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Structure and Function of Leukocyte Integrins

RICHARD S. LARSON & TIMOTHY A. SPRINGER

Cell communication is essential to development, tissue organization and function of all multicellular organisms. Cells communicate with each other and with their environment via soluble mediators and during direct contact. In the immune system leukocytes are involved in a host of cell-soluble mediator, cell-cell and cell-matrix interactions. For instance, an acute inflammatory response is initiated by release of soluble substances at the site of injury that direct leukocyte migration and localization. The circulating leukocytes arriving at the site of injury must directly adhere to the endothelium and basement membrane during extravasation from the circulation. Once in the tissue, leukocytes will migrate in response to gradients of chemoattractants and to adhesive gradients. The leukocytes will adhere and de-adhere to a variety of cells, finally find infected or antigen-presenting cells, adhere to them and deliver the appropriate immunological response.

Discovery of several cell surface receptors involved in cell-cell, cell-substrate, or cell-soluble ligand binding has been a key factor in understanding the mechanisms underlying inflammatory and immune phenomena. Three protein families, the immunoglobulin (Ig) family (Williams & Barclay 1988, Williams 1987), the integrin family (Springer 1990, Kishimoto et al. 1989a, Hynes 1987, Ruoslahti & Pierschbacher 1987, Hemler 1988), and the recently designated selectin family (Tedder et al. 1989, Stoolman 1989, Springer 1990), have been described that are extensively involved in a network of cell-cell and cell-matrix interactions in the immune system. Engagement of these surface receptors can transduce a signal leading to cellular events that change the phenotype, movement, gene expression, or activation state of the cell. On the other hand, cytoplasmic signals regulate the functional activity and surface expression of these receptors. These receptors thus mediate a dialogue, transferring information in

The Center for Blood Research and Department of Pathology, Harvard Medical School, Boston, MA 02115, U.S.A.

both directions across the membrane. To add to the complexity of these interactions, these cytoplasmic signals may be initiated by engagement of other surface receptors. Study of these adhesion molecules and their counter-structures has yielded model systems for cell-cell and cell-matrix interactions, cell signalling, and regulation of cell adhesion.

Members of the Ig superfamily (Williams & Barclay 1988, Williams 1987) include both antigen-specific receptors, such as the T-cell receptor (TCR) and surface immunoglobulin (Ig), as well as antigen-independent receptors and their counter-receptors, such as CD2 and lymphocyte function associated molecule-3 (LFA-3). These molecules have varying numbers of Ig-like domains each consisting of 90 to 100 amino acids and a characteristic intradomain disulfide bond. The Ig domain consists of two β-sheets of anti-parallel β-strands each containing 5–10 amino acids. Ig superfamily members bind soluble and cell surface ligands and function in cell activation, differentiation, and cell-cell interaction. In some cases, both an adhesion receptor and the counter-receptor to which it binds are members of the Ig superfamily.

Three selectin family molecules, endothelial cell adhesion molecule-1 (ELAM-1), leukocyte adhesion molecule (LAM-1, Mel-14), and CD62, also known as platelet activation-dependent granule-external membrane protein (PADGEM) and granule membrane protein of 140 kD (GMP-140), have been implicated in a number of leukocyte adhesion phenomena including leukocyte homing to lymphoid tissue (Tedder et al. 1989, 1990 Stoolman 1989, Johnston et al. 1989, Larsen et al. 1989). All these molecules have an NH_2-terminal domain of 117–120 amino acids which is homologous to C-type lectins (Drickamer 1988) including hepatic galactose receptors and soluble mannose-binding lectins. However, direct carbohydrate binding of this domain has not yet been demonstrated. A single epidermal growth factor precursor motif of 34–40 amino acids and two to nine short consensus repeats (SCR) of 62 amino acids follow the lectin domain. Epidermal growth factor precursor motifs are found in a number of proteins, and the short consensus repeats are present in proteins regulating the complement cascade. Selectins are expressed on both leukocytes and endothelial cells. Cell surface expression of ELAM-1 and GMP-140 is inducible with cytokine or procoagulant factors, respectively, suggesting a role for these molecules in inflammatory processes and coagulation. Interestingly, Mel-14 is released by a protease within 4 minutes of neutrophil activation, suggesting that Mel-14 functions very early in neutrophil-endothelium adhesion (Kishimoto et al. 1989b).

The integrin family consists of at least 14 α/β heterodimers divided into subfamilies according to distinct β subunits. Each subfamily has distinct structural and functional characteristics (Fig. 1). The β_1 or VLA subfamily contains at least 6 distinct members (VLA 1–6) and is found on a variety of cell types (Hynes 1987, Hemler 1988). The β_2 subfamily, or leukocyte integrins, contains

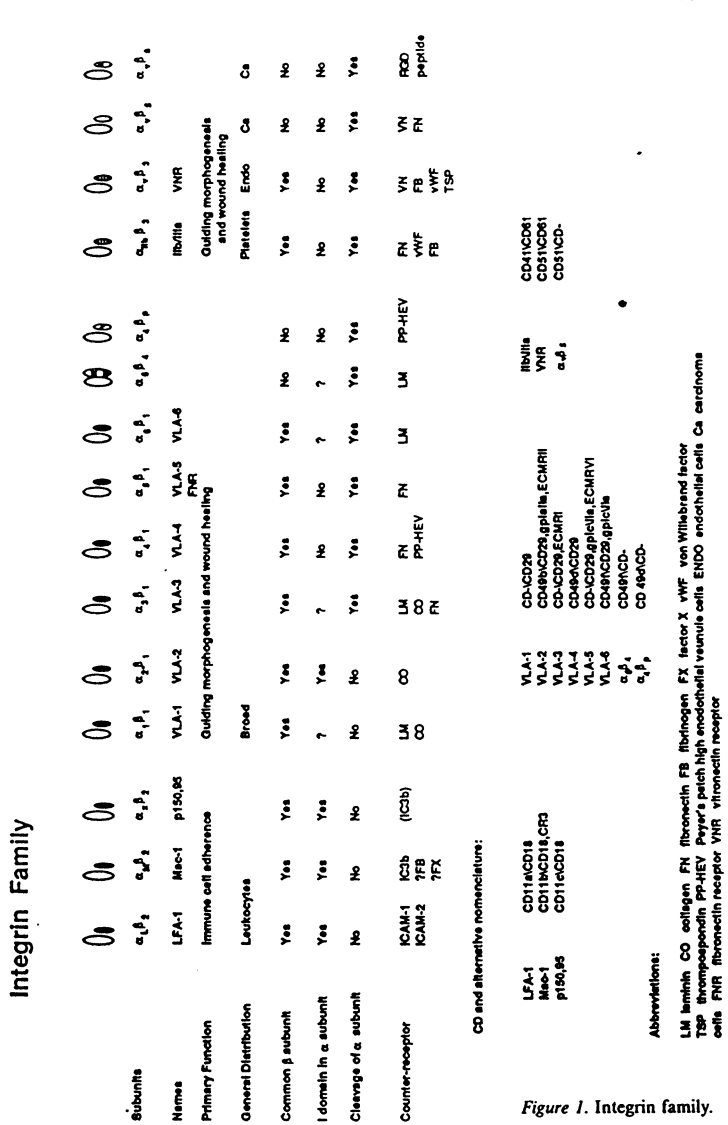

Figure 1. Integrin family.

three members (LFA-1, Mac-1 and p150,95) exclusively expressed on leukocytes (Springer 1990, Kishimoto et al. 1989a), and the β_3 subfamily, or cytoadhesin family, has two members (VNR and platelet glycoprotein IIb/IIIb) expressed primarily on endothelial cells and platelets (Ruoslahti & Pierschbacher 1987). Initial observations indicated that each β subunit associates with a number of distinct α subunits, but each α subunit associates with only one β subunit. Recently, four additional or alternative β subunits have been defined, β_4 (Kajiji et al. 1989, Hemler et al. 1989) β_5 (Cheresh et al. 1989a) β_p (Holzmann & Weissmann 1989) and β_s (Freed et al. 1989). Interestingly α_6 associates with both β_1 and β_4, α_v associates with β_3, β_5, β_s, and α_4 associates with both β_1 and β_p.

The integrin family is phylogenetically ancient. Homologous $\alpha\beta$ heterodimers have been defined in Drosophila (Wilcox & Leptin 1985), Xenopus (Desimone & Hynes 1988), and chicken (Tamkun et al. 1986), indicating that progenitor integrins were present prior to the invertebrate-vertebrate divergence. Immunologically cross-reacting proteins have also been detected in nematode and fungi (Marcantonio & Hynes 1988).

