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The Leukocyte Integrins

TAKASHI K. KISHIMOTO,* RICHARD S. LARSON, ANGEL L. CORBI, MICHAEL L. DUSTIN, DONALD E. STAUNTON, AND TIMOTHY A. SPRINGER

The Center for Blood Research,
Department of Pathology,
Harvard Medical School,
Boston, Massachusetts 02115

I. Introduction

Cellular adhesion and recognition mechanisms are among the most basic requirements for the evolution of multicellular organisms. During the development of an embryo, cellular adhesion proteins can impart position-specific information which guides cell migration, localization, and the transfer of information between cells. As cells are triggered to differentiate to form tissues or organs, adhesion proteins help to maintain the organization and integrity of the body. The immune system is composed of a network of cells in which cellular recognition mechanisms have been highly specialized. The function of the immune system is to distinguish self from nonself and to eliminate the latter. Two major protein families, the integrin family and the immunoglobulin superfamily, have evolved to guide cell-extracellular matrix and cell-cell interactions for both developmental processes and immune function. The immunoglobulin superfamily, which includes the polymorphic antigen-specific receptors of lymphocytes, has recently been reviewed (Williams and Barclay, 1988). This review will focus on the molecular biology of the leukocyte integrins, LFA-1, Mac-1, and p150,95, and on their role in mediating inflammation.

Three recent developments have underscored the importance of the leukocyte integrins as adhesion receptors of the immune system: (1) The recognition that the leukocyte integrins are evolutionarily related to other integrins, such as the fibronectin receptor and platelet glycoprotein IIb/IIIa, which guide cell localization during embryogenesis and wound healing; the leukocyte integrins provide a similar mechanism in the immune system for guiding leukocyte localization during inflammation. (2) Identification of intercellular adhesion molecule-1 (ICAM-1), a ligand for LFA-1, which is induced during inflammation and may regulate

* Present address: Department of Pathology, Stanford University, School of Medicine, Stanford, California 94305.

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leukocyte migration and localization; this receptor–ligand pair demonstrates the first known interaction between a member of the integrin family (LFA-1) and a member of the immunoglobulin superfamily (ICAM-1). (3) Discovery and characterization of immunodeficiency patients who are genetically deficient in their expression of the leukocyte integrins; leukocytes from these patients fail to mobilize during inflammation, and as a consequence these patients suffer from recurrent life-threatening bacterial and fungal infections.

A. Nomenclature

LFA-1 is an acronym for lymphocyte function-associated antigen-1. Mac-1 is an abbreviation for macrophage antigen-1 and is also called Mo-1, OKM-1, and complement receptor type-3 (CR3). In some reports p150,95 is called complement receptor type-4 (CR4) and Leu M5. The Third International Workshop on Human Leukocyte Differentiation Antigens (McMichael, 1987) has designated the α subunits of LFA-1, Mac-1, and p150,95 to be CD11a, b, and c, respectively, and the common β subunit to be CD18.

The LFA-1, Mac-1, and p150,95 family has been called the LFA-1 family, leukocyte adhesion proteins, LeuCAM, and the leukocyte integrins. The homology of this family to other integrin receptors makes the latter name preferable.

B. Initial Characterization of LFA-1, Mac-1, and p150,95

Mac-1 was first defined by monoclonal antibodies (MAbs) as a marker for myeloid cells (Springer *et al.*, 1979). In contrast, LFA-1 was identified independent of Mac-1 by screening MAbs for the ability to inhibit cytotoxic T lymphocyte (CTL)-mediated killing of tumor cell targets (Davignon *et al.*, 1981a). Further analysis showed that the LFA-1 MAbs prevent the Mg^{2+}-dependent conjugate formation step, rather than the actual killing event (Davignon *et al.*, 1981b). Mac-1 and LFA-1 antigens are both high-molecular-weight $\alpha\beta$ heterodimers (Kürzinger *et al.*, 1981). Detailed immunochemical and physiochemical comparison of the two antigens showed that the α subunits of LFA-1 and Mac-1 are unique, but that the β subunit is identical in both proteins (Trowbridge and Omary, 1981; Kürzinger and Springer, 1982; Sanchez-Madrid *et al.*, 1983b). The remarkable structural similarity of LFA-1 and Mac-1 led to the hypothesis that Mac-1 would also function in adhesion. A function for Mac-1 was discovered when Mac-1 MAbs were found to inhibit Mg^{2+}-dependent binding of the C3bi fragment of complement by mouse and human myeloid cells, thus defining Mac-1 as the complement receptor type-3 (CR3) (Beller *et al.*, 1982; Wright *et al.*, 1983).

Analysis with a β-subunit-specific MAb led to the identification of a third heterodimeric protein, p150,95, which shares the common β subunit (Sanchez-Madrid *et al.*, 1983b).

Expression of all three leukocyte integrins is restricted to immune cells. LFA-1 is expressed by virtually all immune cells (Kürzinger and Springer, 1982; Krensky *et al.*, 1983), with the exception of some tissue macrophages (Kürzinger *et al.*, 1981; Strassmann *et al.*, 1985). Mac-1 has a more limited distribution (Springer and Unkeless, 1984); it is found on monocytes, macrophages, granulocytes, large granular lymphocytes, and immature and CD5^{+} B cells (de la Hera *et al.*, 1988). The p150,95 protein has a similar distribution to Mac-1, although it is also expressed on some activated lymphocytes and is a marker for hairy-cell leukemia (Schwarting *et al.*, 1985; Miller *et al.*, 1986).

C. Leukocyte Adhesion Deficiency (LAD) Disease

Since 1974, a number of investigators have identified a class of immunodeficient patients who suffer from recurrent, life-threatening bacterial and fungal infections and who have neutrophils deficient in chemotaxis and phagocytosis (Anderson and Springer, 1987; Todd and Freyer, 1988; Fischer *et al.*, 1988; Anderson *et al.*, 1988; Kishimoto and Springer, 1988). Infected, necrotic lesions in these patients contain few leukocytes, despite the observation that these patients have chronic leukocytosis. One of the earliest reports described a possible actin dysfunction (Boxer *et al.*, 1974). However, Crowley *et al.* (1980) showed that neutrophils from these patients were deficient in a high-molecular-weight surface protein. They further proposed that defects in chemotaxis and phagocytosis were secondary to a defect in adhesion. In 1984, several groups used MAbs to demonstrate that the missing glycoprotein was actually the LFA-1, Mac-1, and p150,95 complex (Anderson *et al.*, 1984; Springer *et al.*, 1984; Beatty *et al.*, 1984; Dana *et al.*, 1984). In every case studied, expression of all three leukocyte integrins was found to be deficient. More recently, we have shown that LAD is due to heterogenous defects in the common β subunit (Kishimoto *et al.*, 1987b). Although LAD is a rare disease, the analysis of this disease has greatly increased our understanding of the biology of the leukocyte integrins and their role in inflammatory responses.

D. LFA-1, Mac-1, and p150,95 Are Members of the Integrin Family

LFA-1, Mac-1, and p150,95 are evolutionarily related to the integrin receptors that mediate cell adhesion to the extracellular matrix during development and wound healing (Fig. 1). There are three subfamilies

	LFA-1	Mac-1	p150,95	VLA-1	VLA-2	VLA-3	VLA-4	FNR(VLA-5)	VLA-6	VLA-7	IIb/IIIa	VNR
Primary Function	Immune cell adherence			Guiding morphogenesis and wound healing							Guiding morphogenesis and wound healing	
General Distribution	Leukocytes			Broad							Platelets	Broad
Structure	$\alpha_1\beta_1$			$\alpha_1\beta_1$							$\alpha_1\beta_1$	
Common β subunit	Yes			Yes							Yes	
Cleavage of α subunit upon reduction	No	No	No	No	No	Yes	No	Yes	Yes	?	Yes	Yes
Ligands	ICAM-1	IC3b Fibrinogen Factor X	(IC3b)	?	Collagen	Laminin Fibronectin Collagen	?	Fibronectin	Laminin	?	Fibronectin Fibrinogen Vitronectin von Willebrand factor	Vitronectin
Recognition Sequence								Arg-Gly-Asp			Arg-Gly-Asp	Arg-Gly-Asp
Interaction with cytoskeleton								Yes				

FIG. 1. A supergene family of adhesion proteins.

of integrins, each defined by a common β subunit which shares multiple distinct α subunits (Hynes, 1987; Kishimoto *et al.*, 1987a; Ruoslahti and Pierschbacher, 1987). The three β subunits are designated $\beta1$, $\beta2$, and $\beta3$. The $\beta1$ and $\beta3$ subfamilies include receptors for extracellular matrix (ECM) components. The $\beta1$ subunit is shared by at least six VLA antigens (VLA-1–VLA-6) (Hemler, 1988), which include the fibronectin receptor (VLA-5). VLA-3 has fibronectin and laminin-binding activity, while VLA-2 has recently been shown to be identical to platelet glycoprotein IaIIa, a collagen-binding receptor. The $\beta2$ subunit is shared by the leukocyte integrins, LFA-1, Mac-1, and p150,95. The $\beta3$ subunit is shared by the vitronectin receptor and platelet glycoprotein IIbIIIa.

