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Human Leukocyte Adhesion Deficiency: Molecular Basis for a Defective Immune Response to Infections of the Skin

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Introduction

The normal function of the immune response in the skin requires the ability of peripheral blood leukocytes to mobilize to the site of infection. In a classical inflammatory reaction, neutrophils and monocytes migrate in response to chemotactic factors released at the infection site. However, until recently, the molecular mechanisms which enable leukocytes to migrate across blood vessels have been largely unknown. These mechanisms have been elucidated, in part, by the characterization of a recently defined disease called human leukocyte adhesion deficiency (LAD). Patients with this heritable disease suffer from severe recurrent bacterial and fungal infections of soft tissues, primarily skin and mucous membranes. Infected, necrotic lesions in these patients contain few leukocytes despite the observation that these patients have chronic leukocytosis. In the early 1980s, several laboratories demonstrated that this disease is due to a cell surface deficiency of the LFA-1, Mac-1, and p150,95 family of leukocyte adhesion proteins. More recently we have shown that LAD is due to heterogenous mutations in the β subunit common to all three of these leukocyte glycoproteins. Although LAD is a rare disease, the analysis of this disease has greatly increased our understanding of the biology of the leukocyte adhesion proteins and the molecular mechanisms involved in the immune response to infections of the skin.

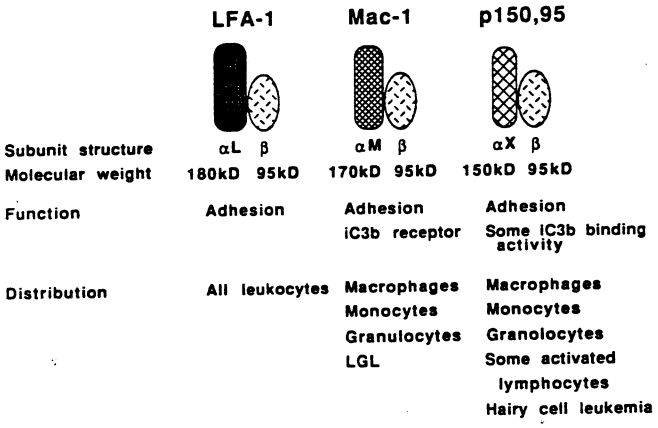

Fig. 1. The LFA-1, Mac-1, p150,95 family of leukocyte integrins.

The LFA-1, Mac-1, and p150,95 Family of Leukocyte Adhesion Proteins

LFA-1, Mac-1, and p150,95 constitute a family of structurally and functionally related $\alpha_i\beta_i$ heterodimers [Sanchez-Madrid et al., 1983] (fig. 1). The α subunits of LFA-1, Mac-1 and p150,95 are distinct (180,000, 170,000, and 150,000 daltons, respectively) and designated CD11a, b, and c, respectively, in the international nomenclature. In contrast, the β subunit of 95,000 daltons (CD18) is identical in all three proteins [Sanchez-Madrid et al., 1983; Kishimoto et al., 1987c]. During the biosynthesis of these proteins, the newly synthesized α subunit precursor associates with the β subunit precursor (fig. 2). The immature αβ heterodimer is transported through the Golgi apparatus, where the N-linked carbohydrates are processed to a mature form, and then the mature protein is exported to the cell surface and to granular compartments [Sanchez-Madrid et al., 1983; Springer et al., 1984].