Integrin family members engage in heterophilic interactions with cell surface ligands and extracellular matrix components (ECM). ECM components including fibronectin, collagen, and laminin have been shown to be ligands for members of the β_1 and β_3 subfamilies (Hemler 1988, Ruoslahti & Pierschbacher 1987, Hynes 1987). Members of these subfamilies are of great significance in embryogenesis, growth and repair, and hemostasis. The leukocyte integrins – or β_2 subfamily – have been shown to be involved in a diverse number of leukocyte adhesion-dependent phenomena, giving them a critical role in inflammatory and immune responses (Kishimoto et al. 1989a, Springer 1990).

The term integrin was initially used to emphasize that these receptors integrate signals from the extracellular environment with the intracellular cytoskeleton. A signal is transduced from outside to inside the cell (Hynes 1987), i.e. "outside-in signalling." Recently, studies on T-cell triggering have demonstrated that LFA-1 functional activity is regulated by intracellular signals, and thus participates in "inside-out" signalling as well (Dustin & Springer 1989). These observations have provided an explanation for how the functional activity of these molecules is regulated, and models for adhesion/deadhesion of cells to other cells or substrates via integrin-ligand interaction can now be suggested.

DISCOVERY AND SIGNIFICANCE OF THE LEUKOCYTE INTEGRINS

Mac-1, the earliest integrin recognized, was initially defined in the mouse (Springer et al. 1979) and later in the human (Springer et al. 1982) as a marker for myeloid cells. This heterodimer consisting of an α subunit ($M_r = 170\,000$) and β subunit ($M_r = 95\,000$) was shown to bind iC3b and to be equivalent to

the complement type 3 receptor (Springer et al. 1982, Wright et al. 1983a, Beller et al. 1982).

Both the murine and human LFA-1 molecules were first defined by the ability of mAb to block CTL-mediated killing and T-cell proliferation (Davignon et al. 1981b, Pierres et al. 1982, Sanchez-Madrid et al. 1982, Hildreth et al. 1983). Subsequent observations demonstrated that anti-LFA-1 mAbs blocked NK killing (Beatty et al. 1983, Hildreth et al. 1983, Krensky et al. 1983, Spits et al. 1983, Springer et al. 1982) and ADCC of granulocytes and monocytes (Kohl et al. 1984, Miedema et al. 1984). Closer analysis suggested that anti-LFA-1 antibodies were inhibiting conjugate formation rather than the killing event (Davignon et al. 1981a, b, Springer et al. 1982, Bongrand et al. 1983, Krensky et al. 1984).

LFA-1 is a heterodimer consisting of an α subunit ($M_r = 180\,000$ human, $M_r = 177\,000$ mouse) and β subunit ($M_r = 95\,000$ (Kürzinger et al. 1981). Subsequent biochemical characterization (Kürzinger et al. 1982, Trowbridge & Omary 1981, Sanchez-Madrid et al. 1983b) showed that the β subunit of the LFA-1 and Mac-1 molecules was identical but the α subunits were distinct, suggesting that a group of molecules might share a common β subunit and have distinct α subunits. Using anti-β subunit antibody, a third member, p150,95 ($M_r = 150\,000$, α subunit), was defined in the human (Springer et al. 1986, Sanchez-Madrid et al. 1983a).

Recent observations have broadened the significance of the LFA-1, Mac-1, and p150,95 family of molecules. First, this family of three glycoproteins was found to be homologous by N-terminus peptide sequencing and cDNA cloning (see below) to receptors binding extracellular matrix components, defining the integrin family.

Second, a group of patients was discovered who have recurrent life-threatening bacterial infections and who have leukocytes that lack cell surface expression of all three leukocyte integrins (Anderson & Springer 1987, Anderson et al. 1989, Fischer et al 1988, Todd & Freyer 1988). The defect in this disease is due to heterogeneous mutations in the shared β subunit. A clinical syndrome, leukocyte adhesion deficiency (LAD), was defined and the study of this disease has demonstrated the functional significance of the leukocyte integrins *in vivo*.

Third, the leukocyte integrins have been shown to be involved in antigen-independent processes such as leukocyte adhesion to endothelial cells, fibroblasts, epidermal keratinocytes, synovial cells, and other leukocytes (Dustin & Springer 1988, Dustin et al. 1986, 1988, Haskard et al. 1987, Mentzer et al. 1988, te Velde et al. 1987, Anderson et al. 1986, Lo et al. 1989). These observations broaden the role of these molecules to include antigen-independent inflammatory and immune processes such as migration and diapedesis.

Finally, LFA-1 has been shown to interact with two counter-receptors, intercellular adhesion molecule-1 (Rothlein et al. 1986a, Marlin & Springer 1987)

and 2 (Staunton et al. 1989b) (ICAM-1 and ICAM-2). The LFA-1/ICAM interactions are novel in that a member of the integrin family interacts with members of the immunoglobulin superfamily. Structural analysis, structure-function studies, and regulation studies have emphasized the complexity of this receptor/counter-receptor interaction and have provided a general model for integrin-ligand interaction.

STRUCTURAL AND MOLECULAR BIOLOGICAL CHARACTERIZATION OF THE LEUKOCYTE INTEGRINS

The leukocyte integrins are noncovalently associated $\alpha_1\beta_1$ membrane glycoproteins. LFA-1, Mac-1 and p150,95 have distinct α subunits (M_r = 180 000, 170 000, 150 000 respectively) and a shared β subunit (M_r = 95 000) as shown by mAb reactivity, tryptic peptide mapping and antigen-preclearing studies (Kürzinger et al. 1981, Trowbridge & Omary 1981, Sanchez-Madrid et al. 1983b, Springer et al. 1986). The leukocyte integrin subunits are synthesized as precursor proteins (αL, M_r = 165 000; αM, M_r = 160 000; αX, M_r = 145 000; β subunit M_r = 89 000 in human) containing high-mannose N-linked oligosaccharides. Association of the α and β subunits occurs in the Golgi apparatus where further processing to complex-type N-linked oligosaccharide occurs (Ho & Springer 1983, Springer et al. 1984). The mature heterodimer is transported to the cell surface or intracellular granules (Bainton et al. 1987, Todd et al. 1984). Biosynthetic studies (Sanchez-Madrid et al. 1983a) and studies using cells from LAD patients (Springer et al. 1984) have suggested that in the normal leukocyte the β subunit precursor is expressed in higher amounts than the α subunit precursor.

The type and amount of glycosylation varies considerably on each leukocyte integrin subunit. The LFA-1 α subunit contains 10 potential N-linked glycosylation sites (Larson et al. 1989a) and considerable heterogeneity in LFA-1 glycosylation on different cell types has been observed. N-linked oligosaccharides on LFA-1 are sulfated on thymocytes and splenic T cells but not on bone marrow cells or splenic B cells (Dahms & Hart 1985). Isoelectrofocusing experiments have shown that LFA-1 on T cells is less acidic than LFA-1 on B cells (Takeda 1987). The difference in isoelectric focusing can be abrogated by treatment of the cells with neuraminidase, indicating differences in sialylation of LFA-1 on different cell types. The human Mac-1, murine Mac-1, and human p150,95 α subunits and the common human β subunit have 18, 17, 12, and 6 potential glycosylation sites respectively (Corbi et al. 1987, 1988b, Pytela 1988, Kishimoto et al. 1987). An unusual carbohydrate moiety, lacto-N-fucopentose II, has been found on a subset of leukocyte integrins on neutrophils (Skubitz & Snook 1987). Since cDNA cloning has revealed that the α subunit peptide backbones are of similar molecular weights, it is likely that glycosylation patterns at least partially account for the differences in apparent molecular weight.

Alpha subunits

cDNA clones for all three human α subunits and murine Mac-1 have been identified (Larson et al. 1989a, Corbi et al. 1987, 1988b, Pytela 1988) and the nucleotide sequence determined. The mRNA for human LFA-1, Mac-1, and p150,95 α subunits are 5.2, 4.8, and 4.7 kb respectively. Murine Mac-1 α subunit has a mRNA of 6.0 kb (Sastre et al. 1986a). The α subunits are typical transmembrane proteins with a short leader sequence of 15 to 25 amino acids, a large extracellular domain of 1063 to 1089 amino acids, a COOH-terminal transmembrane region of about 26 amino acids, and a short cytoplasmic tail (αL 53 amino acids, αM 19 amino acids, αX 29 amino acids, and murine Mac-1 24 amino acids). The three human leukocyte integrin α subunits have striking homology, with 44% +/− 11% amino acid sequence identity, and possess similar overall structural characteristics in that all of the leukocyte integrin α subunits contain putative divalent cation binding domains and an "inserted/interactive" or I domain (discussed below). The murine cDNA contains a very large 5′ untranslated region (at least 573 nucleotides). This region contains a consensus sequence of interferon-regulated genes. Although regulatory sites are typically found upstream of transcription start sites, this may be a bona fide transcription regulatory site since Mac-1 mRNA expression is stimulated by γ-interferon (Sastre et al. 1986a).