The integrin family is ancient in origin. Homologous structures, termed position-specific (PS) antigens, have been implicated in guiding *Drosophila* development (Bogaert *et al.*, 1987; Wilcox and Leptin, 1985). The structure, function, and primary sequence of the integrins have been highly conserved in evolution. The integrins involved in cell matrix interactions recognize a sequence, Arg-Gly-Asp (RGD), which is embedded in numerous, unrelated matrix components (Ruoslahti and Pierschbacher, 1987). The ECM receptor integrins can show exquisite specificity for one matrix component or broad reactivity to multiple ligands. The leukocyte integrins are the first integrins known to mediate cell–cell interactions. The name integrin denotes that these are membrane receptors that integrate the extracellular environment (extracellular matrix or other cells) with the intracellular cytoskeletal network. The evolutionary and functional significance of LFA-1, Mac-1, and p150,95 as integrin proteins will be discussed.

II. Structural Features of the Leukocyte Integrins

A. Basic Structure

The leukocyte integrins are $\alpha_1\beta_1$ heterodimers (Kürzinger and Springer, 1982), in which the α subunit is noncovalently associated with the β subunit. The α subunits of LFA-1, Mac-1, and p150,95 are 180,000, 170,000, and 150,000 Da, respectively. The α subunits have been shown to be distinct by MAb reactivity, antigen-preclearing studies, and tryptic peptide mapping. In contrast, the β subunit, M_r = 95,000, has been shown to be identical in all three proteins by the same criteria (Trowbridge and Omary, 1981; Kürzinger and Springer, 1982; Sanchez-Madrid *et al.*, 1983b). Deglycosylation of the LFA-1, Mac-1, and p150,95 α subunits and the common β subunit reveal polypeptide backbones of 149,000, 137,000, 132,000, and 78,000 Da, respectively (Miller and Springer, 1987;

Kishimoto *et al.*, 1987b; Sastre *et al.*, 1986). Heterogeneity in the glycosylation of LFA-1 has been reported. N-Linked oligosaccharides of LFA-1 on T cells but not B cells or macrophages are sulfated (Dahms and Hart, 1985). Moreover, sialyation patterns of LFA-1 on B and T cells differ, with LFA-1 on B cells being more acidic (Takeda, 1987). Finally, only a subset of leukocyte integrins on neutrophils contain a lacto-*N*-fucopentaose II oligosaccharide moiety (Skubitz and Snook, 1987). The functional significance of this heterogeneity is unknown.

B. Biosynthesis

The α subunits of LFA-1, Mac-1, and p150,95 and the common β subunit are synthesized as distinct precursors of 165,000, 160,000, 146,000, and 89,000 Da, respectively (Sanchez-Madrid *et al.*, 1983b; Sastre *et al.*, 1986; Miller and Springer, 1987). The newly synthesized precursors contain high-mannose N-linked oligosaccharides (Sastre *et al.*, 1986). Association of the α subunit precursor and the β subunit precursor is required for further processing to complex-type N-linked oligosaccharides (Ho and Springer, 1983; Springer *et al.*, 1984). This oligosaccharide modification occurs in the Golgi apparatus (Kornfeld and Kornfeld, 1985) and is evident by a decrease in the electrophoretic mobility of the mature polypeptide and resistance to endoglycosidase H digestion. The mature proteins are then transported to the cell surface or, in some cases, to intracellular granules (Todd *et al.*, 1984; Miller *et al.*, 1987; Bainton *et al.*, 1987).

C. Primary Structure

1. β *Subunit Structure*

a. β *Subunit cDNA*. The cDNA encoding the common β subunit was isolated and characterized by us (Kishimoto *et al.*, 1987a) and independently by Law *et al.* (1987). The deduced 769-amino acid sequence (Fig. 2) has the characteristic features of an integral membrane protein, with a 677-amino acid extracellular domain containing six potential N-glycosylation sites, a 23-amino acid transmembrane domain, and a 46-amino acid cytoplasmic domain. A striking feature of the β subunit is the high cysteine content (7.4% overall). A cysteine-rich (20%) region of 186-amino acid contains a fourfold repeat of an unusual cysteine motif. The high cysteine content is predicted to give the β subunit a very rigid tertiary structure.

Northern blot analysis, Southern blot analysis, and peptide sequence data from the β subunit isolated independently from purified LFA-1, Mac-1, and p150,95 confirm immunochemical evidence that a single

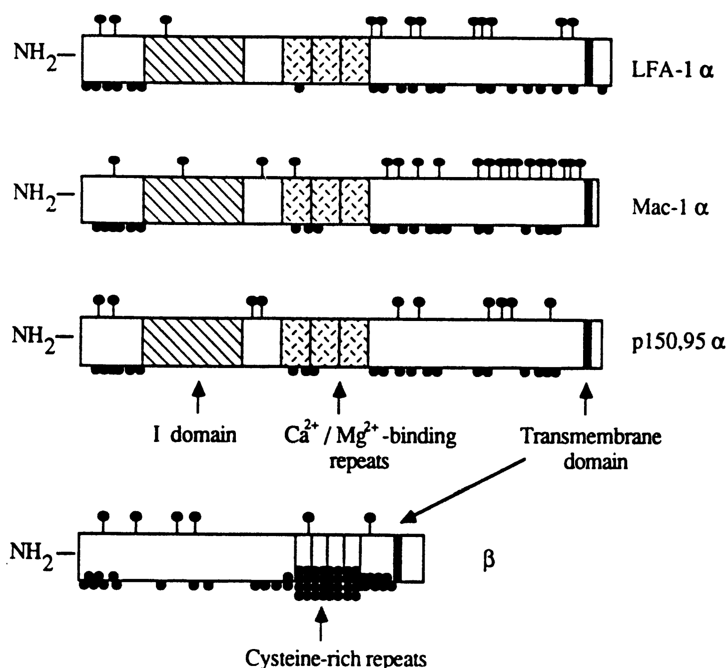

FIG. 2. Schematic representation of primary structure of the LFA-1, Mac-1, and p150,95 α subunits and common β subunit. Black lollipops and circles represent N-linked glycosylation and cysteines, respectively.

gene encodes the β subunit of all three leukocyte integrins (Kishimoto *et al.*, 1987a).

b. Homology to Other Integrin β Subunits. The three β subunits, which define the three integrin subfamilies, share 37–45% amino acid identity, with particularly high conservation of the cytoplasmic domain, the transmembrane domain, and a stretch of 241 amino acids in the extracellular domain (70, 47, and 64%, respectively) (Tamkun *et al.*, 1986; Kishimoto *et al.*, 1987a; Law *et al.*, 1987; Fitzgerald *et al.*, 1987). All 56 cysteine residues are conserved in each of the three β subunits, including the fourfold repeat of the unusual cysteine motif, first described for the chick β1 subunit (Tamkun *et al.*, 1986). This high cysteine content probably gives the β subunits similar tertiary structures and may account for the observed differences in mobility in reducing versus nonreducing gels (Leptin, 1986; Ruoslahti and Pierschbacher, 1987). Interestingly, β1 (Tamkun *et al.*, 1986) and β3 (Fitzgerald *et al.*, 1987) have a consensus tyrosine phosphorylation sequence in the cytoplasmic domain,

which is not found in the leukocyte $\beta 2$ subunit (Kishimoto *et al.*, 1987a; Law *et al.*, 1987).

2. α Subunit Structures

a. α Subunit cDNAs. cDNAs encoding the α subunits of p150,95 (Corbi *et al.*, 1987), Mac-1 (Corbi *et al.*, 1988b; Pytela, 1988; Arnaout *et al.*, 1988), and more recently LFA-1 (Larson *et al.*, 1989) have been cloned and characterized. The deduced amino acid sequences (Fig. 2) define homologous integral membrane proteins with a long extracellular domain (αX, 1081 amino acids; αM, 1092 amino acids; αL, 1063 amino acids), a hydrophobic transmembrane domain (αX, 26 amino acids; αM, 26 amino acids; αL, 29 amino acids), and a short cytoplasmic domain (αX, 29 amino acids; αM, 19 amino acids; αL, 53 amino acids). Although the polypeptide backbones are of similar size, the differences in apparent molecular weight of the mature polypeptides may be due, in part, to differences in the number of potential N-glycosylation sites (αX, 10 potential sites; αM, 19 potential sites; αL, 12 potential sites).

A striking feature of the α subunits is that each contains three homologous repeats that have putative divalent cation-binding sites which are similar to the Ca^{2+}-binding "EF-hand loop" sequences of calmodulin, troponin C, and parvalbumin. These putative metal-binding sites may account for the Mg^{2+} dependency of leukocyte integrin-mediated adhesion. The concept that exogenous divalent cations stabilize the interaction of integrin α and β chains (Jennings and Phillips, 1982) has been used in the immunopurification of leukocyte integrins in functional form (Dustin and Springer, 1989; S. A. Stacker, M. S. Diamond, and T. A. Springer, unpublished). The stabilizing effect of Mg^{2+} and Ca^{2+} on leukocyte integrins is the most direct evidence for binding of divalent cations by these heterodimers.

