plasma leakage.[126] Electron microscopy showed that monoclonal antibody treatment prevented neu-

trophils from spreading on the endothelium and causing endothelial damage, but it did not reduce the number of neutrophils in contact with the endothelium. Anti-CD11a or anti-ICAM-1 monoclonal antibodies inhibited neutrophils from emigrating into phorbol ester-induced inflamed rabbit lungs. In another study, CD18 administration prevented neutrophil emigration into rabbit alveoli following intrabronchial instillation of E. coli endotoxin, but not following a similar injection of *Streptococcus pneumoniae* organisms. The apparent lack of significant protection of lung tissue in some of these models may be a reflection of the importance of other adhesion pathways that mediate PMN emigration in this organ.[1,14,15,128-134]

Septic shock can complicate infectious,neoplastic and traumatic conditions. It is frequently caused by bacterial components that activate cellular and humoral systems of the host. Liberation of inflammatory mediators, together with the bacterial products, induces leukocyte and endothelial activation. Vasodilatation and endothelial damage result in vascular leakage that contributes to diffuse peripheral hypoperfusion. Treatment with monoclonal antibodies directed against leukocyte CD18 can prevent organ failure and increase survival in animals.[133]

Asthma

Asthma is a chronic inflammatory disease characterized by the accumulation of inflammatory cells, particularly neutrophils and eosinophils, in the bronchial airways.[133,134] The combination of the E-selectin monoclonal antibody plus ICAM-1 or CD18 monoclonal antibodies completely inhibits the adhesion of inflammatory cells to activated endothelium. These findings do not exclude the potential role of other adhesion molecules such as P-selectin or VCAM-1. In a rabbit model of lung inflammation, the administration of ICAM-1 or CD18 antibodies also led to a marked decrease in the accumulation of neutrophils in the lungs.[122,126] Similar findings have been obtained in primate models of antigen-specific airway inflammation.[112,113] The administration of monoclonal antibodies to CD18[49] blocked the influx of neutrophils in this model and improved airway obstruction.

Arthritis

Synovial histology of inflammatory arthritis showing infiltrates of granulocytes and mononuclear cells[70] has lead to the hypothesis that leukocytes adhere to the synovium, diapedese through the vascular endothelium and then are presumably retained in the synovial tissue by adhesion to fibroblasts.

ICAM-1 and CD11/CD18 interactions are fundamental for T-cell and phagocyte adhesion to synovial endothelium.[14] Treatment of a rabbit model of chronic antigen-induced arthritis with a monoclonal antibody to CD18 resulted in decreased numbers of phagocytes in the synovial fluid, and in synovial histologic findings similar to those of control animals. The use of anti-adhesion therapies for the treatment of human inflammatory arthritis, such as rheumatoid arthritis, may be beneficial provided chronic administration and non-immunogenic adhesion blocking can be developed.

Prevention of graft rejection in transplantation

The role of the CD11a/CD18 and ICAM-1 receptor-ligand pair in cytotoxic T-cell function has been the rationale for using monoclonal antibodies directed against these adhesion molecules in transplantation.[127] The administration of anti-CD11a/CD18 MoAbs in combination with standard immunosuppressive therapy has been found to improve the survival of transplanted bone marrow. This approach was underlined by the finding that patients with Leu-CAM deficiency who received partially incompatible bone marrow transplants as therapy for type I deficiency showed better tolerance for the transplant than historic controls. Several children with a variety of disorders have since received non-HLA identical bone marrow transplants after combination chemotherapy with cyclophosphamide/busulfan/total body irradiation and pretreatment with anti-CD11a/CD18 MoAbs. Anti-CD11a MoAb was used in the non-HLA identical bone marrow transplantation of 36 children with congenital disease and 16 patients with leukemia.[7,9,15] The rate of engraftment was 73%, with 58% alive with functional

grafts 3-31 months later, as compared with historical controls showing less than 20% survival with a functional graft. However, in another study, anti-CD11a MoAb did not facilitate engraftment of T-depleted allogenic bone marrow in 8 adult leukemic patients.[115]

In vivo anti-CD11a MoAb increased the survival of allogenic tumor grafts in mice.[12,61] Prophylactic administration of the monoclonal antibody against ICAM-1 in cynomolgus monkeys receiving heterotypic renal allografts significantly improved survival. When administered after the onset of acute rejection, it led to reversal of the rejection, mainly by minimizing vascular damage. These last results, although understandable in view of established lymphocytes adhesion pathways, reflect the need for randomized and prospective clinical trials to properly evaluate the role of anti-CD11a/CD18 MoAbs in graft rejection. Further clarification of the structure-function relationships of these molecules, the mechanisms controlling their activation state, and their role in signal transduction will hopefully expand therapeutic targets.[133,134]

A possible role in cancer metastases

Recent studies have suggested that certain tumor cells (e.g. melanoma and carcinoma) may interact with endothelial-leukocyte adhesion molecules during hematogenous metastasis.

Initial studies demonstrated that tumor cells bound in increased numbers to cytokine-activated endothelium, a process reminiscent of endothelial-leukocyte adhesion.[52]

Selectin-carbohydrate interactions have also been implicated in the hematogenous spread of cancer cells. Early papers reported that E-selectin could support the adhesion of human colon cancer cells.[14,130] The carboydrate structures of sLex and sLea are expressed in abundance on most human colon cancers and appear to participate in this adhesive interaction.[131-135] Separate studies have demonstrated that the CD11/CD18 complex and CD44 expressed on hematological tumor cells may alter metastatic capacity and growth.[132] Other endothelial-leukocyte adhesion molecules that participate in the metastatic spreading will probably be found.

Concluding remarks

In this review we have attempted to summarize several functional aspects of CD11/CD18, a member of the integrin family of cell adhesion receptors.

One of the most interesting and least understood mechanisms governing integrin activity involves the rapid alterations in receptor affinity that occur with cellular activation. The precise series of intracellular events responsible for integrin affinity modulation are not known and, to date, the intracellular mediators of this process have not been identified. Cellular activation of integrins occurs concomitantly with increases in intracellular calcium levels, activation of multiple protein kinases, alterations in the lipid composition of the plasma membrane, and rearrangement of the cytoskeleton. Which, if any, of these processes contributes to integrin activation is unknown.

Molecular biology techniques are expected to identify critical regions in CD11/CD18 that mediate various adhesive interactions. Information derived from such studies should provide invaluable insights that could lead to the development of other chemotherapeutic reagents which would be useful in limiting PMN-mediated tissue injury.

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