Divalent cation binding domains. All the leukocyte integrins require Ca^{2+} or Mg^{2+} for ligand binding (Anderson & Springer 1987, Wright et al. 1983b, Micklem & Sim 1985). Three tandem repeats of about 60 amino acids, located between amino acids 422–616 in the extracellular domain, contain putative divalent cation binding sites (Larson et al. 1989, Corbi et al. 1987, 1988b, Pytela 1988) that are similar to the "EF-hand loop" divalent cation binding sites of calmodulin, troponin C, and parvalbumin (Szebenyi et al. 1981). Metal ions can be used to stabilize human LFA-1, Mac-1 and p150,95 during protein purification, yielding functional $\alpha\beta$ complexes, further demonstrating divalent cation-leukocyte integrin interaction (Dustin & Springer 1989) (Stacker, Diamond, and Springer, unpublished observations). Divalent cation binding has been directly demonstrated for the platelet glycoprotein IIb/IIIa (Fujimura & Phillips 1983). Thus, it is likely that the EF-hand loop-like sequences in the integrin α subunits bind divalent cations, reflecting the divalent cation requirements for ligand binding. The classic EF-hand loop structure of metal-ion binding sites has a glutamic acid residue in the −Z position which the X-ray crystallography structures demonstrate is coordinated with metal. This glutamic acid is absent in the leukocyte integrins as well as other integrins. It has been proposed that the aspartic acid residue of the Arg-Gly-Asp (RGD) sequence in several integrin ligands, or an equivalent residue in other ligands, may coordinate with the metal bound to the receptor (Corbi et al. 1987).

I domain. A region of 190 amino acids located toward the N-terminus from the metal ion binding sites is present in all three leukocyte integrins α subunits but is not present in four of five α subunit sequences within the β_1 and β_3 subfamilies published to date (summarized in Larson et al. 1989a and Takada et al. 1989). Structural analysis has revealed homology with domains in von Willebrand factor, complement cascade components (factor B and C2), collagen matrix protein (CMP) (Larson et al. 1989a), collagen type VI (Koller et al. 1989), and VLA-2 (Takada & Hemler 1989). The I domain-like region serves important recognition functions in several of these proteins. The A1 domain of vWF binds glycoprotein Ib and heparin while both the A1 and A3 domains are involved in binding to collagen (Girma et al. 1987). The domain in CMP may also have an important role in interaction with cartilage and proteoglycan (Argraves et al. 1987b). The domain in factor B and C2 is located in a region available for interaction with its ligand C3b (Bently 1986). Furthermore, since this region virtually lacks cysteines and N-glycosylation but is bounded by several N-glycosylation sites and cysteines toward the NH_2-terminus and COOH-terminus, the I domain is probably accessible to ligand and capable of conformational changes which may be important in the regulation of ligand binding (Larson et al. 1989).

Genomic structure. Genomic clones encoding the complete human p150,95 α subunit and partially encoding the human LFA-1 and murine Mac-1 α subunits have been isolated. The human p150,95 gene extends over 25 kb and contains at least 31 exons (Corbi et al. 1989). The partial genomic LFA-1 clone extends over 32 kb and encodes approximately 70% of the residues in the extracellular domain. Nucleotide sequence has been determined for 6 exons within the LFA-1 gene (Larson and Springer, unpublished). The 6 equivalent exons in the LFA-1 and p150,95 genomic clones have splice sites at identical amino acid residue positions as predicted by protein alignment (Larson et al. 1989). One exon corresponding to the N-terminus within the murine Mac-1 gene has been sequenced and the splice sites occur in exactly the same position as in human p150,95 (Sastre et al. 1986b, Corbi et al. 1989). Collectively, this data suggests that the leukocyte integrin α subunit genes will have similar if not identical intron/exon organization.

Each of the three divalent cation-binding repeats in LFA-1 and p150,95 are encoded in single exons (Larson and Springer, unpublished) (Corbi et al. 1989). These divalent cation binding motifs show statistically significant homology to each other. The structural similarity and presence of each repeat in a single exon strongly suggests that these binding sites arose by duplication.

Within the p150,95 gene in an intron following the I domain is an inverted repeat (Corbi et al. 1989). This region is 85% identical to 2 divalent cation binding exons and the intron between them. This inverted repeat is found on more than one genomic clone, demonstrating that it is not a cloning artifact.

Whether this inverted repeat encodes another gene product on the antisense strand or is simply a case of exon duplication is under study.

The I domain of p150,95 is encoded by 4 exons. Comparison of the intron/exon organization of the I domain of p150,95 α subunit with other proteins for which the genomic organization is known (vWF, CMP, factor B) demonstrates variability in the number, length and class of the introns within the I domain. However, the introns at the beginning and end of I domains in all these genes are phase 1 introns.

Evolutionary relationship of leukocyte integrin α subunits. The protein homology, exon/intron boundary similarity, and proximal chromosomal location suggest that the leukocyte integrins arose by gene duplication of an ancestral α subunit gene that contained an I domain (Larson et al. 1989a). This gene duplicated and gave rise to LFA-1 and a Mac-1/p150,95 primordial gene. Further duplication of the Mac-1/p150,95 primordial gene gave rise to p150,95 and Mac-1. Mac-1 and p150,95 would have diverged at a later point than LFA-1 since Mac-1 and p150,95 are more homologous to each other (61%) than to LFA-1 (35.7% and 37.4% respectively). VLA-2 also contains an I domain but is only 22–25% identical (Takada & Hemler 1989) to the leukocyte integrins, implying that it may have diverged from the ancestral I domain-containing integrin prior to its duplication into LFA-1 and Mac-1/p150,95 primordial genes.

Recently, Brown et al. (1989) published the genomic organization of the α subunit of the position-specific antigen 2 (PS2), a Drosophila integrin. It contains 12 exons. Alignment of p150,95 and PS2 α subunit sequences demonstrate significant correlation in their exon structures. The I domain exons 7–10 of p150, 95 have no equivalent in PS2 and, significantly, PS2 exons 5 and 6 between which they would be inserted are separated by a phase 1 intron, which is the correct phase for insertion of I domains. Also, exon boundaries in PS2 correlate in position and phase with exon boundaries in p150,95 with one exception, an exon in PS2 that is variably spliced and does not align with any p150,95 sequence. These comparisons demonstrate significant conservation in exon organization of integrin α subunits, despite the evolutionary distance between insects and mammals and the likely disparity in function between PS2 and p150,95.

Chromosomal location. Initial studies using mouse-human somatic cell hybrids demonstrated that the human LFA-1 α subunit gene was located on chromosome 16 (Marlin et al. 1986). Southern analysis using DNA from these hybrids later demonstrated that all three leukocyte integrin α subunits were located on chromosome 16 and *in situ* hybridization further localized all three subunits to the same band, 16p11.1–p13, defining a cluster of genes involved in cell adhesion (Corbi et al. 1988a). Interestingly, this region of chromosome 16 has been implicated in chromosomal abnormalities in 25% of patients with acute myelomonocytic leuke-

mia (AMMoL). These patients have an inversion on chromosome 16, inv(16) (p13;q22) or reciprocal translocation of both chromosomes 16, t(16:16) (p13;q22). The breakpoint on 16q22 is located within the metallothionein gene cluster. Although the precise role these rearrangements play in the production of AMMoL is unknown, the location of the leukocyte integrins raises the possibility that the leukocyte integrin α subunit gene cluster may be involved in the pathogenesis of this disease.

Beta subunit

The mature β subunit is a typical type I transmembrane protein consisting of 747 amino acids (Kishimoto et al. 1987, Law et al. 1987). There is an extracellular domain of 678 amino acids, a hydrophobic transmembrane region of 23 amino acids and a cytoplasmic tail of 46 amino acids. Four cystein-rich domains are located near the C-terminus of the extracellular domain. This 186-amino acid region contains an unusual cysteine motif with an extremely high content of cysteine (20%), suggesting that this region of the molecule has a rigid tertiary structure. The mRNA is 3.0 kb and is induced by phorbol esters in myelomonocytic cells, similarly to Mac-1 and p150,95 α subunit mRNA (Corbi et al. 1988b).