The Mac-1 and p150,95 α subunits share 63% amino acid identity with each other but only 35% identity with the LFA-1 α subunit. The transmembrane domain and the three homologous repeats containing putative divalent cation-binding sites are the most highly conserved regions (88 and 87% amino acid identity, respectively, between Mac-1 and p150,95).

b. Homology to the Extracellular Matrix Receptor Integrins. The α subunits of the three subfamilies of integrins share 25-63% amino acid identity (Corbi *et al.*, 1987, 1988b; Pytela, 1988; Arnaout *et al.*, 1988; Larson *et al.*, 1989; Argraves *et al.*, 1987b; Poncz *et al.*, 1987; Suzuki *et al.*, 1987). The evolutionary relationships among integrin α subunits can be assessed by percent amino acid sequence identity (Fig. 3). The integrins may be divided into two functional groups, those which bind extracellular matrix ligands (ECM receptors) and those involved in cell-cell

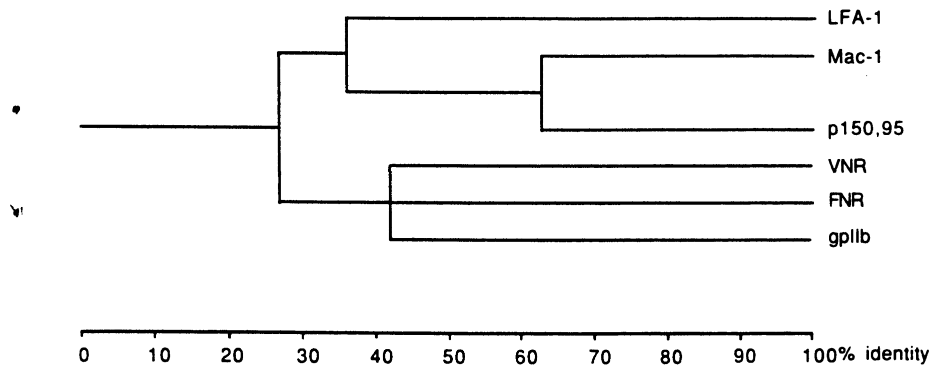

FIG. 3. Evolutionary relationship of integrin α subunits. A pathway of evolution is suggested by the percentage identity among α subunits, indicated by the scale above.

interactions and expressed on leukocytes (the leukocyte integrins). The ECM receptor integrins utilize both $\beta 1$ and $\beta 3$ subunits whereas the leukocyte integrins use the $\beta 2$ subunit. The α subunits of the leukocyte integrins are more similar to each other ($\bar{x}$ = 47% identity) than to those of the ECM receptor integrins ($\bar{x}$ = 27% identity). Moreover, the ECM receptor integrin α subunits are more related to one another ($\bar{x}$ = 42% identity) than to the leukocyte integrins. The α subunits of the ECM integrins, VLA-3, VLA-5 (fibronectin receptor), vitronectin receptor, and glycoprotein IIb/IIIa (gpIIb/IIIa) share a sequence in the extracellular domain which is posttranslationally cleaved, with resulting fragments bridged by a disulfide bond (Ruoslahti and Pierschbacher, 1987). This sequence is not found in the α subunits of leukocyte integrins (Corbi *et al.*, 1987, 1988b; Pytela, 1988; Arnaout *et al.*, 1988; Larson *et al.*, 1989). This explains the increased electrophoretic mobility in reducing gels observed for the α subunits of the ECM receptor integrins but not the leukocyte integrins (Ruoslahti and Pierschbacher, 1987). The regions of highest conservation are the transmembrane domain and the putative metal-binding domains. Like leukocyte integrins, the ECM receptor integrins are dependent upon divalent cations for activity; however, the metal specificity is for Ca^{2+}, Mg^{2+}, or Mn^{2+}. Radioactive calcium has been shown to bind directly to the α subunit of gpIIb/IIIa (Fujimura and Phillips, 1983) and the fibronectin receptor (Gailit and Ruoslahti, 1988).

c. I Domain. All three leukocyte integrin α subunits have a 200-amino acid segment in the extracellular domain which has a counterpart in only one of five known ECM receptor integrin α subunit sequences (Takada and Hemler, 1989). This domain, termed the I domain for

inserted/interactive domain, is homologous to the three A domains of von Willebrand factor (vWF), to a domain in the complement cascade proteins C2 and factor B, and to two domains in the cartilage matrix protein (CMP) (Corbi *et al.*, 1988b; Arnaout *et al.*, 1988; Larson *et al.*, 1989; Pytela, 1988).

The relationships among the α subunits suggest the evolutionary scheme depicted in Fig. 3. A primordial α integrin gene duplicated and gave rise to at least two branches of integrin α subunits, the leukocyte integrins and the ECM receptor integrins. The primordial leukocyte integrin α subunit gene, containing an I domain, then duplicated and gave rise to LFA-1 and Mac-1/p150,95 primordial α subunit genes. Further duplication of the Mac-1/p150,95 primordial gene gave rise to the Mac-1 and p150,95 α subunits.

d. Expression. As a first step to uncover the structural basis for the adhesive activities displayed by LFA-1, Mac-1, and p150,95, their corresponding cDNAs (α and β subunits) have been inserted in the vector CDM8 (Seed, 1987) to obtain transient expression of the three heterodimers. After DEAE-dextran mediated cotransfection of α and β cDNAs, LFA-1, Mac-1, and p150,95 α/β complexes have been detected on the surface of COS cells by immunofluorescence (Larson *et al.*, 1990). Immunoprecipitation studies have demonstrated that the α and β subunits of LFA-1, Mac-1, and p150,95 are noncovalently associated. Moreover, the LFA-1 and Mac-1 heterodimers expressed by the transfected cells were functional. COS cells expressing LFA-1 bound to purified ICAM-1 absorbed to plastic, and this binding was inhibited by anti-LFA-1, anti-ICAM-1, and EDTA (R. S. Larson and T. A. Springer, unpublished). COS cells expressing Mac-1 specifically bound to erythrocytes sensitized with human or mouse C3bi, and this was inhibited with anti-Mac-1α OKM10 MAb and anti-β 60.3 MAb (A. L. Corbi and T. A. Springer, unpublished).

D. Relation of Structure to Ligand Binding

The three-dimensional structure of the leukocyte integrins and the individual contributions of the α and β subunits to ligand binding remain to be determined. The ECM receptor integrins recognize the RGD sequence and related sequences such as KQAGD within their ligands (Ruoslahti and Pierschbacher, 1986; Ruggeri *et al.*, 1986). Because the integrins within a given subfamily share the same β subunit but have distinct ligand-binding specificity, it seems reasonable to propose that the α subunits impart this specificity. The α subunits may influence recognition by changing the conformation of the β subunit to recognize different conformations of the RGD sequence present in different matrix proteins, or by binding to further sites on the matrix protein. Complementation studies with fragments of fibronectin suggest that a second site distinct

from RGD is required for binding to the fibronectin receptor (Obara *et al.*, 1988).

Chemical cross-linking studies show that although radiolabeled RGDS tetrapeptide binds predominately to a highly conserved region of the β subunit of gpIIb/IIIa, there is significant labeling of the α subunit as well (D'Souza *et al.*, 1988). The RGD binding site of the β subunit maps to the N-terminal portion of a highly conserved 241-amino acid segment of the extracellular domain. The RGD binding site of the α subunit has not been determined. One model is that divalent cations held in the metal-binding domains of the α subunits may help to stabilize interaction with the RGD sequence (Corbi *et al.*, 1987). X-Ray crystallography of the Ca^{2+}-binding EF-hand loops shows that amino acids with oxygen-containing side groups form the coordination axes for ligating the metal (Szebenyi *et al.*, 1981). The putative metal-binding domains of all integrins differ from these classical EF-hand loops in that a glutamic acid in the $-Z$ position of the coordination axes is missing from the former. This may leave the metal free to coordinate in the $-Z$ position with a residue on the ligand. It is tempting to speculate that the metal bound to the receptor may coordinate with the aspartic acid (D) of the RGD recognition sequence. This sequence appears suited for metal binding because GD and DG sequences appear frequently both in the EF-hand loop and in the metal-binding domains of the integrins.

The I domain appears as a functional unit in diverse proteins. The A1 domain of vWF binds glycoprotein Ib and heparin, while both A1 and A3 domains are involved in binding collagen (Girma *et al.*, 1987). Partial sequence data from the cartilage matrix protein reveals two I domainlike repeats separated by an epidermal growth factorlike sequence (Argraves *et al.*, 1987a). The I domainlike region in factor B and C2 is clearly demarcated on the N-terminal side by the cleavage site that gives the active Bb factor, and on the C-terminal side by the serine protease domain (Bently, 1986). Interestingly, both factor B and the homologous protein C2 bind C3b, whereas Mac-1 and p150,95 are receptors for the C3bi fragment of C3. It is tempting to speculate that the I domains of Mac-1 and p150,95 contribute to this specificity. The ECM integrins which recognize RGD do not contain I domains. The I domain may impart distinctive and additional recognition specificities to leukocyte integrins.

