LEUKOCYTE ADHESION PROTEINS: THEIR ROLE IN NEUTROPHIL FUNCTION*

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INTRODUCTION

Since the era of Metchnikoff it has been known that phagocytes respond to tissue inflammation by adhering to post capillary venule endothelium and exit the circulation by squeezing between endothelial cells (diapdesis) in response to chemotactic stimuli (1). While the past 30 years has witnessed a remarkable growth in the understanding of mechanisms of chemotaxis, phagocytosis and killing of microorganisms, definitive knowledge of the mechanisms responsible for adherence of phagocytes to endothelial cells, other leukocytes and to a variety of surfaces has been gathered only relatively recently. Over the past decade the developing story of the role of a family of plasma membrane glycoproteins in these events has been an exciting chapter for students of leukocyte biology. These glycoproteins are now known to belong to a highly conserved superfamily of proteins termed "integrins." Named for their ability to integrate functions of extracellular and cytoplasmic compartments, integrins are involved in a wide variety of phenomena that depend upon intimate cell-cell or cell-surface contact (2). The characterization of a unique group of patients with recurrent infections together with the application of the tools of monoclonal antibody technology and molecular biology have been central to defining the role of these proteins in inflammation and host defense.

LEUKOCYTE ADHESION DEFICIENCY (LAD)

In the early 1980's, several groups of investigators described infants and children who had an unusual constellation of symptoms and signs including delayed separation of the umbilical cord, recurrent necrotizing bacterial infections of the soft tissues that were devoid of pus, respiratory tract infections, severe periodontitis and gingivitis, and delayed wound healing. A persistent polymorphonuclear leukocytosis was characteristic with some patients exhibiting counts in excess of 100,000/μl when

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infected. Biopsies of infected sites disclosed no intralesional polymorphonuclear leukocytes but these cells were often found in increased numbers in vessels leading to and from the lesions. Neutrophils and monocytes failed to enter Rebuck skin windows (3–7).

To date, about 60 such patients have been identified in the literature. Some of the patients have died in infancy of the complications of progressive infection including septicemias whereas others have had a milder clinical course and have reached adulthood. The sex incidence of the disorder is about equal, consanguinity has been involved in a number of parents and an autosomal pattern of inheritance has been defined, with the heterozygotes usually exhibiting no clinical illness (8).

In vitro functional deficiencies of neutrophils (PMNs) have included marked impairment in adherence to plastic or lipopolysaccharide coated glass slides, or endothelial cell monolayers; deficient PMN aggregation, depressed chemotaxis; minimal or absent binding and phagocytosis of C3bi coated particles with a subsequent failure of triggering of the leukocyte respiratory burst and depressed granulocyte or monocyte antibody-dependent, cellular cytotoxicity (3–7). These defects of cellular adherence have led to the name "leukocyte adhesion deficiency" (LAD) for patients with this syndrome (8). In contrast, PMNs from most patients display near normal stimulation of the respiratory burst, changes in cell shape and lysosomal degranulation when exposed to soluble stimuli such as F-met-leu-phe or phorbol myristate acetate. Abnormalities of lymphocyte function have also been found including T-lymphocyte mediated killing, proliferative responses to mitogens or other cells, natural killing activity and antibody-dependent cytotoxicity (8, 9). Despite these abnormalities viral and other intracellular infections have not been prominent nor have repeated infections with encapsulated organisms. Serum complement, immunoglobulins, antibody responses and cutaneous delayed hypersensitivity reactions have been reported as normal in most patients (7–9). Recently, LAD in a dog has been described raising the possibility that an animal model of the syndrome can be developed (10).

IDENTIFICATION AND CHARACTERIZATION OF LEUKOCYTE ADHESION PROTEINS

The possibility that abnormal adherence-dependent neutrophil functions could be due to alterations or deletions in surface glycoproteins was first reported in 1980 by Crowley and colleagues. PMNs from a patient with recurrent infections, had defective PMN adherence to surfaces and lacked a glycoprotein of MW 110,000 Kd (4). Shortly thereafter, Arnaout et al. (5) and Bowen et al. (6) reported studies on other patients with a