Chromosomal location. The common β subunit has been localized in the human to chromosome band 21q22.3 using somatic cell hybrids (Marlin et al. 1986), Southern analysis, *in situ* hybridization (Corbi et al. 1988a), deletion mutants and restriction fragment length polymorphism linkage (Gardiner et al. 1988). This band, 21q22, has been identified as a breakpoint in chromosomal translocations (t(3;21)(q26;q22) associated with the blast phase of chronic myelogenous leukemia (CML) (Corbi et al. 1988a). Interestingly, hematopoietic progenitor cells in CML show abnormal adhesive interactions with bone marrow stroma (Gordan et al. 1987). However, the precise role, if any, of the leukocyte integrins in CML is unknown.

Relation of leukocyte integrins to integrin family

All members of the integrin family are $\alpha\beta$ heterodimers and have been divided into subfamilies on the basis of distinct but homologous β subunits, initially termed β_1, β_2, and β_3. Members of the β_1 and β_3 subfamilies are expressed on a broad number of lymphoid and non-lymphoid cells (Table I). The VNR, FNR, and platelet glycoprotein IIb/IIIa have been shown to recognize the sequence Arg-Gly-Asp in binding to ligand. The β_2 subfamily has a more restricted cell surface expression, solely on leukocytes (reviewed in Kishimoto et al. 1989a, Hynes 1987).

Relationship of β subunit structures. The complete primary structure of β_1 (Tamkun et al. 1986), β_2 (Kishimoto et al 1987, Law et al. 1987), and β_3 (Fitzgerald et al. 1987) has been determined while only N-terminal sequence is known for β_4 (Kajiji et al. 1989) and β_5 (Cheresh et al. 1989a). The β_1, β_2, β_3, β_5, β_p, and β_s subunits have similar apparent molecular weight (M_r = 95 000–105 000). The higher apparent molecular weight of the β_4 subunit (M_r = 205 000) is reported to be due to sialylation (Kajiji et al. 1989). cDNA cloning of the β_1, β_2, and β_3 subunits has indicated that these transmembrane proteins are highly homologous (37–45% identity) and all contain four cysteine-rich repeats in the C-terminal region of the extracellular domain. A region of 200 amino acids located near the N-terminus is highly conserved among the three β subunits (70, 47, and 64% respectively). The β_1 and β_3 subunits have a consensus tyrosine phosphorylation sequence in the cytoplasmic domain, which is not found in the β_2 subunit. The β_4 subunit has been shown to associate with α_6 on epithelial cells (Kajiji et al. 1989, Hemler et al. 1989). Recently, two alternative β subunits, β_5 and β_s, have been shown to be present on epithelial cells and tumor cells in association with a_v (Cheresh et al. 1989a, Freed et al. 1989). A direct comparison of β_5 and β_s will be necessary to determine their relationship. Both β_4 and β_5 have homologous N-termini to the other β subunits. β_p associates with α_4 and like α_4/β_1 is a Peyer's patch-specific homing receptor (Holzmann & Weissman 1989). The functional significance of these alternative β subunits is unknown.

Structural relationships of integrin α subunits. The complete primary structure of eight α subunits (VNR (Suzuki et al. 1987), FNR (Argraves et al. 1987a), gp IIb (Poncz et al. 1987), VLA-2 (Takada & Hemler 1989), LFA-1 (Larson et al. 1989a), Mac-1 (Corbi et al. 1988b, Arnaout et al. 1988, Pytela 1988), p150,95 (Corbi et al. 1987), and VLA-4 (Takada et al. 1989)) is known. The α subunits are of three structural types. The first structural type is characteristic of the VNR, FNR, and platelet glycoprotein IIb/IIIa. These α subunits have four putative metal ion binding sites and a protease cleavage site near the C-terminus of the extracellular domain. The second structural type found in VLA-2, LFA-1, Mac-1, and p150,95 does not have this proteolytic cleavage site, has three cation binding sites, and possesses an I domain of 190 amino acids located near the N-terminus. A third structural type is indicated by the VLA-4 α subunit. This α subunit does not contain an I domain, has three divalent cation binding sites and possesses a putative proteolytic cleavage in the middle of the extracellular region.

Electron microscopy. The structures of gpIIb/IIIa (Carrell et al. 1985) and the FNR (Nermut et al. 1988) have been studied by electron microscopy. The $\alpha\beta$ complex has a globular head with two tails. Nermut et al. propose that the globular NH_2-terminal region of each subunit interacts and the region toward the COOH-terminal of each subunit forms one of the tails. Although similarities

are expected with the leukocyte integrins, it is of interest to know how the presence of an I domain will influence the tertiary structure.

COUNTER-RECEPTORS AND FUNCTION OF THE LEUKOCYTE INTEGRINS

LFA-1 counter-structures

ICAM-1. Intercellular adhesion molecule-1 (ICAM-1) is an inducible cell surface ligand for LFA-1. This membrane glycoprotein is a single chain glycoprotein (M_r = 90 000) with a peptide backbone of 55 kD. cDNA cloning in the human (Staunton et al. 1988, Simmons et al. 1988) and mouse (Horley et al. 1989) has shown that ICAM-1 is a member of the immunoglobulin superfamily, consisting of 5 immunoglobulin-like domains. The neural cell adhesion molecule (NCAM) and myelin-associated glycoprotein (MAG) also have 5 Ig-like domains. ICAM-1 is expressed or induced by inflammatory mediators on many cell types, including endothelial cells, epithelial cells, keratinocytes, synovial cells, lymphocytes, and monocytes (Dustin & Springer 1988, Dustin et al. 1986, 1988, Haskard et al. 1987, Mentzer et al. 1988, te Velde et al. 1987). Deletion studies have localized the LFA-1 binding site to the first two immunoglobulin domains. Extensive single amino acid mutation of the predicted β turns withing the first two domains has indicated that the primary sites of contacts between ICAM-1 and LFA-1 are discontinuous amino acids in the first Ig domain (Staunton, Dustin, Erickson & Springer, submitted).

LFA-1-dependent antigen-specific responses can be completely inhibited, unaffected, or partially inhibited by ICAM-1 mAb (Rothlein et al. 1986a, Dustin & Springer 1988, Makgoba et al. 1988) depending on the target cell used. This phenomenon is due to the existence of at least one alternative counter-receptor for LFA-1, ICAM-2. Furthermore, transfection of ICAM-1 into murine L-cells expressing human HLA-DR greatly augments T-lymphocyte antigen-specific responses to these cells, and in the case of low density HLA-DR expression converts unresponsiveness to responsiveness (Altmann et al. 1989).

LFA-1/ICAM-1 cross-species interaction has been studied recently (Johnston and Springer, in preparation). Interestingly, human LFA-1 will interact with murine ICAM-1 but murine LFA-1 does not interact with human ICAM-1. This restriction in cross-species binding is due to the LFA-1 α subunit, since mouse-human hybrids expressing human α chain and murine β chain will bind human ICAM-1.

ICAM-1 has recently been shown to be the surface receptor for the major group of rhinovirus serotypes by using purified protein, ICAM-1 transfected cells, and inhibition by mAb to ICAM-1 of virus binding and infection of cells *in vitro* (Staunton et al. 1989, Greve et al. 1989). More recently, soluble ICAM-1 has been shown to be a potent and specific inhibitor of major rhinovirus infection (Marlin et al. 1990). Mutagenesis has indicated that there is overlap in the

sequences within the first domain of ICAM-1 that interact with LFA-1 and rhinovirus (HRV). Additionally, some amino acid changes within the first domain can eliminate binding of rhinovirus but maintain the ability to interact with LFA-1 (Staunton, Dustin, Erickson, and Springer, submitted).

Several other adhesion molecules are used as receptors for viruses. CD4, the complement receptor type 2 (CR2), and a third surface molecule containing an Ig-like domain are used as receptors for the human immunodeficiency virus-1 (HIV-1), Epstein-Barr virus (EBV), and polio virus, respectively. Interestingly, HRV, HIV-1, and EBV bind to a region of their receptor similar to that bound by the cell adhesion counter-receptor. HRV and LFA-1 bind to the first domain of ICAM-1 (Staunton, Dustin, Erickson, and Springer, submitted), HIV-1 and major histocompatibility complexes (MHC) type II molecules interact with similar regions in CD4, and EBV and C3d interact with the same region of CR2 (Clayton et al. 1989, Landau et al. 1988, Peterson & Seed 1988, Rao et al. 1985, Nemerow et al. 1989). The cell adhesion counter-receptor binding site may not be able to mutate in the host species without deleterious effects.

ICAM-1 has been shown be one of two receptors for Plasmodium falciparum-infected erythrocytes (Berendt et al. 1989). The primary event in the pathogenesis of malaria is the adherence of infected erythrocytes (RBCs) to endothelium in the liver. Increased levels of cytokines have been detected in acute malaria. Thus, induction of ICAM-1 expression on endothelium resulting from cytokines may allow *P. falciparum*-infected RBCs to bind endothelial cells.