III. Chromosomal Localization

A. β Subunit Gene on Chromosome 21

The gene encoding the common β subunit was first mapped to chromosome 21 by gene complementation in somatic cell hybrids (Marlin *et al.*, 1986; Suomalainen *et al.*, 1983; Akao *et al.*, 1987). With the

availability of the β subunit cDNA, the gene has been further localized to band 21q22 by *in situ* hybridization to metaphase chromosomes (Corbi *et al.*, 1988a). More recently, the β subunit has been shown to be the most distal marker known on the long arm of chromosome 21 (21q22.3) by hybridization to a panel of chromosome 21 deletion mutants and linkage of restriction fragment length polymorphisms (Gardner *et al.*, 1988). The β subunit gene may be a very useful marker for analysis of trisomy 21 in Down's syndrome. In addition, band 21q22 has been identified as a breakpoint in chromosomal translocations [t(3;21)(q25;q22)] associated with the blast phase of chronic myelogenous leukemia (CML) (Rubin *et al.*, 1987). Hematopoietic progenitor cells in CML show abnormal adhesive interactions with bone marrow stroma (Gordan *et al.*, 1987). Further studies are required to determine if the β subunit gene is involved in this translocation and contributes to the progression of CML.

B. α Subunit Gene Cluster on Chromosome 16

The LFA-1 α subunit gene has been mapped to chromosome 16 by gene complementation in somatic cell hybrids (Marlin *et al.*, 1986). Subsequent Southern blot analysis of DNA from LFA-1 α hybrid cells containing human chromosome 16 has shown that the genes encoding Mac-1 and p150,95 are on the same chromosome (Corbi *et al.*, 1988a). Furthermore, *in situ* hybridization data show that all three α subunit genes are clustered between bands p11–p13.1 on chromosome 16, defining a gene cluster involved in cell adhesion (Corbi *et al.*, 1988a). The close proximity of the α subunit genes provides further support for their evolution by gene duplication.

Inversions [inv(16)(p13q22)] and translocations [t(16;16)(p13;q22)] involving this region of chromosome 16 are frequently observed in patients with acute myelomonocytic leukemia (Le Beau *et al.*, 1985). Further investigation is required to determine if the genes encoding the leukocyte integrin α subunits are involved in these rearrangements.

IV. The Leukocyte Integrins in Inflammation

A. The Role of Leukocytes in the Inflammatory Process

The acute inflammatory reaction provides a rapid host defense response to contain and eliminate infectious agents in extravascular tissues. The peripheral blood leukocytes constitute the recruitable force which infiltrates the infected tissues. Neutrophils and monocytes migrate in response to chemotactic factors released at the infection site. Chemotactic stimulation causes leukocyte cell polarization, granule release, and increased adhesiveness. Diapedesis requires the ability of leukocytes to

bind vascular endothelial cells, cross the basement membrane, and enter the infected tissues. Regulation of the inflammatory response must be exquisite, so that leukocytes only enter the infected area and do not damage healthy tissues. Until recently, the molecular mechanisms which regulate and mediate leukocyte extravasation have been largely unknown. The role of the leukocyte integrins in this process has been elucidated by the characterization of human leukocyte adhesion deficiency disease.

B. Insights from the Study of Leukocyte Adhesion Deficiency

The clinical hallmarks of LAD are recurrent necrotic and indolent infections of soft tissues, such as the skin, mucous membranes, and intestinal tract (Anderson and Springer, 1987). The infectious microbes include a wide spectrum of fungi and bacteria, but most commonly staphylococcal or gram-negative enteric bacteria. A key feature of LAD is that infected skin lesions are largely devoid of granulocytes, despite chronic peripheral blood leukocytosis (5-20 times normal levels). This failure of leukocytes to mobilize is also observed in Rebuck skin window assays. Transfused normal leukocytes are capable of mobilization to the infected tissues (Bowen *et al.*, 1982). Adhesion-independent responses, such as cell polarization in response to chemotactic factors, fMLP-binding, and respiratory burst from soluble stimuli, are normal. These observations demonstrate a direct involvement of the leukocyte integrins in the extravasation of leukocytes during an inflammatory response.

In vitro, neutrophils and monocytes from LAD patients show profound defects in adhesion-related functions (Anderson and Springer, 1987). Mac-1 and p150,95 are deficient both on the cell surface and in intracellular granules. Chemoattractants induce granule mobilization, but not up-regulation of leukocyte integrin expression or homotypic cell aggregation. Neutrophils and monocytes fail to adhere and spread on artificial substrates, such as glass and plastic, and on endothelial cell monolayers. As a consequence, leukocytes have impaired directed motility in response to chemoattractants. C3bi-coated particles fail to induce phagocytosis or subsequent respiratory burst. Antibody-dependent cellular cytotoxicity mediated by granulocytes and monocytes is also abnormal (Kohl *et al.*, 1984, 1986).

C. Functional Studies

1. *LFA-1 Function*

LFA-1 serves to mediate cellular adhesion events in a wide spectrum of both antigen-dependent and antigen-independent interactions of immune cells (Springer *et al.*, 1987; Martz, 1986). The role of LFA-1 in conjugate formation during CTL- and NK-mediated cytolysis has been

extensively reviewed (Springer *et al.*, 1987; Martz, 1986) and will not be discussed here. Lymphocyte localization to lymphoid organs, sites of inflammation, and grafts is dependent upon specific interaction with the vascular endothelium. T lymphocyte adherence to endothelial cells (Mentzer *et al.*, 1986b; Haskard *et al.*, 1986; Dustin and Springer, 1988), fibroblasts (Dustin *et al.*, 1986), epidermal keratinocytes (Dustin *et al.*, 1988), synovial cells (Mentzer *et al.*, 1988), and hepatocytes (Roos and Roossien, 1987) is inhibitable partially or totally by LFA-1 MAb. Typically adherence is greatly increased by stimulation of the nonhematopoietic cells with cytokines such as TNF, LPS, IFN-γ, and IL-1 and by activation of the lymphocytes.

T lymphocyte and lymphoblast adherence to cultured endothelial cells has both LFA-1-dependent and LFA-1-independent components (Haskard *et al.*, 1986; Dustin and Springer, 1988). Both pathways are increased upon stimulation of the endothelial monolayer with cytokines. LFA-1$^-$ lymphoblasts from LAD patients exhibit the LFA-1-independent pathway, but not the LFA-1-dependent pathway (Dustin and Springer, 1988). The impaired primary responses of LAD lymphocytes to specific antigen, but near-normal secondary responses (Krensky *et al.*, 1985; Mentzer *et al.*, 1986a), may reflect selective involvement of LFA-1-independent and LFA-1-dependent pathways. LAD patients display essentially normal delayed-type hypersensitivity responses. Inflammatory tissues, which are devoid of granulocytes, do contain lymphocytes. The nature of the LFA-1-independent pathway of adhesion to endothelium is unknown, but does not appear to be the CD2/LFA-3 pathway. In the case of lymphocyte binding to high endothelial venules of lymphoid organs, adherence is partially inhibitable by LFA-1 MAb (Hamann *et al.*, 1988); however, organ specificity is mediated by lymphocyte homing receptors (Butcher, 1986).

An *in vitro* model of LFA-1-dependent adhesion is the homotypic aggregation of lymphocytes and lymphoid cell lines in response to phorbol ester stimulation (Patarroyo *et al.*, 1985; Mentzer *et al.*, 1985; Rothlein and Springer, 1986). Time-lapse videomicroscopy (Rothlein and Springer, 1986) shows that phorbol ester-activated peripheral blood lymphocytes are motile and show uropod formation and extensive membrane ruffling. Contact between pseudopodia of adjacent cells leads to adhesion, followed by mass aggregation. This aggregation event is both inhibitable and reversible by LFA-1 MAbs. Moreover, cells from patients who are genetically deficient in LFA-1 expression do not aggregate (Rothlein and Springer, 1986). Although phorbol ester-induced aggregation is an *in vitro* phenomenon, it has been a useful model to study the cell biology of LFA-1 function and probably correlates with adhesion events *in vivo*.

The requirements for aggregation are remarkably similar to the adhesion phase of CTL conjugate formation. Both events are Mg^{2+}, energy, and temperature dependent and require an intact cytoskeleton (Martz, 1977; Patarroyo *et al.*, 1983; Rothlein and Springer, 1986). Aggregation-like cluster formation is also observed shortly after T helper cell activation by antigen presentation (Werdelin, 1980). Homotypic aggregation of activated lymphocytes may be an important step in extravasation of lymphocytes.