similar clinical picture and missing PMN surface glycoproteins of 150,000 Kd and 180,000 Kd, respectively. The nature of these missing proteins and their relationship to leukocyte functions was assisted enormously by the parallel development in the late 1970's and early 1980's of monoclonal antibodies (MoAb) against several key membrane proteins involved in leukocyte differentiation and function. Springer and colleagues demonstrated that antibodies against a protein, which they termed LFA-1, that is present on murine and human lymphocytes, monocytes and PMNs, blocked T-cell cytotoxicity and mitogenic responses (Reviewed in 9). Antibodies directed against a macrophage differentiation protein, Mac-1, were then used to define its presence on murine and human PMNs and confirm its relationship to the C3bi receptor (CR3) (11). Monoclonal antibodies which identified and blocked CR3 functions on human monocytes, macrophages and PMNs were also developed at around the same time by Todd et al. (Mol) (12, 13), Wright et al. (IB4) (14), and Beatty and colleagues (60.1 and 60.3) (15, 16). In contrast to antiMo1, antiMac-1 and OKM10, which bound only to PMNs monocyte/macrophages and NK cells, IB4 and 60.3, bound to all leukocytes and could inhibit lymphocyte-mediated functions in addition to those belonging to CR3 (14, 15). Schwarting et al. developed an antibody against spleen cells from a patient with hairy cell leukemia which identified an antigen (Leu M5) present on myeloid precursor monocytes, macrophages, PMNs and NK cells but not normal B or T lymphocytes (17). Using several of these MoAbs, Sanchez-Madrid and colleagues purified the proteins that these antibodies identified and clarified their structural and functional relationship to each other (18). A family of 3 leukocyte glycoproteins involved in a wide array of cell-cell and cell-surface contact mediated functions of human leukocytes was defined (LFA-1, Mac-1/Mo1, and p150.95) (18). These have been classified by the Third International Workshop on Leukocyte Differentiation Antigens as members of the CD11/CD18 complex (19).

The properties identifying MoAbs, cell distribution, functions and known ligands of the CD11/CD18 proteins are summarized in Table I. The family consists of three molecules with distinct alpha-subunits now designated as CD11a, CD11b and CD11c (formerly αL, αM and αX), respectively, which are bound in non-covalent linkage to a common β subunit, CD18 in $\alpha 1/\beta 1$, configuration. The gene for the β subunit has been cloned; a 45% homology between the β subunit sequence and band III of integrin, a chick fibronectin and laminin receptor has been defined (20). By peptide mapping and aminoacid sequence analysis, the α subunits of CD11b and CD11c are 60% identical, whereas they share approximately 35% sequence homology with CD11a (21). There is a common transmembrane-spanning region as well as extracellular domains

TABLE I
Leukocyte Adhesion Proteins of the CD11/CD18 Complex

Protein	CD11a/CD18	CD11b/CD18	CD11c/CD18
Other names	LFA-1	MAC-1/Mo1/OKM1 CR3	P150-95 Leu/M5
Subunits	α (αL)/β	α (αM)/β	α (αX)/β
Mr $\times 10^{-3}$	180/95	170/95	150/95
Binding of monoclonal antibodies	TS1/22 60.3^{-1}	antiMac-1/M1/70	anti-LeuM5
	L1 IB4	antiMo1-44	KiM1
	TA-1 TS-1/18	OKM1	L29
	L11 MHM23	OKM9	
	10F12	OKM10	
		60.1	
		MN-41	
Cell distribution	T cells	PMNs	PMNs
	NK cells	Monocytes	Monocytes
	PMNs, Monocytes	NK cells	Hairy cell leukemia
	EBV-B cells		
	Myeloid precursors in marrow		
Increased expression with cell activation	NO	YES	YES
Functions	Leukocyte-cell adherence	CR3 mediated functions, PMN adherences, spreading, aggregation, chemotaxis	PMN adherence
	T-cell cytotoxicity		
	NK cell cytotoxicity		
	T-cell mitogenic responses		
	Antibody dependent cytotoxicity		
Ligands	ICAM-1	ELAM-1, C3bi, fibrinogen, leishmania GP63, zymosan	C3bi, ELAM-1
Recognizes RGD sequences	NO	YES	YES

1-MoAb listed in this column bind to CD18 epitopes which are found in all three adhesion proteins

that bind cations (Ca^{++} or Mg^{++}). The genes for the CD11 proteins reside on chromosome 16; the gene for CD18 is on chromosome 21 (22). Patients with LAD either fail to synthesize any of the proteins (severe phenotype), or minimal amounts (5–7%); heterozygotes synthesize about 50% of the proteins (7, 8). The molecular mechanisms for the defect are in the process of being defined. Available evidence suggests that mutations which involved products of the common β subunit play a central role in inhibiting normal assembly and expression of the protein complexes in all types of leukocytes (22a).

With characterization of the structure and cloning of their genes, it is now apparent that the CD11-CD18 complex belongs to the superfamily of transmembrane proteins termed the integrins (2). These heterodimeric transmembrane proteins include the receptors for the matrix proteins fibronectin, laminin, collagen and vitronectin as well as the platelet glycoprotein, gp^{IIb}/IIIa (23). The β subunits among this family share 40–50% sequence homology; the α chains are 30–50% identical. Many α chains of the integrins contain a recognition or binding sequence in their extracellular domains for the tripeptide arginine-glycine-aspartic acid (RGD sequence) (24). CD11b contains such a binding domain (25). The cytoplasmic domains bind to proteins in the cytoskeleton responsible for changing cell shape and other contractile functions. As a family, the integrins are intimately involved in embryological development, hemostasis, wound healing and perhaps oncogenic transformation in addition to their role in leukocyte inflammatory responses and immune host defense (2, 23, 24).