ICAM-2. A second ligand, ICAM-2, has recently been identified by selecting for cDNA clones that, when expressed, would promote cell adherence to purified LFA-1 in the presence of mAb to ICAM-1 (Staunton et al. 1989b). ICAM-2 is an integral membrane protein with two immunoglobulin-like domains and is closely related to ICAM-1 (34% identity with the first two domains of ICAM-1). The ICAM-2 gene has 4 exons (Aguilar and Springer, in preparation). There is one exon for the signal sequence, one for each immunoglobulin domain, and one for the transmembrane/cytoplasmic tail sequences. This intron/exon organization is characteristic of many immunoglobulin superfamily members, but differs from that of NCAM. The Ig-like domains of NCAM are each encoded by 2 exons. Unlike ICAM-1, the expression of ICAM-2 is not affected by cytokines (Staunton et al. 1989b). ICAM-2 does not bind to the major or minor group of HRV (Staunton and Springer, unpublished).

Function of LFA-1. LFA-1 mediates a wide range of adhesion-dependent functions both in antigen-dependent and antigen-independent processes (Kishimoto et al. 1989a, Martz 1987). LFA-1 was first defined by screening for mAbs that block CTL-mediated killing (Davignon et al. 1981b). mAbs directed against LFA-1 have since been shown to inhibit virtually every immune phenomenon that involves T-

lymphocyte adhesion, including CTL-mediated killing, generation of CTL in culture, allo- and xenogenic mixed lymphocyte reactions (MLR), antigen-specific and Con A-induced T-cell proliferation, and T cell-dependent antibody responses. Several other functions that involve cell-cell adhesion are also inhibited, such as NK activity, LAK activity, γ-IFN induced monocyte homotypic adhesion, and spontaneous and PMA-induced homotypic adhesion of B lymphoblastoid cells and T-cell lines (reviewed in Springer et al. 1987 and Martz 1987).

Several immunofluorescence and microscopic studies have provided evidence that anti-LFA-1 prevents conjugate formation between CTL and target cells, and NK and target cells (Davignon et al. 1981a, b, Bongrand et al. 1983, Krensky et al. 1983). Kinetic immunofluorescence studies suggest that LFA-1 redistributes earlier than the TCR to the site of adhesion between T cells and antigen-presenting B cells (Kupfer & Singer 1989a, b). Redistribution occurs only in presence of specific antigen. In macrophage tumor cell adhesion systems, adhesions are initially weak, but become stronger over a period of 1.5 h. Anti-LFA-1 prevents strengthening but not initial, weak adhesions (Strassmann et al. 1986). As a whole, the data suggest LFA-1 functions early in T-cell target cell adhesion but an earlier adhesion event may occur in macrophage-tumor cell adhesion.

During the acute inflammatory response LFA-1 is involved in a number of antigen-independent processes such as interaction of leukocytes with endothelium, epithelial cells, synovial cells, keratinocytes, and fibroblasts (Dustin & Springer 1988, Dustin et al. 1986, Haskard et al. 1987, Mentzer et al. 1988, te Velde et al. 1987). These sort of interactions are necessary for localization, diapedesis, and migration of leukocytes.

Of these interactions, leukocyte-endothelial cell interaction has been studied most extensively (Dustin & Springer 1988, Haskard et al. 1986). Leukocyte adhesion to endothelial cells has revealed three pathways used in adhesion of PBLs, a B-lymphoid cell line and T-lymphoblasts to endothelial cells. The three pathways are: a) an LFA-1/ICAM-1-dependent pathway that provides weak basal adhesion but increases significantly in the presence of monokines or LPS, b) an LFA-1-dependent, ICAM-1-independent pathway that is basally present, not influenced by cytokines or LPS, and is consistent with a LFA-1/ICAM-2 interaction since ICAM-2 is basally expressed and not induced by cytokines on endothelial cells (Staunton et al. 1989b), and c) an LFA-1-independent pathway.

Mac-1

Complement receptor type 3. Mac-1 is a multifunctional receptor that binds an array of ligands. Mac-1 was first shown to bind iC3b-coated erythrocytes and to be equivalent to the complement receptor type 3 (Beller et al. 1982). Mac-1 mediates both adherence and phagocytosis of these E-iC3b particles on myeloid cells (Rothlein & Springer 1985), and on NK cells Mac-1 contributes to elevated NK activity against iC3b-coated targets (Ramos et al. 1988).

Microorganism interactions. Mac-1 has been implicated in a number of macrophage-microorganism interactions including binding to E. coli (Wright & Jong 1986) Histoplasma (Bullock & Wright 1987), and gp63 on Leishmania (Mosser & Edelson 1985, Russell & Wright 1988).

Adhesive interactions on myeloid cells. Mac-1 also mediates cell adhesive interactions on myeloid cells. MAbs directed against Mac-1 inhibit phorbol ester-induced neutrophil aggregation, chemotaxis, and adherence to protein-coated glass and plastic (Anderson et al. 1986, Dana et al. 1986). Experiments in laminar flow (Smith et al. 1989a) and $1 \times g$ inversion wash adhesion assays (Smith et al. 1989b) have suggested that LFA-1 and Mac-1 are both involved in neutrophil and monocyte binding to endothelial cells. The binding of unstimulated neutrophils to IL-1-stimulated endothelial cells is inhibited by only LFA-1 mAbs. In contrast, the binding of f-Met-Leu-Phe (fMLP)-stimulated neutrophils to IL-1-stimulated endothelial cells is inhibitable with both Mac-1 and LFA-1 mAbs. When the interaction of neutrophils to purified ICAM-1 on planar membranes was tested, fMLP-stimulated but not unstimulated neutrophil binding was inhibitable with both LFA-1 and Mac-1 mAbs. Therefore, unstimulated neutrophils exhibit LFA-1-dependent attachment to ICAM-1 and endothelial cells. Chemotactic stimulation with fMLP enhances the attachment of neutrophils to ICAM-1 by a Mac-1-dependent process. Additionally, it has been demonstrated that under appropriate conditions COS cells transfected with Mac-1 or ICAM-1 can bind purified ICAM-1 or Mac-1, respectively, coated on plastic (Diamond and Springer, unpublished data). However, interaction of ICAM-1 with LFA-1 can be demonstrated under conditions where there is no interaction of ICAM-1 with MAC-1, and thus the latter interaction is weaker (Larson and Springer, submitted). These observations suggest that ICAM-1 under certain conditions may act as a counter-structure for Mac-1. Consistent with this observation, activated PNMs form homoaggregates that are Mac-1-dependent, ICAM-1-dependent but LFA-1-independent (Anderson et al. 1986) (Diamond and Springer, unpublished data). Antibodies to Mac-1 have also been shown to block Fc-receptor-mediated phagocytosis, suggesting that these two molecules may be in close proximity (Brown et al. 1988).

Other ligands. Several other soluble ligands have been reported to bind Mac-1, including factor X (Altieri et al. 1988b) and fibrinogen (Altieri et al. 1988a, Wright et al. 1988). It is notable that a fibrinogen peptide inhibits both fibrinogen and iC3b binding to Mac-1, suggesting that a single binding site is used for at least two ligands for Mac-1 (Wright et al. 1988). However, all ligands do not use the same binding sites as the cell surface ligand and iC3b are blocked by different mAbs.

p150,95

Compelement receptor type 4. p150,95 has been reported to bind to iC3b coupled to Sepharose columns (Micklem & Sim 1985, Malhotra & Sim 1985) and anti-p150,95 mAb partially blocks binding of E-iC3b particles to macrophages (Myones et al. 1988). As a result, p150,95 has been termed the complement receptor type 4 (CR4). However, the authenticity of the iC3b activity is unclear since COS cells transfected with p150,95 do not bind E-iC3b (Corbi and Springer, unpublished data). It may be relevant that other C3 binding proteins (Cole et al. 1985) have been purified by C3 and C3b affinity chromatography that do not mediate rosetting of complement-sensitized particles.

Role in cell-cell adhesion. p150,95, like the other leukocyte integrins, has been implicated in a number of cell adhesion-dependent processes. p150,95 mAb inhibit peripheral blood monocyte and neutrophil adhesion to endothelium (Lo et al. 1989, Dianzani et al. 1989, Anderson et al. 1986), phagocytosis, and chemotaxis (Keizer et al. 1987a, te Velde et al. 1987).

p150,95 has also been reported on some activated T cells and lymphoid cell lines. Subsequently, anti-p150,95 mAb was found to inhibit conjugate formation by CTLs expressing high amounts of p150,95. These CTLs also expressed LFA-1 and the inhibitory effect was additive with LFA-1 and p150,95 mAb (Keizer et al. 1987b). Another group has reported no inhibition of CTL activity by p150,95 (Lanier et al. 1985). However, the level of p150,95 expression was not determined and differing expression could account for these results.