Improved isolation procedures have allowed functional studies on purified LFA-1 (Dustin and Springer, 1989). LFA-1 purified in the presence of Mg^{2+} remains associated in an $\alpha\beta$ complex and is functional because it mediates adhesion of several human cell lines when reconstituted into planar membranes or absorbed directly to plastic. This binding is blocked by pretreatment of cells with ICAM-1 MAbs, pretreatment of the monolayer with LFA-1 MAbs, and removal of divalent cations. The major difference between LFA-1-ICAM-1 mediated cell–cell adhesion and adhesion of cells to purified LFA-1 is that the former requires high temperature and metabolic activity, while the latter does not. In contrast, binding of cells to ICAM-1 monolayers is temperature and energy dependent (Marlin and Springer, 1987). This suggests that the energy and temperature requirements are met on the LFA-1-bearing cell side of the interaction, consistent with cellular regulation of LFA-1 avidity for its ligand.

2. *Mac-1 Function*

A functional role for Mac-1 was first demonstrated by the ability of Mac-1 MAbs to inhibit monocyte and granulocyte binding of C3bi-coated erythrocytes (Beller *et al.*, 1982). Thus Mac-1 is equivalent to the complement receptor type-3. Mac-1 can mediate phagocytosis and lysis of C3bi-coated erythrocytes (Rothlein and Springer, 1985) and contributes to elevated natural killer (NK) activity against C3bi-coated target cells (Ramos *et al.*, 1988). Mac-1 has also been implicated in the ability of macrophages to bind *Leishmania* promastigotes (Mosser and Edelson, 1985; Russell and Wright, 1988), *Escherichia coli* (Wright and Jong, 1986), and *Histoplasma capsulatum* (Bullock and Wright, 1987).

Recent evidence suggests that Mac-1, like LFA-1, may play a more general role in mediating adhesive interactions of myeloid cells. Activated neutrophils, like phorbol ester-stimulated lymphocytes, form homotypic aggregates *in vitro*. Neutrophil aggregation is inhibitable by Mac-1 MAbs, and not by LFA-1 MAbs (Anderson *et al.*, 1986). Neutrophil and monocyte chemotaxis (Anderson *et al.*, 1986; Dana *et al.*, 1986) and adherence to glass and plastic (Anderson *et al.*, 1986; Dana *et al.*, 1986)

and to endothelial (Wallis *et al.*, 1986) and epithelial (Simon *et al.*, 1986) monolayers also involve Mac-1. Differential MAb blocking of CR3 activity and general adhesion suggest that Mac-1 may be a multifunctional receptor (Anderson *et al.*, 1986; Dana *et al.*, 1986).

3. *p150,95 Function*

A role for p150,95 as a complement receptor was first discovered when both Mac-1 and p150,95 were found to elute specifically from a C3bi affinity column (Micklem and Sim, 1985; Malhotra *et al.*, 1986). The physiological relevance of this finding was tested with p150,95 on intact cells. The contribution of p150,95 to C3bi binding by neutrophils and monocytes could only be assessed after antibody blockade of CR1 and Mac-1, which are expressed in 10-fold excess of p150,95. The remaining C3bi-binding activity on these antibody-treated cells could be blocked with the p150,95 MAbs. These results suggest that p150,95, like Mac-1, may have some C3bi-binding activity, and it has thus been designated as CR4 (Myones *et al.*, 1988). However, p150,95 expressed in COS cells does not bind C3bi (J. Garcia-Aguilar, A. L. Corbi, and T. A. Springer, unpublished).

p150,95, like Mac-1, probably has a broad role as a general adhesion protein. Anderson *et al.* (1986) showed that p150,95 MAbs could partially inhibit the adhesion of neutrophils to substrates. However, Mac-1 appears to have a more important role. In contrast, Figdor and his colleagues reported that p150,95 is a major component of peripheral blood monocyte adhesion to substrates and endothelial cells, phagocytosis of latex particles, and chemotaxis (Keizer *et al.*, 1987b; te Velde *et al.*, 1987). These results were somewhat unexpected, because p150,95 is expressed only at low levels on blood monocytes but at high levels on tissue macrophages (Schwarting *et al.*, 1985).

Recently p150,95 expression has been reported on some activated lymphocytes and lymphocytic cell lines (Miller *et al.*, 1986; Keizer *et al.*, 1987a). The p150,95 MAb was found to inhibit conjugate formation by cytotoxic T lymphocytes (CTL) expressing comparable amounts of p150,95 and LFA-1 equally as well as LFA-1 MAbs (Keizer *et al.*, 1987a). The inhibitory effects of the p150,95 MAb and LFA-1 MAb are additive. One group (Lanier *et al.*, 1985) reported no effect of the p150,95 MAb on CTL activity. This discrepancy may reflect differences in p150,95 expression by CTL clones.

D. Functional Redundancy among the Leukocyte Integrins

All three leukocyte integrins appear to function as general adhesion proteins for immune cell function. MAbs directed to the α subunits have provided powerful tools to dissect the functions of the individual proteins.

While the relative importance of LFA-1, Mac-1, and p150,95 may vary with different systems, there is clearly some redundancy, particularly with neutrophil and monocyte functions. Comparative studies have shown that all three leukocyte integrins contribute to neutrophil and monocyte adhesion to endothelial cells and artificial substrates (Anderson *et al.*, 1986; te Velde *et al.*, 1987). These results are consistent with the finding that MAbs to the common β subunit are often the most potent inhibitors of adhesion-related functions. Moreover, none of the leukocyte adhesion deficiency patients examined to date have been found to have a selective deficiency in the expression of only one of the leukocyte integrins (reviewed in Anderson and Springer, 1987), suggesting that the immune system might be able to compensate for the loss of one but not all three proteins.

E. Animal Models

Whereas the study of human LAD has provided tremendous insight into the role of the leukocyte integrins in inflammation, the next stage of investigation—experimental manipulation *in vivo*—requires a suitable animal model. One possibility is a canine LAD model (Giger *et al.*, 1987), which appears completely analogous to human LAD. A serious limitation is the difficulty and expense of maintaining a stable colony.

A second approach has been to mimic the LAD state by *in vivo* administration of MAbs directed against the leukocyte integrins. An anti-β subunit MAb given intravenously to rabbits inhibits leukocyte extravasation and leukocyte-dependent plasma leakage in response to intradermal administration of chemotactic agents fMLP, leukotriene B4, and C5a (Arfors *et al.*, 1987). Intravital microscopy of the rabbit tenuissimus muscle revealed that the MAb inhibited adherence of leukocytes to the vascular endothelium but not rolling of leukocytes along the venule wall (Arfors *et al.*, 1987). In another system, mice given an intravenous injection of Mac-1 MAb fail to mobilize monocytes in response to thioglycolate-induced inflammation of the peritoneum (Rosen and Gordon, 1987). These results suggest that of the three leukocyte integrins, Mac-1 may play the major role in myeloid cell extravasation.

F. Potential Therapeutic Value of Leukocyte Integrin MAb

Inflammation is an integral part of the host defense to infection and injury of extravascular tissue. However, an inappropriate or uncontrolled inflammatory response may contribute to the pathogenesis of chronic disease states, such as arthritis, and acute ischemic shock followed by reperfusion. Leukocytes are thought to be a major culprit in promoting tissue damage following ischemia-reperfusion injury, perhaps by generating reactive oxygen metabolites, proteases, and phospholipases. Animals depleted of peripheral blood leukocytes show significantly reduced damage from myocardial ischemia and reperfusion.

Two recent studies have investigated the use of leukocyte integrin MAbs to reduce ischemia-reperfusion injury. In a dog model of myocardial infarction, arterial flow is interrupted, then reestablished. Reperfusion injury, measured as infarct size as a percentage of area at risk, was reduced twofold by *in vivo* administration of Mac-1 MAbs (Simpson *et al.*, 1988). Histological studies show that Mac-1 MAb-treated dogs had fewer neutrophils in the myocardium. A rabbit model of hemorrhagic shock and resuscitation showed similar protective effects of an anti-β subunit MAb against liver and gastrointestinal injury but not lung injury (Vedder and Harlan, 1988). All MAb-treated animals survived 5 days, compared to a 29% survival rate among control animals. These results suggest potential therapeutic value for leukocyte integrin MAbs in controlling tissue and organ injury following myocardial infarction, hemorrhagic shock, and other trauma that cause ischemia and are followed by reestablishment of normal circulatory flow. Of particular relevance is treatment of myocardial infarction with streptokinase or tissue plasminogen activator (TPA), both of which dissolve clots and allow circulation to be reestablished in affected areas of the heart. Administration of leukocyte integrin MAbs or ICAM-1 MAbs together with anticlotting agents promises to significantly reduce the amount of damaged cardiac tissue.