SURFACE EXPRESSION OF CD11/CD18 PROTEINS ON PMNs

The use of fluorescent or I^{125} labelled congeners of a variety of MoAbs has permitted an analysis of the cellular distribution and expression of the members of the CD11/18 complex under a variety of conditions. Mature PMNs and monocytes from all species examined express all three glycoproteins on their cell-surface. On PMNs CD11b/CD18 is most abundant followed by CD11c/CD18 and CD11a/CD18 (18). CD18 is expressed on ~80% of bone marrow mononuclear cells (15). When myeloid precursor cell lines (e.g. HL 60 or U937) are examined, CD18, CD11a and CD11c but not CD11b are expressed at the promyelocyte (or promonocytic) stage of differentiation (18, 26–28). Induced maturation of cell lines to PMNs or mature monocytes leads to the *de novo* synthesis and expression of CD11b; CD11c is also increased (Figure 1). Cells from patients with chronic myelogenous leukemia (CML) show a similar display of the three glycoproteins with little or no expression of CD11b

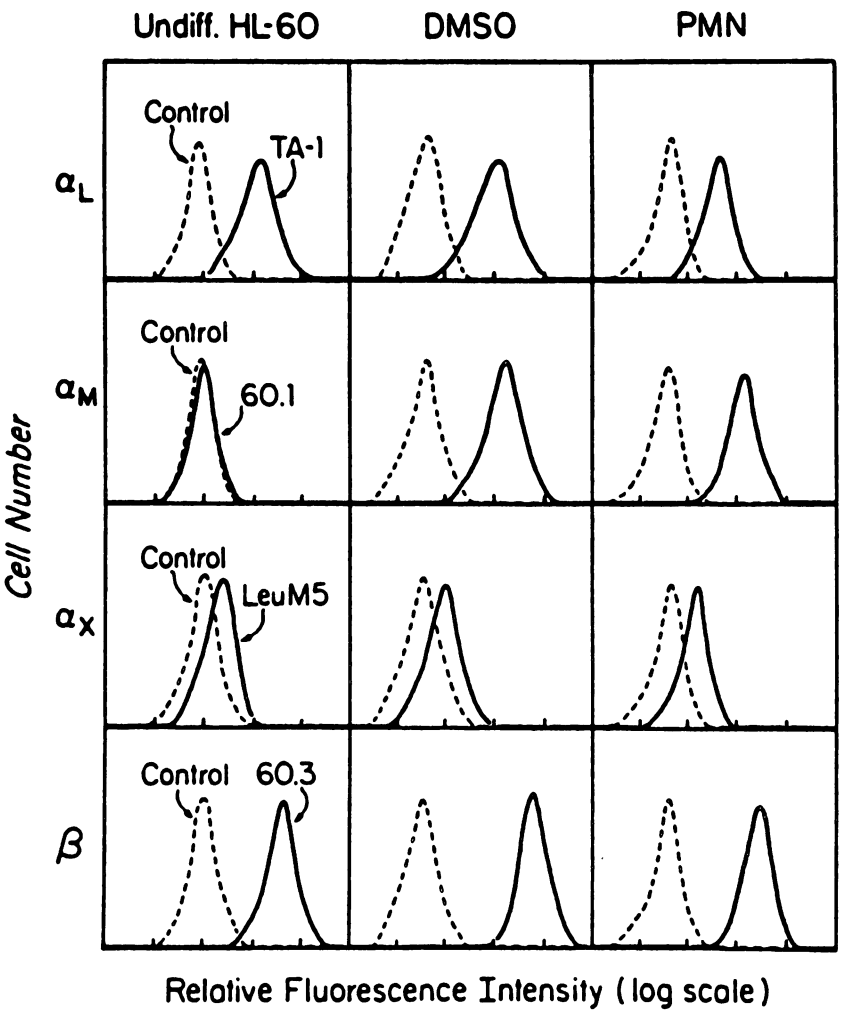

FIG. 1. Immunofluorescence flow cytometry of undifferentiated and DMSO-differentiated HL-60 cells compared with normal human neutrophils. HL-60 cells were induced to differentiate for 4 days with DMSO (1.25%). Cell populations were incubated with the control MAb 9E8 (dashed lines) or with MAb specific for αL (CD11a, TA-1), αM (CD11b, 60.1), αX (CD11c, Leu-M5), or β-chain (CD18, 60.3) before incubation with fluoresceinated F(ab')$_2$ sheep anti-mouse antibody. Cells were examined by using flow cytometry. Relative fluorescence intensity on a log scale is shown on the abscissa and cell number on the ordinate. From Reference 28 with permission from the authors and The American Association of Immunologists.

until the metamyelocyte stage (28). Thus, CD11b is a classic differentiation antigen for myelomonocytic cells.