Functional redundancy and ancillary function of the leukocyte integrins

All three leukocyte integrins function in cell-cell adhesion in the immune system. The above discussion demonstrates a number of overlapping functions attributed to the leukocyte integrins, particularly on neutrophils and monocytes. They may even share the same ligand under certain conditions, i.e. LFA-1 and Mac-1 can both bind ICAM-1. However, the relative affinities differ and the physiological importance of the Mac-1/ICAM-1 and LFA-1/ICAM-1 interactions may also differ. Use of cells in a laminar flow system and purified functional protein may give some additional understanding to these interactions.

STRUCTURE-FUNCTION RELATIONSHIPS OF THE LEUKOCYTE INTEGRINS

Distinct binding sites for ligands. The leukocyte integrins are multifunctional and bind an array of ligands. Studies using several mAbs have mapped the functional domains of Mac-1 (Anderson et al. 1986, Dana et al. 1986). The mAbs 903, Leu-15, OKM10, and LM2/1 have been shown to inhibit iC3b binding to granulocytes

but do not inhibit granulocyte spreading or chemotaxis. The mAb 904 has reverse effects, inhibiting granulocyte spreading and chemotaxis but not influencing iC3b binding. These studies suggest the presence of two distinct functional domains, one for iC3b binding and one involved in granulocyte adhesion-dependent phenomena. Other ligands share the same binding site (see below).

Peptide studies. Binding of murine iC3b and fibrinogen to Mac-1 can be inhibited using a peptide containing a modified Arg-Gly-Asp (RGD) sequence (Wright et al. 1988). Namely, a dodecapeptide from fibrinogen containing the sequence Lys-X-X-Gly-Asp can inhibit both iC3b and fibrinogen interaction with Mac-1. Furthermore, this data suggests that at least two ligands of Mac-1, iC3b and fibrinogen, use a single binding site. In contrast, ICAM-1 does not contain an RGD sequence (Staunton et al. 1988, Simmons et al. 1988) and, correspondingly, RGD-containing or related peptides do not inhibit LFA-1/ICAM-1 interaction (Marlin & Springer 1987).

Transient cell surface expression of the leukocyte integrins. The cDNAs encoding the LFA-1, Mac-1, and p150,95 α subunits and the common β subunit have been inserted in the expression vector, CDM8. Cotransfection of an α subunit and the common β subunit cDNAs leads to cell surface expression of antigenically intact LFA-1, Mac-1, and p150,95 (Larson et al. 1989c). Immunoprecipitation studies have indicated that the subunits are associated, and the functional integrity of these molecules is maintained in that cells expressing LFA-1 and Mac-1 bind purified ICAM-1 bound to plastic and iC3b respectively (Larson et al. 1990a), Aguilar, Corbi and Springer, in preparation). The cDNA encoding the α or β subunit, when transfected alone, will lead to surface expression of the single chain at significantly lower levels than the $\alpha\beta$ heterodimers (Larson et al. 1990b). These α and β chains on the cell surface are not recognized by all subunit-specific mAb and may be associated with an inappropriate integrin VLA-α or β_1 subunit that normally is expressed in these cells.

Studies using chimaeric leukocyte integrin α subunits. Several Mac-1/p150,95 and LFA-1/Mac-1 chimaeric α subunit cDNAs have been recently constructed and transiently expressed on the cell surface or intracellularly in COS cells (Larson and Springer, unpublished, Aguilar, Corbi and Springer, manuscript in preparation). Twelve LFA-1/Mac-1 chimaeric α subunit molecules are not expressed on the cell surface when transfected into COS cells with the β subunit. Biosynthetic and immunoprecipitation studies have demonstrated that the chimaeric α subunit will not associate with the common β subunit. The LFA-1 and Mac-1 α subunits are only 35% identical (Larson et al. 1989a) and possibly both the α and β subunits have a globular region in the N-terminal half of the extracellular domain where they associate. Switching regions of the α subunits may disrupt folding interactions

and prevent proper folding of the α subunit, thereby affecting association with the β subunit and expression on the cell surface.

Eight of 10 chimaeric Mac-1/p150,95 α subunits are expressed on the cell surface of COS cells in association with the β subunit (Aguilar, Corbi, and Springer, in preparation). These molecules have been used to map the epitopes of a panel of antibodies to regions of each α subunit. The majority of mAbs tested are directed against the I domain of each α subunit. However, mAbs against the cation binding domains as well as the C-terminal region have been defined.

Structure-function studies using the β_1 subunit. Recently, stable murine 3T3 cells expressing the avian β_1 subunit have been produced (Solowska et al. 1989). The avian β_1 subunit is expressed on the cell surface as a heterodimer with either a murine α_3 or α_5 subunit. These interspecies hybrid heterodimers bind the fragment of fibronectin containing the cell attachment domain and localize in focal contacts where both extracellular matrix and cytoskeletal elements associate with the plasma membrane. A mutant form of the avian β_1 subunit lacking the cytoplasmic tail will also associate with murine α subunits and be expressed on the cell surface. However, this cytoplasmic tail-deleted form will not localize efficiently to focal contacts, indicating that the cytoplasmic domain is required for interaction with the cytoskeleton.

Domains in β_1 and β_3 subfamilies involved in ligand binding. Cross-linking of ligand peptides has been employed to localize the binding domains of the β_1 and β_3 integrins. Cross-linking of ^{125}I-labelled RGD-containing peptides to gpIIb/IIIa (D'Souza et al. 1988) and VNR (Smith & Cheresh 1988) has demonstrated that β_3 residues 109 to 171 and β_1 residues 61 to 203 are labelled most strongly. Interestingly, these residues fall within the region of 200 amino acids which is highly conserved among the three β subunits. The α subunit is labelled as well, and the RGD binding site has been localized to amino acid residues 294 to 314 (D'Souza et al. 1990).

Ligand recognition sequence. Peptide blocking studies have demonstrated that a variety of RGD-containing and RGD-related sequences can inhibit a number of integrin receptor/ligand interactions. Peptide fragmentation studies have examined integrin-dependent interactions of platelets and endothelial cells with fibrinogen (Cheresh et al. 1989b). There are two RGD sequences on fibrinogen. Endothelial cell and platelet integrins do not recognize the same RGD-containing sequence on fibrinogen. Furthermore, peptides containing RGD-related sequences have differential effects on blocking fibrinogen binding to either endothelial cells or platelets (Tranqui et al. 1989). Collectively, this data suggests that slight differences in RGD-containing recognition sites may account for specificity of ligand recognition. This further suggests a mechanism in which platelets and

endothelial cells can interact with distinct sites on fibrinogen during hemostasis and wound healing. Other peptide fragmentation studies have demonstrated the use of non-RGD-containing sites as well. Whereas VLA-5 binds to the RGD site, VLA-4 binds to distinct site(s) in an alternatively spliced segment of fibronectin (Humphries et al. 1989, Wayner et al. 1989). Fibrinogen binding to gpIIb/IIIa has also been shown to involve an RGD recognition site as well as a dodecapeptide sequence on the γ-chain of fibrinogen (Kloczewiak et al. 1984).

CELL SURFACE EXPRESSION, SIGNALLING AND REGULATION OF FUNCTIONAL ACTIVITY

As leukocytes differentiate, migrate and localize to tissue, the size, motility and adhesive properties of these cells change. Accordingly, cells will express different membrane proteins. A complex network of intracellular signals and protein interactions influences the expression and functional activity of these surface receptors. Leukocyte integrin expression and functional activity is regulated by numerous processes on different cell types, presumably fulfilling particular cell requirements in varying microenvironments.

Expression during differentiation

Leukocyte integrin cell surface expression and functional activity are regulated via several different processes. LFA-1 is expressed on virtually all leukocytes (Krensky et al. 1983) with the exception of some murine macrophages (Kürzinger et al. 1982, Strassmann et al. 1985a). Mac-1 and p150,95 are primarily expressed on cells of the myeloid lineage (Schwarting et al. 1985, Kürzinger et al. 1981). In addition, p150,95 is found on the cell surface of some activated lymphocytes (Miller et al. 1986, Schwarting et al. 1985, Keizer et al. 1987b, Lanier et al. 1985). Mac-1 is expressed on CD5$^+$ B cells (de la Hera et al. 1988) and lymphokine-activated killer cell precursors (Dianzani et al. 1989).