G. Regulation of Expression and Functional Activity

1. De novo Synthesis

Cell-surface leukocyte integrin expression is regulated both by *de novo* biosynthesis and by up-regulation of preformed material. Change in biosynthetic rate is a relatively slow and inefficient means of responding to rapid changes in the environment, such as in an infection, and is primarily associated with immune cell differentiation. LFA-1 is expressed during the differentiation of hematopoietic stem cells. In the B cell and myeloid lineages, LFA-1 expression is first detected in the cytoplasmic μ-chain-positive pre-B cells and late myeloblasts, respectively (Campana *et al.*, 1986). Mac-1 expression is associated with committed granulocyte and monocyte precursors in the bone marrow (Miller *et al.*, 1985). These observations concur with the *in vitro* differentiation of the promyeloblastic cell line HL-60. Undifferentiated HL-60 expresses only LFA-1. Differentiation to the monocytic lineage, induced with phorbol esters, or to the granulocytic lineage, induced with retinoic acid, results in expression of both Mac-1 and p150,95 (Miller *et al.*, 1986; Corbi *et al.*, 1987, 1988b). Peripheral blood monocytes express high levels of Mac-1 and low levels of p150,95. However, upon extravasation and maturation

to tissue macrophages, the pattern of expression is reversed to low levels of Mac-1 and high levels of p150,95 (Schwarting *et al.*, 1985). Work in the murine system shows that many peripheral monocytes lose expression of LFA-1 after differentiation to tissue macrophages (Kürzinger and Springer, 1982; Strassmann *et al.*, 1985). These results suggest some role for p150,95 in tissue macrophage function.

2. *Mobilization of an Intracellular Pool*

In contrast to the slow time course for up-regulation by *de novo* synthesis, Mac-1 (Todd *et al.*, 1984; Berger *et al.*, 1984; Springer *et al.*, 1984; Miller *et al.*, 1987a) and p150,95 (Lanier *et al.*, 1985; Springer *et al.*, 1986; Miller *et al.*, 1987) expression on phagocytic cells can be dramatically up-regulated in a matter of minutes. Most of the Mac-1 and p150,95 proteins are stored in intracellular pools of neutrophils (Todd *et al.*, 1984; Miller *et al.*, 1987; Bainton *et al.* 1987) and monocytes (Miller *et al.*, 1987). Electron microscopy shows that leukocyte integrins are associated with peroxidase-negative granules (Miller *et al.*, 1987; Bainton *et al.*, 1987). This latent pool is mobilized in response to chemotactic factors, including fMLP, C5a, and leukotriene B4, and results in up to a 10-fold increase in surface expression of Mac-1 and p150,95. LFA-1 is up-regulated twofold in monocytes and none in neutrophils. Up-regulation presumably aids in the rapid mobilization of monocytes and neutrophils to inflammatory sites.

3. *Receptor Activation*

The regulation of the functional activity of leukocyte integrins is more complex than changing the gross level of surface expression. Peripheral blood lymphocytes express LFA-1, but do not spontaneously adhere to each other. Phorbol ester stimulation induces a rapid homotypic aggregation event. Aggregation is inhibitable by MAbs against LFA-1 (Patarroyo *et al.*, 1985; Mentzer *et al.*, 1985; Rothlein and Springer, 1986) and ICAM-1, an LFA-1 ligand (Rothlein *et al.*, 1986). Aggregation is not associated with quantitative changes in the surface expression of either LFA-1 or ICAM-1. These results suggest that cell activation induces some change in either LFA-1 or ICAM-1 molecules.

Mac-1 functional activity appears to be similarly regulated on neutrophils. Neutrophils also form homotypic aggregates upon stimulation. Buyon *et al.* (1988) were able to dissociate up-regulation of the latent pool of Mac-1 from the homotypic aggregation event. Mac-1 MAbs can effectively block neutrophil aggregation. Freshly isolated neutrophils were pretreated with Mac-1 MAbs to coat surface Mac-1, washed, and then activated. Precoating the surface Mac-1 did not affect up-regulation

and expression of the latent pool; however, it did effectively inhibit neutrophil aggregation. Furthermore, up-regulation and aggregation were dissociated kinetically and pharmacologically. In contrast, Vedder and Harlan (1988) found that treating neutrophils with an anion channel-blocking agent, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), inhibited both Mac-1 up-regulation and neutrophil aggregation. Interestingly, DIDS did not inhibit Mac-1-dependent binding of neutrophils to endothelial cells.

Phorbol esters have a biphasic effect on the C3bi-binding activity of Mac-1 that is independent of up-regulation (Wright and Meyer, 1986). First, a rapid increase in C3bi-binding activity is observed. However, after 1 hour, binding activity is reduced below resting levels, despite the increased expression of Mac-1. Interferon-γ depressed C3bi-binding activity without affecting Mac-1 expression. Binding activity is restored by adherence to fibronectin-coated surfaces (Wright *et al.*, 1986). These results suggest that qualitative changes in Mac-1 are important for functional activity. The quantitative changes as a result of up-regulation of intracellular pools may augment the response.

a. Activation Epitopes. The molecular mechanisms which regulate functional activity are not clear. The simplest explanation is a conformational change which unmasks the ligand binding site. However most MAbs that block adhesion of activated cells also bind to leukocyte integrins on resting cells. One MAb against a novel epitope of LFA-1 has been shown to induce homotypic aggregation of lymphoid cells, which is inhibitable by other LFA-1 MAbs (Keizer *et al.*, 1988). This and several other MAbs appear to define activation epitopes on LFA-1 (Pircher *et al.*, 1986; Morimoto *et al.*, 1987).

b. Interaction with Other Receptors. Interaction of the leukocyte integrins with other membrane proteins may also influence binding activity. Pytowski *et al.* (1988) recently raised a MAb against a 157,000-Da cell-surface protein on neutrophils, which specifically inhibits the C3bi-binding activity of Mac-1. The antigen is distinct from the leukocyte integrins and is expressed on cells from LAD patients. Another report suggests that a subset of Mac-1 on monocyte cell surfaces is associated with the Fc receptor and shows no lateral diffusion in the membrane (Brown *et al.*, 1988). Antibody against the nondiffusable Mac-1 blocks IgG-mediated phagocytosis.

c. Receptor Clustering. Binding activity may also be regulated by cross-linking of receptors. Phorbol ester-induced C3bi-binding activity of Mac-1 correlates with aggregation of Mac-1 in small clusters on the cell surface. Prolonged exposure to phorbol myristate acetate (PMA) reverses C3bi-binding activity and results in dispersal of the Mac-1 clusters (Detmers *et al.* 1987).

d. Phosphorylation. Receptor activity is likely to be regulated by intracellular events. Phorbol esters, which induce homotypic aggregation, are known to activate protein kinase C. Phosphorylation of the LFA-1 β subunit is induced by phorbol ester stimulation of peripheral blood lymphocytes (Hara and Fu, 1986). Neutrophils loaded with thiophosphate, which renders phosphorylated proteins resistant to phosphatases, show enhanced C3bi-binding activity upon phorbol ester stimulation. Moreover, thiophosphate-loaded neutrophils do not display the characteristic deactivation of C3bi-binding activity after prolonged exposure to phorbol esters (Wright and Meyer, 1986). These results suggest that phosphorylation may play an important role in regulating leukocyte integrin function.

e. Interaction with the Cytoskeleton. Cell activation may also induce association of the leukocyte integrins with cytoskeletal elements. LFA-1-dependent adhesion is disrupted by cytochalasin B (Rothlein and Springer, 1986). Furthermore, LFA-1 and actin filaments colocalize to the site of contact between NK cells and target cells (Carpen *et al.*, 1986). More interestingly, the fibronectin receptor, an evolutionarily related integrin, has been shown to interact directly with talin, a cytoskeletal protein (Horwitz *et al.*, 1986). LFA-1 and talin also colocalize in activated, but not resting, lymphocytes (Burn *et al.*, 1988), and redistribute to sites of adhesion with specific antigen-bearing cells (Kupfer and Singer, 1988). Redistribution of LFA-1 and talin is one of the earlier events, preceding redistribution of the T cell antigen receptor and reorientation of the Golgi and microtubule organizing center. Redistribution of LFA-1 may be triggered by initial antigen receptor engagement, a mechanism earlier proposed to regulate adhesion strengthening via LFA-1 based on experiments with phorbol esters (Rothlein and Springer, 1986; Springer *et al.*, 1987). An unglycosylated intracellular protein of 86,000 Da has been found in association with cell-surface LFA-1 (van Agthoven *et al.*, 1985). The functional significance is unknown, although this protein may be part of a cytoskeletal linkage. Association and disassociation of the leukocyte integrins with the cytoskeleton would provide a mechanism for rapid adhesion and deadhesion events, as required for CTL-mediated cytolysis and other immune functions.

V. Ligand Molecules for the Leukocyte Integrins

A. LFA-1 Ligands

1. ICAM-1

a. Identification and Characterization of an LFA-1 Ligand. Phorbol ester-induced homotypic aggregation of lymphoid cells is an LFA-1-dependent phenomenon. Rothlein and Springer (1986) showed that

LFA-1$^-$ cells from LAD patients do not form homotypic aggregates; however, LFA-1$^+$ control cells can form aggregates with LFA-1$^-$ cells from LAD patients. These results suggested that LFA-1 binds a ligand distinct from itself, and that cells from LAD patients should express the ligand. Rothlein *et al.* (1986) raised MAb against LFA-1$^-$ cells from LAD patients and screened them for the ability to inhibit LFA-1-dependent homotypic aggregation, in the hopes of identifying the putative LFA-1 ligand. One MAb (RR1/1) defined a 76,000- to 114,000-Da heavily glycosylated molecule, ICAM-1, which fit these criteria.