Exposure of PMNs to chemotactic, phagocytic or other stimuli that induce lysosomal degranulation such as phorbol myristate acetate (PMA) increase the surface expression of CD18 (Figure 2) (29) in concert with CD11b and CD11c but not CD11a (28). Similar effects have been observed after incubation of PMNs with cytokines including tumor necrosis factor α (30) and granulocyte-monocyte colony stimulating factor (31, 32) and

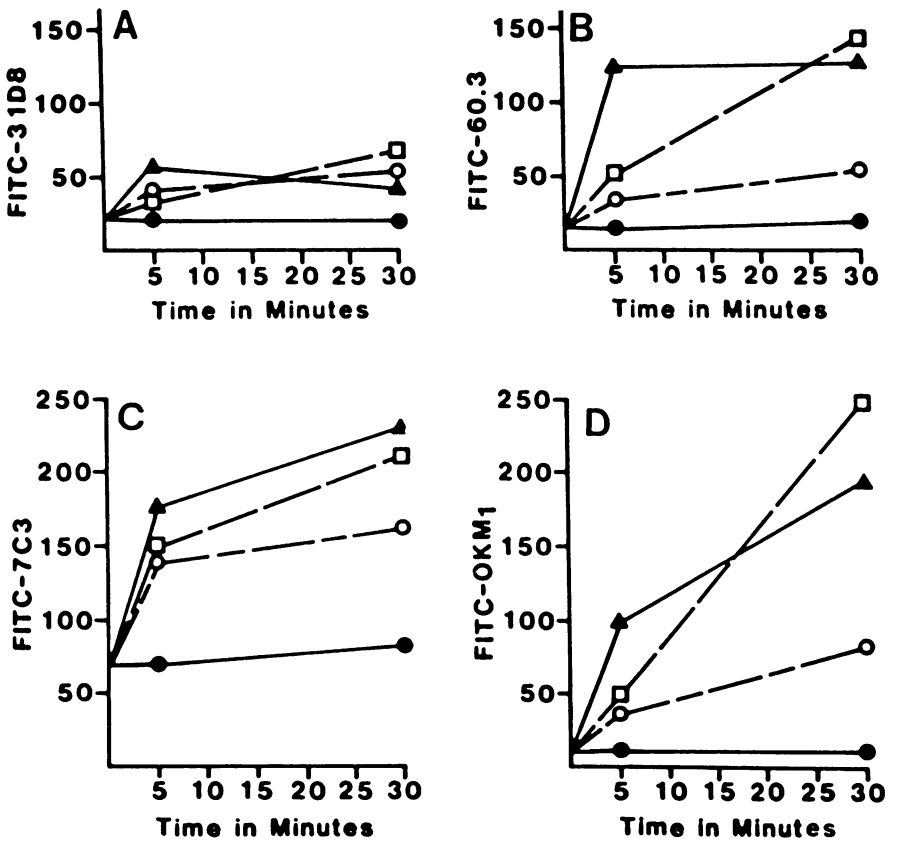

FIG. 2. Appearance of new antigen sites on antibody-pretreated PMNs incubated under resting conditions at 4°C (●——●) or 37°C (○——○) with PMA (▲) or opsonized zymosan (□). Percentage of maximal fluorescence is shown on the ordinates. Cells were saturated with nonfluorescent antibody at 4°C and washed free of unbound antibody before incubation and subsequent exposure to FITC-antibody. Data shown are the mean of three experiments. Antibody body 31D8 is a control not binding to CD11/CD18 (Panel A); antibody 60.3 binds to CD18 (Panel B); antibody 7C3 binds to CD11b/CD18 in addition to another unidentified antigen (Panel C); antibody OKM1 binds to CD11b (Panel D). From Reference 29 with permission from the authors and Grune & Stratton, Inc.

C5a (33). An intracellular pool, localized to specific and/or gelatinase containing granules can be defined for these proteins which are translocated to the cell membrane upon stimulation (34–37). Differentiated HL60 cells which lack specific granules also show augmented membrane expression of these proteins with PMA-stimulation indicating that other latent subcellular sites may exist (Table II). The increased expression of the surface proteins with stimulation can be correlated with increases in the functional properties of PMNs and was initially thought to be important in augmenting plasma membrane CR3 and adherence-mediated activities in response to inflammatory stimuli. Recent data suggest that this analysis requires revision.

Consistent with a failure to produce complete protein complexes, patients with the severe forms of LAD do not express any of the CD11/18 molecules on the surface of PMNs or any other leukocytes, even when the cells are stimulated with various activating agents (7, 8). Minimal expression of CD11/18 (~5–10%) is seen in subjects with milder forms of the disease; heterozygotes express about 50% of the amount of the protein (7, 37).