The leukocyte integrins are first expressed during differentiation of hematopoietic stem cells (Campana et al. 1986, Miller et al. 1985). LFA-1 has been detected as early as μ chain-positive pre-B cells and late myeloblasts (Campana et al. 1986). Mac-1 and p150,95 have been detected on committed granulocyte and monocyte precursors in the bone marrow (Miller et al. 1985).

These *in vivo* observations correlate with *in vitro* differentiation studies using the promyelomonocytic cell line, HL-60 (Miller et al. 1986, Corbi et al. 1987, 1988b). Unstimulated HL-60 cells express LFA-1 but not Mac-1 or p150,95. Phorbol ester or retinoic acid will induce HL-60 cells to differentiate toward monocytes or granulocyte lineages respectively. In both cases, Mac-1 and p150,95 *de novo* synthesis occurs as indicated by an increase in cell surface expression, protein synthesis, and mRNA.

In the circulation, peripheral blood monocytes have been shown to change their relative expression of Mac-1 and p150,95 when they extravasate from the blood vessel and differentiate into tissue macrophages. Mac-1 is more highly expressed than p150,95 on peripheral blood mononuclear cells but with extravasation Mac-1 expression decreases and p150,95 increases (Miller et al. 1986, Schwarting et al. 1985).

Although virtually all leukocytes express LFA-1, the level of expression is influenced by several factors. Resting peritoneal macrophages do not express LFA-1 but *in vivo* Bacillus Calmette-Guerin (BCG) elicitation greatly increases LFA-1 expression (Strassmann et al. 1985a). The conversion from naive to memory T cells increases the expression levels of several adhesion molecules, including LFA-1 (Sanders et al. 1989). Cytokine stimulation *in vitro*, including γ-interferon treatment of murine macrophages (Strassmann et al. 1985b) and IL-4 treatment of B cell lymphoma cell lines (Rousset et al. 1989), increases LFA-1 expression, demonstrating regulation by cytokines. Viral infection can also influence LFA-1 expression as the latent infection membrane protein (LMP) gene of the EBV virus will increase the level of mRNA and cell surface expression of LFA-1 (Wang et al. 1988).

Upregulation events

After *de novo* synthesis, Mac-1 and p150,95 are stored in intracellular pools in monocytes and polymorphonuclear cells (Todd et al. 1984, Miller et al. 1987, Bainton et al. 1987). This pool can be rapidly mobilized in response to a variety of chemoattractants including fMLP, C5a, and leukotriene B4 resulting in a 10-fold increase in Mac-1 and p150,95 cell surface expression within minutes (Springer et al. 1984, Lanier et al. 1985, Berger et al. 1984, Todd et al. 1984, Miller et al. 1987, Bainton et al. 1987). LFA-1 expression increases only 2-fold in monocytes and does not change on neutrophils. The intracellular pool of Mac-1 and p150,95 has been shown to localize to peroxidase-negative granules in monocytes and granulocytes (Miller et al. 1987, Bainton et al. 1987).

Regulation of functional activity and cell signaling

1. *LFA-1.* a. Intracellular signalling. Some LFA-1- and ICAM-1-expressing B-cell lines will not, or will only mildly, aggregate spontaneously when grown in culture (Mentzer et al. 1985). However, phorbol ester treatment of these cell lines will cause a rapid increase in homotypic adhesion (Rothlein et al. 1986a, Rothlein & Springer 1986b, Patarroyo et al. 1985). These aggregates are inhibitable with mAb against LFA-1 (Rothlein & Springer 1986b, Patarroyo et al. 1985) or ICAM-1 (Rothlein et al. 1986a). Furthermore, this aggregation is not associated with significant quantitative changes in the cell surface expression of LFA-1 and

ICAM-1 (Rothlein et al. 1986a). In a homotypic adhesion assay, avidity regulation of ICAM-1 or LFA-1 cannot be distinguished. Recent studies with purified LFA-1 or ICAM-1 have allowed regulation of the binding activity of these cellular counter-receptors to be individually assayed. This has shown that LFA-1, but not ICAM-1, functional activity is regulated by phorbol ester treatment (Dustin & Springer 1989).

A linkage via intracellular signals between the TCR and LFA-1 has recently been demonstrated (Dustin & Springer 1989). Cross-linking the TCR transiently increases the strength of the LFA-1/ICAM-1 interaction by regulating the adhesiveness of LFA-1. PMA treatment of T cells causes a sustained increase in LFA-1 avidity. Treatment of T cells with cyclic AMP analogues prior to cross-linking of the antigen receptor strongly inhibits TCR-stimulated LFA-1 adhesion to ICAM-1 but has no effect on PMA-stimulated adhesion. This observation suggests that intracellular signals control LFA-1 avidity, that these signals link the TCR and LFA-1, and that LFA-1 can transduce signals from the cytoplasm to the extracellular environment, i.e. "inside-out signalling."

These observations have led to a model of cooperation between LFA-1 and the TCR in mediating antigen recognition (Dustin & Springer 1989). Resting T cells express LFA-1 in a low avidity state. When a CTL or helper T cell contacts appropriate antigen in association with MHC, an intracellular signalling event leads to the conversion of LFA-1 from a low to high avidity state. The TCR triggers a cascade of kinetically staggered second messengers such that an early signal leads to an increase in LFA-1 avidity while a later signal lowers LFA-1 activity. The kinetics of this cascade will be influenced by the number of TCR engaged. This provides a mechanism for regulating adhesion and de-adhesion. Additionally, it is important to remember that this adhesion strengthening role is necessary for T-cell stimulation (see LFA-1 function section).

b. Co-stimulatory signal. Human T-cell clones can be activated with mAb to certain surface glycoproteins, and this may be enhanced when two different surface glycoproteins are crosslinked together. These combinations include CD3 and HLA-A,B,C, CD3 and CD4/CD8, HLA-A,B,C and CD4/CD8, and CD3 and LFA-1 (Wacholtz et al. 1989). The combination of mAbs against CD3 and LFA-1 followed by crosslinking results in a prolonged increase in calcium flux and an enhancement in IL-2 production and proliferation. MAbs against LFA-1 alone do not have any of these effects. LFA-1 mAb may also regulate proliferative responses to CD3 mAb on substrates (van Noesel et al. 1988), and a mAb to an LFA-1 activation epitope can by itself stimulate T-cell responses (Pircher et al. 1986). LFA-1 may be acting as a co-stimulatory signal for T cells when the CD3 complex is engaged. The ability of LFA-1 and CD3 to be co-stimulatory and for the LFA-1 avidity state to be influenced by intracellular signals demonstrates how LFA-1 participates in both "inside-out" and "outside-in" signalling.

c. Phosphorylation. Phorbol esters induce LFA-1-dependent homotypic ad-

hesion of leukocytes and activate protein kinase C. Phorbol esters have also been shown to cause a transient phosphorylation of the LFA-1 β chain in peripheral blood lymphocytes and $CD4^+$ and $CD8^+$ cells (Hara & Fu 1986) (Gallis, Larson, and Springer, unpublished data).

d. Activation epitopes. Unlike most mAbs against LFA-1 that typically block adhesion, the mAb NKI-L16 induces homotypic adhesion of B-lymphoblastoid cells (Keizer et al. 1988). This mAb was shown to be directed against the LFA-1 α subunit. This induction by NKI-L16 is inhibitable by LFA-1 blocking antibodies, and NKI-L16 activation is equivalent to phorbol ester treatment. MAb to another activation epitope on LFA-1 can induce proliferation and lymphokine release in cloned T cells (Pircher et al. 1986). Morimoto et al. have also reported two LFA-1 mAbs, one of which can distinguish killer effector and suppressor cells (Morimoto et al. 1987), and one which will enhance $T11_2/T11_3$ T-cell stimulation (Morimoto and Schlossman, personal communication).

e. Cytoskeleton. Several studies have suggested an interaction of leukocyte integrins with cytoskeletal elements. LFA-1-dependent adhesion is disrupted by cytochalasin B (Rothlein & Springer 1986b). LFA-1 and actin filaments or talin colocalize to the site of contact between NK cells or T cells and their target cells (Carpen et al. 1986, Burn et al. 1988, Kupfer & Singer 1989a, b). Redistribution of LFA-1 and talin to the site of adhesion is an early event and may be signalled by antigen receptor engagement.