The receptor–ligand relationship of LFA-1 and ICAM-1 was formally proved with purified ICAM-1 incorporated into planar lipid membranes (Marlin and Springer, 1987) and by gene transfection (Simmons *et al.*, 1988). All of these systems can mediate LFA-1-dependent adhesion of lymphoblasts, which is inhibitable by pretreatment of the lymphoblast with LFA-1 MAbs or pretreatment of the ICAM-1 surface with ICAM-1 MAbs. Moreover, LFA-1$^-$ cells from LAD patients do not bind to purified ICAM-1 (Marlin and Springer, 1987). Confirming studies with ICAM-1 cross-linked to artificial substrates extended these findings and demonstrated that three independent MAbs defined ICAM-1, one of which had been used to map it to chromosome 19 (Makgoba *et al.*, 1988b).

Reciprocal studies have been done using purified LFA-1 (Dustin and Springer, 1989). Binding of some cell types to purified LFA-1 in planar membranes was inhibited by ICAM-1 MAbs. Furthermore, purified LFA-1 binds to purified ICAM-1 when ICAM-1 is presented in planar membranes, confirming that LFA-1-dependent adhesion can be accounted for by direct interaction of LFA-1 with ICAM-1 and other ligands (see below).

ICAM-1 is a widely distributed molecule whose expression is highly regulated. Basal ICAM-1 expression on nonhematopoietic cells is normally low, but can be up-regulated by a variety of cytokines, including interleukin-1, tumor necrosis factor, and interferon-γ (Dustin *et al.*, 1986, 1988; Pober *et al.*, 1986, 1987; Dustin and Springer, 1988). ICAM-1 expression is prominent on cytokine-activated endothelial cells during inflammation (Dustin *et al.*, 1986; Cotran *et al.*, 1988). Increased ICAM-1 expression correlates directly with increased LFA-1-dependent adhesion of lymphoblasts to induced cells (Dustin *et al.*, 1986, 1988; Dustin and Springer, 1988). These results suggest that ICAM-1 provides dynamic "position-specific" information to guide lymphocyte and leukocyte localization during the course of the immune response. ICAM-1 is expressed only weakly on resting peripheral blood leukocytes, but expression is increased upon cell activation. A molecule identical to ICAM-1 (Makgoba *et al.*, 1988b) was defined as a B cell activation

molecule (Clark *et al.*, 1986). The LFA-1/ICAM-1 adhesion pathway has been implicated in lymphocyte adhesion to endothelial cells (Dustin and Springer, 1988), fibroblasts (Dustin *et al.*, 1986), epidermal keratinocytes (Dustin *et al.*, 1988), synovial cells (Mentzer *et al.*, 1988), and other lymphoid cells (Rothlein *et al.*, 1986; Makgoba *et al.*, 1988a).

ICAM-1 has been reported to localize to uropods of the T cell line HSB-2 (Dougherty *et al.*, 1988). By indirect immunofluorescence, ICAM-1 expressed in COS cells (D. E. Staunton and T. A. Springer, unpublished) and endothelial cells (Smith *et al.*, 1989) demonstrates a punctate staining. This localized cell membrane distribution may facilitate initial interaction with LFA-1. Spreading of cells on surfaces coated with extracellular matrix components or adhesion ligands is an indication of cytoskeletal association. A Reid-Sternberg cell line expressing high levels of ICAM-1 spreads dramatically on LFA-1 bearing planar membranes. This raised the possibility that at least in some cells, ICAM-1 is associated with the cytoskeleton. A truncated form of ICAM-1, which completely lacks a cytoplasmic tail was generated by oligonucleotide mutagenesis (Staunton *et al.*, unpublished). The truncated protein immunoprecipitated from transfected COS cells demonstrates a mobility on SDS-PAGE which is consistent with its predicted 3-kDa reduction in molecular weight. Resistance to cleavage by a phosphatidyl inositol (PI)-specific phospholipase C suggests that it does not acquire a PI membrane anchor. This truncated form of ICAM-1 still demonstrates its characteristic cell-surface localization. Thus, if localization is through cytoskeletal interaction, this must occur in the ICAM-1 transmembrane region, or by association of the extracellular domain with an anchored protein.

b. Gene Cloning. ICAM-1, which has recently been cloned and sequenced (Simmons *et al.*, 1988; Staunton *et al.*, 1988), has been shown to be a member of the immunoglobulin superfamily, with its closest relationship being to the neural cell adhesion molecule (NCAM) and myelin-associated glycoprotein (MAG), another neural adhesion protein. All three proteins contain five immunoglobulin domains. Interestingly, NCAM has been shown to participate in homophilic (like-like) interactions (Edelman, 1986), whereas ICAM-1 participates in heterophilic interactions. LFA-1 is the only known member of the integrin superfamily which binds to a member of the immunoglobulin superfamily. Members of the other two integrin subfamilies bind a conserved Arg-Gly-Asp (RGD) sequence in unrelated extracellular matrix proteins. ICAM-1 does not have an RGD sequence (Simmons *et al.*, 1988; Staunton *et al.*, 1988) and neither does ICAM-2 (see Section V,A,2; Staunton *et al.*, 1989), suggesting that leukocyte integrin binding specificity has diverged from that of other integrins. Preliminary results from mutational studies reveal

that at least two sites in ICAM-1 are involved in binding LFA-1 (Staunton *et al.*, unpublished).

2. *Other Ligands*

There is evidence that ICAM-1 is not the only ligand for LFA-1. Phorbol ester-induced homotypic aggregation of the SKW3 T cell line is inhibited by LFA-1 MAbs but not by ICAM-1 MAbs (Makgoba *et al.*, 1988a). Similarly, some heterotypic cell interactions, such as CTL adhesion to B lymphoblastoid cell lines, is inhibitable by LFA-1 MAbs but not by ICAM-1 MAbs (Makgoba *et al.*, 1988b). LFA-1-dependent T cell adherence to endothelial cells has both an ICAM-1-dependent and an ICAM-independent component (Dustin and Springer, 1988). The ICAM-1-dependent pathway is inducible with cytokines, such as tumor necrosis factor (TNF), whereas the ICAM-1-independent pathway is unaffected. These results suggest that multiple ligands may serve to mediate distinct adhesion requirements of lymphocytes during different stages of the immune response. Cell binding to purified LFA-1 in glass-supported planar membranes or immobilized directly on plastic also suggests the presence of other ligands. The most striking example of this is the T cell line SKW3, which expresses virtually no ICAM-1 yet binds strongly to purified LFA-1. This binding is blocked by LFA-1 MAbs but not by any single ICAM-1 MAb or combination of ICAM-1 MAbs (Dustin and Springer, 1989). Other cell lines show different degrees of inhibition with ICAM-1 MAbs, depending on the LFA-1 density of purified LFA-1 on the monolayer. With these cell lines, ICAM-1 MAbs are inhibitory at low LFA-1 density but not at high LFA-1 density, whereas LFA-1 MAbs are inhibitory at all densities. These results suggest that both ICAM-1 and the alternative LFA-1 ligand are present on these cells, and that interaction is stronger with ICAM-1 than with the alternative ligand. The only nonhematopoietic cells we have encountered that express ICAM-1 in the absence of other LFA-1 ligands are epithelial cells from several tissues and dermal fibroblasts (Dustin *et al.*, 1986, 1988). The existence of multiple ligands for a single integrin is not unprecedented. Other integrins, such as platelet glycoprotein IIb/IIIa, have as many as four distinct ligands.

Recently, a second LFA-1 ligand, designated ICAM-2, was cloned based on its functional properties (Staunton *et al.*, 1989). A cDNA expression library was screened for ability to confer on COS cells the ability to bind to purified LFA-1 coated on Petri dishes. Screening was in the presence of ICAM-1 MAb. ICAM-2 has two Ig-like domains, in contrast to ICAM-1, which has five, and these are 35% identical to the first two domains of ICAM-1. ICAM-1 and ICAM-2 are much more similar to one another than to other members of the Ig superfamily, and thus represent an Ig

subfamily specialized to interact with LFA-1. The functional cDNA isolation approach should have wide application for identifying other adhesion counterstructures.

B. Mac-1 AND p150,95 LIGANDS

1. C3bi

Both Mac-1 and p150,95 bind the C3bi fragment of complement (Micklem and Sim, 1985). A peptide fragment of C3bi which contains an RGD sequence binds to macrophages in a Mac-1 MAb-inhibitable fashion (Wright *et al.*, 1987). However, it is not clear whether the RGD sequence is critical for binding.