ROLE OF CD11/CD18 ADHESION PROTEINS IN PARTICLE BINDING AND PHAGOCYTOSIS BY PMNS

Panels of MoAb have been used to define functional epitopes on PMNs involved in the binding and ingestion of phagocytic particles. MoAb directed against CD11a and CD11c have little effect on particle binding and phagocytosis and specifically have not been found to inhibit the ingestion of opsonized zymosan, the binding and ingestion of erythrocytes coated with C3bi and the ingestion of *Staphylococcus aureus* or oil-red-o particles coated with C3bi (38). Multiple antibodies that bind to CD11b inhibit these functions which are all mediated by the CR3 complement receptor (Table II) (11, 13, 14, 16, 28, 38, 39). The degree of inhibition varies with the MoAb used and no inhibition has been observed with MoAb OKM1, OKM9 (14, 38, 39), nor 904 (40) indicating that the

TABLE II
Effect of Binding of Monoclonal Antibodies to CD11/CD18 Epitopes on Neutrophil CR3-Mediated Functions

	antiCD18	antiCD11b
INHIBIT	60.3	antiMac-1-M1/70, antiMo-1-44, OKM10, 60.1, MN41
DO NOT INHIBIT	IB4 TS 1/18	OKM1, OKM9 antiMo-1-904

1) Functions measured in different studies include rosette formation with EC3bi, adherence and phagocytosis of opsonized zymosan.

epitopes identified apparently are not involved in C3bi binding by CR3. The most potent inhibitory antibodies (antiMac-1, M1/70, OKMIO, antiMol-44 and 60.1) suppress CR3 functions to the levels observed with cells from patients with LAD (11, 13, 16, 39). Binding of MAb 60.3 to CD18 also completely inhibits CR3 function (16), whereas antibodies IB4 and TS 1/18 have little or no effects (14, 38). These data support the concept that the major functional chain of CR3 is CD11b. Whether the inhibitory effects of the antiCD18 MAb 60.3 are due to alterations in a configurational arrangement of CD18 with CD11b or indicate direct participation of a CD18 epitope recognized by 60.3 in CR3 function is not clear.

Under some conditions, antiCD11b MoAb also inhibit functions mediated by PMN and monocyte Fc receptors (FcR) (i.e., ingestion but not binding of EARBC) indicating that CR3 and FcR apparently cooperate in the phagocytic process (13, 41). Furthermore, antiCD11b MoAb can inhibit ingestion of unopsonized zymosan (42) and leishmania (43). Evidence has been presented that lectin binding (zymosan) or binding to RGD sequences (leishmania) with similarities to those found in C3bi (25) are involved in the binding of these molecules to CD11b.

Typical of all integrins, CR3 mediated functions require divalent cations (Mg^{++} Ca^{++}) and warm temperature (37°C optimum) for their expression. They are transiently up-regulated by stimuli which promote translocation of subcellular CD11b/CD18 to the plasma membrane. However, enhanced CR3 activity declines over a period of several hours despite increased quantities of plasma membrane–CD11b/CD18, indicating that qualitative changes in the complex must alter its activity (44). The nature and significance of these changes requires clarification, but may relate to the importance of releasing particles from a membrane bound site for ultimate intracellular processing within phagosomes.

ROLE OF CD11/CD18 PROTEINS IN ADHERENCE AND MIGRATION OF PMNS THROUGH ENDOTHELIUM

A variety of surfaces have been employed to measure the adherence properties of PMNs, including glass slides, plastic and nylon fiber (7, 33, 38–40). Studies using monolayers of endothelial cells (EC) usually derived from bovine or human umbilical veins (HUVEC) are thought to be most representative of conditions occurring during endothelial adherence and diapedesis *in vivo*. PMNs adhere readily to HUVEC and the degree of adherence can be increased either by pretreatment of the PMNs with agents which cause translocation of CD11b/18 to the plasma membrane (33, 39, 45) or pretreatment of the HUVEC with the cytokines, IL1 or TNF, bacterial LPS or phorbol esters (46–48). PMNs placed on stimu-

lated HUVEC not only display increased adherence but migrate through the monolayer (49). PMNs from LAD patients have minimal adherence to unstimulated HUVEC and reduced adherence to stimulated HUVEC with almost no migration through the endothelial cell monolayers (45, 48, 49). This underscores the importance of the CD11/18 complex in both processes. MoAbs have been employed to dissect out the role of different functional epitopes on the specific proteins.

Multiple antibodies directed against CD18 markedly inhibit adherence of unstimulated or stimulated PMNs to endothelium to levels equivalent to PMNs from LAD patients and also to block transendothelial migration (Table III) (33, 39, 45, 49). When HUVEC are stimulated with cytokines, antiCD18 antibodies also inhibit adherence but the degree of inhibition is not as complete as seen with unstimulated endothelium (48, 50). Similar findings have been observed with LAD PMNs and indicate the expression of both CD18 dependent and independent adherence mechanisms when cytokines are used to stimulate the HUVEC. MoAb directed against CD11a and CD11b but not antiCD11c inhibit the adherence of

TABLE III
Effect of Binding of Monoclonal Antibodies to CD11/CD18 Epitopes on Neutrophil Adherence to Endothelium

Treatment	Inhibition by MoAB							
	AntiCD18		AntiCD11a		AntiCD11b		AntiCD11c	
	YES	NO	YES	NO	YES	NO	YES	NO
None	60.3	—	—	—	—	—	—	—[3]
	TS 1/	—	—	—	—	—	—	—
	18	—	—	—	—	—	—	—
	IB4							
Stimulated PMN[1]	60.3	—	TS 1/22	—	60.1	OKM1	—	L29
	TS 1/	—	—	—	antiMo1-44			antiLeuM5
	18							
	IB4	—			antiMo1-			
					904			
	MH-	—						
	M23							
Stimulated	60.3		TS 1/22		antiMo1-44	OKM1		antiLeu
endothelium[2]							L29	M5
	TS 1/	—	L11			60.1		
	18	—						
	10F12							

[1] PMNs exposed to phorbol myristate acetate, F-met-leu-phe, A23187, TNF or C5a during adherence assays. Unstimulated endothelial cells were employed.