2. *Mac-1.* a. Dissociation of upregulation and functional activity. Neutrophils form aggregates upon stimulation which are inhibitable with anti-Mac-1 mAbs. Interestingly, pretreatment of these neutrophils with Mac-1 mAbs to coat surface Mac-1 did not affect upregulation; the surface expression is increased 5- to 10-fold but neutrophil aggregation did not occur. This experiment has led to the suggestion that Mac-1 in the intracellular granules is not functionally active (Buyon et al. 1988), and that stimulation leads to a qualitative change in cell surface Mac-1. Consistent with these observations, a study using a laminar flow system indicated that fMLP-stimulated but not -unstimulated polymorphonuclear cells (PMNs) could bind endothelial cells (Smith et al. 1989a). Transient fMLP-stimulated adhesion of neutrophils to endothelium has been reported (Tonnesen et al. 1984, Lo et al. 1989). Although a recent report implied that transience was due to regulation of Mac-1 (Lo et al. 1989), it failed to show any inhibition of transient adhesion by Mac-1 mAb. Other studies demonstrated that treatment of neutrophils with 4,4′-diisothiocyanostilbene-2,2′ disulfonic acid (DIDS) inhibited Mac-1 upregulation and neutrophil aggregation (Vedder & Harlan 1988). DIDS did not affect Mac-1-dependent binding of neutrophils to endothelial cells. Collectively, this data suggests that upregulation and functional activity of Mac-1 are dissociated events; however, the precise nature of the regulation of Mac-1 functional activity is unclear.

b. Receptor clustering. Activity may also be regulated by cross-linking of receptors. Phorbol-ester-induced iC3b activity of Mac-1 correlates with aggregation of Mac-1 in small clusters on the cell surface. However, the chemoattractant fMLP induces iC3b binding activity but fails to induce clustering, shading the significance of this observation (Detmers et al. 1987).

c. Association with other cell surface receptors on the cell surface. Several studies have implicated an interaction of leukocyte integrins with other membrane proteins which may influence binding activity. Neutrophils have an antigen of 157kd. MAb directed against this antigen inhibit the iC3b receptor activity of Mac-1, but this inhibition is largely overcome if protease inhibitors are added. This antigen is present on cells from patients with LAD (Pytowski et al. 1988).

MAb against Mac-1 can inhibit Fc receptor-mediated phagocytosis, suggesting a relationship of these two molecules. Brown et al. (1988) further characterized surface Mac-1 in two populations, freely diffusible (60%) and non-diffusible (40%) fractions on the membrane surface, suggesting a relationship of a population of Mac-1 with the cytoskeleton.

DISEASE STATES INVOLVING THE LEUKOCYTE INTEGRINS

LAD deficiency

Patients with the clinical syndrome, leukocyte adhesion deficiency (LAD), suffer from chronic granulocytosis, recurrent gingivitis and lack of pus formation. Leukocytes from these patients lack expression of all three leukocyte integrins on the cell surface due to heterogeneous defects in the β subunit. Patients with LAD are categorized as severely (<1% of the normal level of cell surface expression) and moderately deficient (3–10% of the normal level of expression). Level of cell surface expression of the leukocyte integrins correlates with the severity of disease as moderately deficient patients may survive to adulthood with treatment of recurrent infections while severely deficient patients will die early in childhood due to overwhelming infection unless they receive a bone marrow transplant (reviewed in Anderson & Springer 1987).

A number of biosynthetic and mouse-human hybrid studies initially showed that the lack of cell surface expression was due to defects in the β subunit (Springer et al. 1984, Lisowska-Grospierre et al. 1986). In early studies, mouse-human lymphocyte hybrids showed that interspecies complexes could form and surface expression of the human α subunit but not the β subunit could be rescued from patient cells, indicating that the α subunit but not the β subunit is normal and competent for cell surface expression (Marlin et al. 1986). Recently, EBV-transformed cell lines from 4 patients have been transfected with β subunit cDNA. In all cases, cell surface expression of the LFA-1 heterodimer is rescued and functional activity as measured by homotypic adhesion and binding to purified ICAM-1 was regained (Hibbs et al. 1990).

Five mutant phenotypes of β subunit expression and structure have been characterized in LAD (Springer et al. 1984, Kishimoto et al. 1989a). The first two classes are possibly due to defects in β subunit gene expression. In one class of mutation, no mRNA or protein precursor is detectable. Southern analysis has suggested that no large deletions in the β subunit gene are present. In a second class of mutation, trace amounts of both mRNA and protein precursor are synthesized. Other mutations affect the β subunit protein structure. One patient with an aberrantly small β subunit has been extensively studied. This patient has a 90 base pair in-frame deletion corresponding to a single exon. This patient has a G to C substitution in the 5′ splice site, suggesting that abnormal splicing occurs, leading to the deletion of this region of the molecule. The final class of mutations is characterized by a normal size β subunit mRNA and protein precursor. cDNAs cloned from 2 of these patients show different point mutations, both occurring in the highly conserved 180 amino acid region of the β subunit (Wardlaw et al. 1990). Expression and mutagenesis studies using these cDNAs have suggested that these point mutations are responsible for the lack of $\alpha\beta$ association and are not phenotypic differences.

The molecular basis for some of the severe and moderate deficiency phenotypes have been clarified. In the case of deficiency of β subunit mRNA and protein precursor, severe and moderate deficiency correlates with no detectable or decreased amounts of mRNA and protein precursor, respectively. One of the moderately deficient patients has a small deletion in the β subunit due to aberrant splicing of the β subunit gene (see above) (Kishimoto et al. 1989c), leading to a form of the β subunit that cannot associate with the α subunit. However, a small amount of message is spliced normally as determined by S1 nuclease protection studies, indicating that enough normal β subunit may be produced to account for the low levels of surface expression and moderate phenotype of this patient.

Animal models for LAD

Animal models for LAD, allowing for experimental manipulation *in vivo*, will be of significant use in studying the *in vivo* function of the leukocyte integrins. A canine LAD model appearing completely analogous to the human has been identified (Giger et al. 1987). As a second approach, mAbs against the leukocyte integrins have been administered to duplicate a LAD state. Peritoneal macrophages cannot be elicited with thioglycolate in mice injected with anti-Mac-1 (Rosen & Gordon 1987). Leukocytes from rabbits injected with anti-β subunit mAb do not bind to endothelium and their extravasation is prevented (Arfors et al. 1987). Both these studies suggest that the leukocyte integrins and possibly Mac-1 alone play an important role in leukocyte extravasation.

Importance in immunosurveillance and metastasis

LFA-1 mAbs have been shown to block virtually all immune responses requiring cell-cell adhesion. Recently, it has been demonstrated that a proportion of lymphomas including the majority of fresh Burkitt's lymphomas fail to express LFA-1 on the cell surface (Clayberger et al. 1987). These Burkett's lymphoma cells are poor stimulators of both autologous and allogeneic T-cell responses, suggesting that these tumor cells lacking LFA-1 cannot initiate efficient immune responses which might contribute to the escape of these tumors from immunosurveillance. Some Burkitt's lymphoma cells have been shown to be deficient in ICAM-1 and LFA-3 as well (Billaud et al. 1987). Taken together, these observations imply that a decrease in one or a combination of adhesion molecules might be a general means of escape from immunosurveillance.

Overexpression of β subunit in Down's syndrome

Patient's with trisomy 21, Down's Syndrome, have a number of abnormalities in immune function. Since the β subunit gene is located on chromosome 21, the cell surface expression and function of LFA-1 on EBV-immortalized cells from Down's syndrome patients has been studied (Taylor et al. 1986, 1988a). These cells aggregate in response to phorbol ester more readily than normal cells. The aggregation was inhibited by mAb against both the LFA-1 α and β chains. Taylor et al. inferred that the increased adhesiveness was a result of over-expression due to the additional gene (Taylor et al. 1988b).

AIDS

Recently, mAbs directed against LFA-1 have been shown to strikingly inhibit syncytia formation induced by HIV (Hildreth & Orentas 1989).

POTENTIAL THERAPEUTIC APPLICATIONS

Kidney allograft rejection

Anti-ICAM-1 mAb has been used successfully in conjunction with cyclosporine in prolonging kidney allografts acceptance in monkeys. Presumably, ICAM-1 mAbs are blocking the leukocyte-endothelium interaction and preventing leukocyte extravasation into the tissues (Cosimi et al. 1989).

Reperfusion injury

Leukocytes promote much tissue damage following ischemia-reperfusion injury. As a result, the effect of the leukocyte adhesion molecules in preventing leukocyte-

mediated damage has been studied in models of ischemia-induced myocardial infarct and shock. Damage to cardiac tissue is inhibited 50% by anti-Mac-1 antibodies in a dog model of myocardial infarct (Simpson et al. 1988) and survival is greatly increased by administration of anti-β subunit mAb in a rabbit model of ischemic shock (Vedder et al. 1988). These findings again demonstrate the critical *in vivo* role of the leukocyte integrins and their ligands, and the potential of these molecules as targets for therapeutic intervention in a number of human diseases.

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