2. Other Ligands

The ability of Mac-1 to mediate a number of cell-cell interactions in which C3bi is not involved, such as neutrophil aggregation, suggests putative cell surface ligand(s) for Mac-1. The epitopes on Mac-1 involved in C3bi-binding activity and general adhesion have been distinguished by MAb-inhibition studies (Anderson *et al.*, 1986; Dana *et al.*, 1986; Sanchez-Madrid *et al.*, 1983a; Beatty *et al.*, 1983, 1984). Some MAbs show differential effects on neutrophil homotypic aggregation and adhesion to endothelial monolayers as compared to C3bi binding, while other MAbs block both.

VI. Molecular Basis of Leukocyte Adhesion Deficiency Disease

A. EARLY STUDIES

Every LAD patient analyzed to date has been found to be deficient in the expression of all three leukocyte integrins (Anderson and Springer, 1987). The simplest hypothesis is that a defect in the common β subunit could account for LAD. This hypothesis was tested in biosynthesis and human X mouse lymphocyte hybridization experiments.

Biosynthesis experiments utilized Epstein-Barr virus (EBV)-transformed B lymphocyte and mitogen-stimulated T lymphocyte cell lines, which, in healthy individuals, synthsize the LFA-1 $\alpha(\alpha L)$ subunit and the common β subunit and express the LFA-1 $\alpha\beta$ complex on cell surfaces. Early studies showed that patient cell lines synthesize an apparently normal αL subunit precursor, but that the αL precursor does not undergo carbohydrate processing, does not associate in an $\alpha\beta$ complex, and neither subunit is expressed on the cell surface (Springer *et al.*, 1984; Lisowska-Grospierre *et al.*, 1986). In these studies, the use of available anti-β subunit monoclonal antibodies (MAb) did not allow for the immunoprecipitation of β subunit precursors from either control cells or LAD patient cells.

In human X mouse lymphocyte hybrids, human LFA-1 α and β subunits from normal cells were shown to associate with mouse LFA-1 subunits to form interspecies $\alpha\beta$ complexes. Surface expression of the α but not the β subunit of patient cells can be rescued by the formation of interspecies complexes (Marlin *et al.*, 1986). These studies showed that the LFA-1 α subunit in genetically deficient cells is competent for surface expression in the presence of an appropriate mouse β subunit. Taken together, these results suggest that leukocyte adhesion deficiency is secondary to a defect in the common β subunit.

B. Identification of Heterogeneous Mutations in the Common β Subunit

The acquisition of two molecular probes, the β subunit cDNA (Kishimoto *et al.*, 1987a; Law *et al.*, 1987) and a rabbit antiserum that recognizes the precursor form of the β subunit (Kishimoto *et al.*, 1987b; Dana *et al.*, 1987), allowed analysis of the β subunit from LAD patients. Five phenotypes of β subunit expression and structure were identified by Kishimoto *et al* (1987b). One class of mutations resulted in no detectable mRNA or protein precursor. Southern analysis of genomic DNA from these patients showed no gross deletions of the β subunit gene. A second class of mutation is represented by a moderately deficient patient, whose cells synthesized trace amounts of the β subunit precursor and low levels of mRNA message. Dimanche *et al.* (1987) studied two patients with no apparent β subunit precursor synthesis, and this may fall into one of these two classes; however, further analysis at the RNA level is required.

Two other classes of mutations affect the structure of the common β subunit. In one, the patient synthesizes an aberrantly large β subunit precursor. However, after endoglycosidase H digestion, the protein backbone appears about normal in size. One hypothesis is that a point mutation causes an amino acid change, which creates a novel consensus N-glycosylation site (Asn-X-Ser/Thr). In the other class of mutation, four moderately deficient patients, who are all related, synthesized an aberrantly small precursor that was degraded. The pedigree analysis of 14 members of this kindred show that inheritance of the aberrant precursor correlates with the expected disease state and surface expression of LFA-1. Endoglycosidase H digestion of N-linked carbohydrates from the precursor shows that the defect is in the protein backbone rather than in glycosylation.

Finally, three unrelated patients studied by us (Kishimoto *et al.*, 1987b), a group of four patients studied by Dana *et al.* (Dana *et al.*, 1987), and one patient studied by Dimanche *et al.* (Dimanche *et al.*, 1987), synthesized both a normal size β subunit precursor and a normal

size α subunit precursor. Neither subunit was processed or transported to the cell surface. Although it is likely that there is a point mutation in the β subunit, we cannot exclude the possiblity of α subunit mutations.

C. Molecular Basis of the Severe and Moderate Deficiency Phenotypes

Heterogeneity in the defect causing LAD disease was first observed in the extent of the leukocyte integrin deficiency at the cell surface. Patients are classified as severely deficient (<0.5% normal levels of expression) and moderately deficient (3-10% normal levels of expression) (Anderson *et al.*, 1985). Survival of LAD patients is greatly influenced by the extent of deficiency of the leukocyte integrins. Severely deficient patients often die in the first 2 years of childhood, while moderately deficient patients are less prone to life-threatening infections and can survive to adulthood. However, even those patients who survive to adulthood can suddenly die of complications from severe infections.

The molecular basis for this heterogeneity is unclear. In the case of deficiency of β subunit mRNA and protein precursor, the extent of deficiency correlates with moderate and severe phenotype (Kishimoto *et al.*, 1987b). Two severely deficient patients had no detectable β subunit mRNA expression or protein precursor synthesis, while one moderately deficient patient had low levels of mRNA expression and precursor synthesis (Kishimoto *et al.*, 1987b). Apparently, this low level of expression is sufficient to account for the moderate phenotype and less severe clinical complications. However, among 13 patients synthesizing normal quantities of mRNA and protein precursor, it has been unclear why some are of the moderate and some of the severe deficiency phenotype (Kishimoto *et al.*, 1987b; Dimanche *et al.*, 1987; Dana *et al.*, 1987) (Wardlaw and T. A. Springer, unpublished).

We have analyzed four related patients with the moderate deficiency phenotype (Kishimoto *et al.*, 1989). The predominant form of the β subunit precursor synthesized by these patients is several thousand daltons smaller than normal and is degraded before transport to the Golgi apparatus. However, ^{125}I cell-surface labeling shows that the small amount of LFA-1 that reaches the surface contains a normal size β subunit. At the RNA level, Sl nuclease protection studies define a 90-nt deletion in the β subunit message from these patients. The Taq polymerase chain reaction (PCR) was adapted to amplify the aberrant mRNA. Sequence analysis shows an in-frame 90-nt deletion in the region encoding the extracellular domain. This 90-bp region is also shown to be encoded on a separate exon in both the normal and patient genome. Sequence analysis of genomic DNA from these patients shows a single

G-to-C substitution in the sequence of the 5′ splice site, suggesting aberrant RNA splicing. A small amount of normally spliced message, detected by S1 nuclease protection analysis and Taq PCR, encodes a normal size β subunit and accounts for the low levels (3% of normal) of cell-surface expression of the leukocyte integrins observed in these patients, hence the moderate deficiency phenotype. The 30 amino acids encoded by the deletion region share 63% amino acid identity with the corresponding region of the fibronectin receptor. The high conservation suggests some functional significance of this region, perhaps in $\alpha\beta$ subunit association.

VII. Future Avenues of Research

The first leukocyte integrin to be functionally characterized was LFA-1, in 1981 (Davignon *et al.*, 1981a). Rapid progress in recent years in the study of leukocyte adhesion reflects an ever-increasing appreciation of the diverse functions of leukocyte integrins. The leukocyte integrins, like other members of the integrin superfamily, play a dynamic role in transmitting positional information and linking the extracellular environment with intracellular processes. The interaction of LFA-1 and ICAM-1 represents one of the few defined heterotypic receptor–ligand relationships of cell-surface molecules. LFA-1-dependent adhesive interactions are exquisitely regulated at both the level of receptor and ligand. We have an understanding of the role of leukocyte integrins *in vivo* in both immune and nonimmune inflammatory responses. The recent advances hold promise for many new avenues of research.

Precise structure–function relationships of the leukocyte integrins and ICAM-1 can now be directly tested by *in vitro* mutagenesis of the appropriate cDNAs. Domain-swapping among the leukocyte integrin α subunits may allow dissection of LFA-1, Mac-1, and p150,95 functional specificity. Domains that are defined as functionally important can be synthesized and tested both *in vitro* and *in vivo*. Similar mutagenesis approaches may reveal how integrin receptor activation occurs, what kinds of signals are transmitted to the cell by leukocyte integrins, and the nature of cytoskeletal interaction. In addition, the acquisition of the leukocyte integrin and ICAM-1 cDNAs allows analysis of the basis for gene regulation by a variety of cytokines.

There is also substantial evidence that other ligands for LFA-1, Mac-1, and p150,95 exist. Rational strategies must be designed to identify these ligands and to assess their contributions in different phases of the immune response. Multiple ligands may provide quite distinct signals and positional information to leukocytes.

The answers to these questions of structure, function, and regulation of receptor activity have profound therapeutic implications, as suggested

by recent animal models of ischemia and reperfusion injury. The control of pathologic tissue damage by neutrophils during inflammation can be approached by inhibition of receptor activation, modulation of ICAM-1 expression, or by using MAbs or fragments of leukocyte integrins or their ligands to block acute adhesive events.

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