[2] Endothelial cells pretreated with LPS, IL1 or TNF for four hours before adding untreated PMNs

[3] = not reported

stimulated PMN to unstimulated HUVEC (33, 39, 51, 52)). In contrast, when HUVEC are activated with ctyokines, antibodies directed against all three α chains are inhibitory. Their effects are additive and equivalent to that observed with antiCD18 treated or LAD PMN (48, 50, 51). These findings and the pattern of inhibiting MoAb indicate that the functional domains of the three proteins for endothelial adherence differ from those involved in particle binding and phagocytosis. They also support the concept of up-regulation by inflammatory cytokines of ligands on endothelial cells which recognize the α and β chains of all three proteins.

To date, two such ligands have been identified and characterized: intracellular adhesion molecule-1 (ICAM-1) and endothelial leukocyte adhesion molecule-1 (ELAM-1) (Table IV). ICAM-1 was discovered as a specific adhesion molecule for LFA-1 (CD11a/CD18) by Springer and colleagues who used Ebstein-Barr transformed lymphocytes from LAD patients to raise monoclonal antibodies which inhibited phorbol ester-induced aggregation (9, 53). ICAM-1 is a 90,000 dalton monopeptide that is widely distributed on a variety of cell types including fibroblasts epithelial cells in addition to leukocytes and endothelial cells (54). ICAM-1 serves as a major ligand for CD11a/CD18 (LFA-1) bound to lipid membranes, and cellular binding is not inhibited by antiCD11b or CD11c (55). Present in the basal state on endothelial cells, ICAM-1 expression is up-regulated by IL-1, TNF and IFN γ at a rapid pace for four hours and enhanced expression is observed for several days in the continuous presence of cytokines in the medium (54). ELAM-1 was identified by Beviliqua and Gimbrone and coworkers as a protein which appears on endothelial cell surfaces following stimulation with IL1, TNF or LPS (but not IFNγ) (46). Adherence-inhibiting MoAb against endothelial cells were used to isolate and purify ELAM-1 which consists of a polypeptide of MW 115,000 as the major species synthesized from a precursor polypeptide of MW 97,000 (56, 57). Unlike ICAM-1, ELAM-1 is not present in the basal state and its expression on stimulated endo-

TABLE IV
Adherence Proteins of Human Umbilical Vein Endothelial Cells (HUVEC):

Proteins	Present Basal Conditions	Up-regulated[1]
ICAM-1[2]	Yes	Yes
ELAM-1[3]	No	Yes
OTHERS[4]	No	Yes

[1] Increased or appear upon stimulation of cells with cytokines

[2] Intracellular adhesion molecule-1

[3] Endothelial-leukocyte adhesion molecule-1

[4] These unidentified binding sites are CD11/CD18 independent and appear on HUVEC only with stimulation

thelial cells requires transcription of new specific mRNA and new protein synthesis. Peak expression is reached by four hours of incubation with stimulants following which the protein is rapidly degraded so that expression declines to baseline by 24–48 hours (46, 56–58). ELAM-1 is expressed only on endothelial cells and may be detected in tissues undergoing *in vivo* inflammation (59). Neither ICAM-1 nor ELAM-1 contain RGD sequences (55, 57).

Anti-ICAM-1 MoAb partially inhibit the adherence of PMNs to unstimulated HUVEC to an extent similar to that seen when antiCD11a MoAb are employed (49, 52). Additional inhibition by antiCD18 or anti-CD11b MoAb indicates the presence of another ligand for CD11b/CD18 which is not ICAM-1 (52). In cytokine stimulated HUVEC, both anti-ICAM-1 and anti-ELAM-1 MoAb inhibit adherence during peak expression of both proteins (4 hrs) (49, 50). The relative contributions of ICAM-1 and ELAM-1 to CD11/18 mediated adherence at this time have not been reported, although an additive effect has been observed with anti-CD11a, b and c/18 and anti-ELAM MoAb, supporting participation of both (50). Suppression of adherence in 24 hr cultures of HUVEC by anti-CD11/CD18 antibodies (50), a time at which ELAM expression is markedly diminished, presumably reflects inhibition in part of binding to ICAM-1 as well as other endothelial proteins but this has not been directly assessed. Furthermore the nature of non-CD11/18 dependent endothelial ligands has yet to be defined.

The above data support a role for multiple factors that regulate and increase PMN endothelial adherence and migration during exposure to inflammatory stimuli, including those that augment PMN adhesion to almost any surface as well as those that increase the expression of specific ligands on endothelial cells. The presence of CD11a/18 ligands, such as ICAM-1 in the basal state on endothelial cells, may promote immediate PMN adhesion and diapedisis. The up-regulation of ICAM-1 and *de novo* expression of additional CD11/18 ligands (i.e. ELAM-1) during inflammation presumably enhance PMN inflammatory responses. With respect to the PMN adhesion proteins, the mechanism of the up-regulation of adherence by various stimuli cannot be correlated with quantitative translocation of CD11/18 to the cell membrane and in fact can be separated from this event by the use of cytoplasts or inhibitors which prevent translocation (60). This implies that qualitative changes in function of the complex are induced by exposure to the stimulants, the nature of which remains to be determined. Furthermore, increased adherence is a reversible process despite continued expression of ICAM-1 and CD11/18 as documented in experiments with prolonged incubation with HUVEC or when PMN are incubated for varying times with stimuli that promote transfer of CD11/18 to the plasma membrane (33, 50).

From a teleological standpoint, reversible adherence of PMNs to endothelial ligands may be essential to permit the transendothelial migration of these cells, an event in which both PMN CD18 and endothelial cell ICAM-1 appear to play important roles (49).

IN VIVO STUDIES WITH ANTICD11 AND ANTICD18 MoAb

While all the mechanisms governing PMN-endothelial adherence and diapedesis remain to be defined, the importance of the role of CD18 and CD11b in this process *in vivo* has been highlighted by the observations on LAD patients and the effects of administration of antiCD11b or CD18 antibodies to experimental animals. The antiCD18 MoAb 60.3 binds avidly to rabbit as well as human PMN; its administration to rabbits blocked accumulation of PMN in LPS treated polyvinyl sponges implanted subcutaneously (61). Administration of 60.3 to rabbits also blocked the intradermal accumulation of PMNs in response to placement of chemotactic factors FMLP, leukotriene B4 and C5a as well as histamine (62). Plasma leakage of albumin also was inhibited suggesting participation of PMNs in this event as well. Reperfusion injury in rabbits made hypotensive by phlebotomy was reduced and survival prolonged following MoAb 60.3 administration (63). MoAb 904 binds to an epitope on CD11b of human or dog PMN which is involved in adherence to surfaces and chemotaxis but not CR3 activity (10, 40) (Table II). In a dog model, administration of MoAb 904 reduced infarct size and PMN accumulation in ischemic then reperfused myocardium (64). This antibody has also been utilized to inhibit lung injury by human PMNs in an isolated perfused rat lung model (65). These studies demonstrate the utility of specific antiCD11/18 MoAbs to dissect a role for PMNs in inducing vascular and organ injury *in vivo* and suggest potential therapeutic applications. Similar investigations using MoAbs directed against ICAM-1 and ELAM-1 will be of interest.

CORRECTION OF LEUKOCYTE ADHESION DEFICIENCY

Lymphocytes from a patient with LAD which were completely deficient in formation of the β subunit (CD18) have been induced to express intact CD11a/CD18 by the creation of mouse-human hybridomas (66). The murine cells supplied the CD18 to the patient lymphocytes for successful assembly and expression of the $\alpha\beta$ complexes. Extension of these approaches to myeloid cells with reconstitution of CD11b/CD18 and CD11c/CD18 has yet to be reported. The observations suggest, however, that introduction of normal cloned β subunit gene (20) could provide definitive correction for some patients with severe LAD, although the complexity of the molecular mechanisms involved in the defect (22a, 67)

indicate that this approach cannot be universally employed. Nevertheless, from studies of patients with the milder phenotype of LAD (7, 8, 37), expression of as little as 5 to 10% of normal CD11/CD18 complexes could markedly ameliorate the clinical manifestations.

Until such approaches have been better defined and the molecular mechanisms of LAD further unraveled, the only definitive therapy of LAD has been bone marrow transplantation. Since patients with the severe phenotype have a high incidence of death before the age of 2 (7, 8) transplantation has been attempted only in this group. Several successful chimeras have been established with restoration of PMN function and amelioration of symptoms using both HLA matched and mismatched donors. Others have died of graft vs host disease, but the overall success rate in a small group of patients is about 80% (68, 69).

SUMMARY AND CONCLUSIONS

In conclusion, the leukocyte proteins of the CD11/18 complex are highly conserved members of the integrin family in mammalian species. They play a key role in phagocytic and adherence mediated activities of neutrophils and appear to be centrally involved in adhesion to endothelial cells as well as transendothelial migration. Their importance in these processes has been documented by the occurrence of the disease now called leukocyte adhesion deficiency and the functional effects of a variety of monoclonal antibodies directed at different epitopes on the heterodimeric glycoprotein chains. These antibodies, as well as those directed at endothelial cell ligands for leukocyte adhesion proteins or peptides which mimic the functional epitopes, offer opportunities to manipulate or modify the inflammatory response *in vivo* where neutrophil accumulation or action can be harmful. They can also be employed to dissect out the role of PMNs in various repair processes such as wound healing. While bone marrow transplantation can ameliorate the deleterious consequences of severe LAD, continued elucidation of the multiple molecular mechanisms responsible for this disease will pave the way for its future genetic correction.

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DISCUSSION

Nathan (Boston): Thank you very much for this important review. I want to tell you that the day before I came down here, at morning report, they reported that a woman from Puerto Rico entered the Children's Hospital the previous night with her infant with omphalitis and a white count of 60,000. The story was that she'd lost a child two years before and she'd brought her child to attention in Puerto Rico for this omphalitis. They had treated the infant for the omphalitis and the white count was noted to be 65,000. The white count then dropped to 30,000 on treatment, but when the infant got sick again and the white count was now 75,000, she got scared and flew to Boston. That's the way we get most of our admissions. They just sort of fly there and walk in uninsured. I'm very excited. I think they'll know when I get back to Boston. Tim Springer is running these cells. I'm sure that she has a beta chain deficiency. The interesting point is that the first observation of this was incorrectly made by us. Tom Stossel in our group thought that this was a problem in contractile proteins and we presented the first case as actin binding protein deficiency because in this child, according to a phagocytosis assay, the cells did not ingest and it looked like a contractile protein problem but obviously that wasn't the case. We

missed that diagnosis and now we know the right one. It's an interesting history of this and those diseases are still around.

Root: It's a classic wedding between experiments of nature and the development of a technology which will allow identification of the type of defect and that's been very exciting.

Clark (Iowa City): Thanks for that lovely review. This has been an exciting area. Are you aware of any patients with alpha chain deficiency? It seems as if such patients are likely to exist, but have they been identified to your knowledge?

Root: It would seem that such patients ought to exist but to my knowledge there have been no reports in the literature and I have not heard them presented at meetings. I don't have a good explanation for that but some molecular biologist might.

Clark (Iowa City): I have a couple of questions on the regulation of these molecules. You mentioned the up-regulation of surface expression on stimulation of cells and alluded to the complexity of whether that constitutes a mechanism for functional up-regulation since there are experiments suggesting that one can disassociate that kind of event with changes in affinity. I'm not up-to-date on the latest on that.

Root: Probably the latest chapter on that has been written by Michael Gimbrone's group. I showed in one of my slides that there's an increased expression of these proteins. I was using, as an example, the beta chain on the cell surface which occurs very abruptly and after exposure to phorbol myristate acetate. Yet if you carry out your adherence assay for a period of time, despite the presence of increased expression of adhesion proteins on the neutrophil surface, they no longer adhere as efficiently to endothelial cells. So something else is regulating and reversing the phenomenon of increased adherence. The implication is that these molecules are very importantly involved in normal diapedesis. You would think that if they adhered permanently to endothelial cells in an inflammatory stimulus and never let go, then they would never leave the circulation. From a teleologic standpoint, it makes sense to have transient adherence and then letting go. Part of this is related to some disappearance of the ELAM molecule from the endothelial cell itself. What the ligand is that they are adhering to still needs to be worked out in more detail.

Sande (San Francisco): As you know the inflammatory response is not always our best friend. Another twist on this story, which I think is fascinating is the potential therapeutic value of these monoclonals. If you give these monoclonals to rabbits and then give them pneumococcal meningitis, you get absolutely no inflammatory response in the CSF. You get no brain edema, the pressure doesn't go up and in fact there is almost no damage done to the brain itself. It calls into focus, I think, the role of the poly in a destructive process and the potential way of blocking this with monoclonals against these adhesion molecules.

Hazzard (Winston-Salem): Two other clinical conditions I'd be interested in. One would be metastasis and the other would be malnutrition. Can you comment on these adhesion molecules under these circumstances?

Root: I can't comment on them since most of my interest is focused on the neutrophil adhesion molecule and how they function as far as neutrophils are concerned. Neutrophils are probably not involved in the metastatic process. On the other hand, it seems quite likely that the other members of the endocrine family could be involved in metastasis to other sites, adherence to matrix proteins, for example, and other sites. Perhaps, in the case of lymphocytic malignancies, the NFA 1 molecule could metastasize to sites which bear its ligand; namely, the ICAM molecule. I think this is a subject for speculation and certainly could be tested experimentally.

Krevans (San Francisco): I think it is very appropriate for Dr. Allen to make the last comment since it allows me again to express my own gratitude and the Association's to Jim and Claire for what they do for this Association.

Allen (Charleston): I'm sure I'm getting this dispensation from the president because I

have his hotel bill in my pocket for remuneration. Dick, I'm interested if there have been any studies of the time course of up-regulation of the endothelial ligand and how that may relate to the very early phase of stickiness which we see in the face of infection.

Root: Again, the group that's done the most work in this is Michael Gimbrone's group in Boston. They specifically described this endothelial leukocyte adhesion molecule. It is expressed very early, within minutes after exposure of cells to any number of stimuli including PMA itself, tumor necrosis factor, and IL-1 just as an example of the range of stimuli that bring up the ligand as well as what the cell adheres to.