Monoclonal antibodies directed against the LFA-1, Mac-1, and p150,95 family can inhibit a wide spectrum of adhesion-related functions of immune cells. LFA-1, which is expressed by virtually all immune cells, is a general adhesion protein which helps to mediate both antigen-specific interactions (i.e. CTL-mediated cytolysis and antigen presentation) as well as antigen-independent interactions (i.e. phorbol ester-induced aggregation and binding to endothelial cells) [reviewed in Springer et al., 1987]. A natural ligand for LFA-1 is ICAM-1 [Marlin and Springer, 1987], a widely distributed molecule

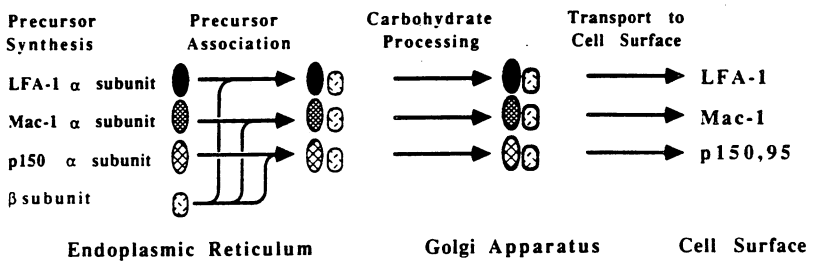

Fig. 2. Biosynthesis of the leukocyte integrins.

which is prominent on activated endothelial cells during inflammation [Rothlein et al., 1986; Dustin et al., 1986]. Mac-1 and p150,95 expression is limited to monocytes, macrophages, granulocytes, and large granular lymphocytes [Sanchez-Madrid et al., 1983], although p150,95 is also found on some activated lymphocytes [Miller et al., 1986]. Mac-1 and p150,95 are present in intracellular pools in monocytes and granulocytes as well as on the cell surface [Todd et al., 1984; Miller et al., 1987]. Chemotactic factors, such as fMLP, induce mobilization of this intracellular pool to the cell surface. This 10-fold increase in surface levels of Mac-1 and p150,95 may aid in the migration of leukocytes from the blood into infected tissues. Cell activation also leads to apparent qualitative changes in Mac-1 which induces binding activity [Wright and Meyer, 1986; Vedder and Harlan, 1988]. Both Mac-1 and p150,95 have been shown to bind the iC3b fragment of the complement cascade [Beller et al., 1982; Micklem and Sim, 1985], although there are probably other natural ligands as well.

Recently the genes encoding the leukocyte adhesion proteins have been cloned and sequenced. The β subunit is a cysteine-rich integral membrane polypeptide [Kishimoto et al., 1987c; Law et al., 1987). There is a 4-fold repeat of an unusual cysteine motif, which probably gives the β subunit a very rigid structure. The α subunits of p150,95 [Corbi et al., 1987], Mac-1 [Corbi et al., manuscript in preparation], and LFA-1 [Larson et al., manuscript in preparation] share 35–63% amino acid identity. All three α subunits contain repetitive sequences which are homologous to the metal binding domains of calmodulin. These domains are likely to serve a similar function for the leukocyte adhesion proteins, since adhesion is Mg^{++}-dependent [Martz, 1977]. The β subunit has been mapped to chromosome 21q22, and all three α subunits have been mapped to chromosome 16p11–16p13.1 [Corbi et al., 1988].

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Gene cloning has revealed that the LFA-1, Mac-1, and p150,95 are members of the integrin superfamily of adhesion proteins [Kishimoto et al., 1987b,c; Hynes, 1987]. There are three integrin subfamilies, each defined by a common β subunit which shares multiple distinct α subunits. Hence, LFA-1, Mac-1, and p150,95 represent one subfamily, the leukocyte integrins. A second subfamily is comprised of at least five VLA antigens [Hemler et al., 1987], which includes the human fibronectin receptor. The third subfamily includes the vitronectin receptor and platelet glycoprotein IIb/IIIa [Ginsburg et al., 1987]. The latter two subfamiles are receptors involved primarily in cell-matrix interactions, and are thought to play a role in guiding embryogenesis, development, and wound healing. The term integrin denotes that these receptors are thought to bridge the interaction of the extracellular environment (matrix and other cells) with the intracellular cytoskeletal network [Tamkun et al., 1986;Hynes, 1987]. The relationship of the LFA-1, Mac-1, and p150,95 molecules to the integrin superfamily underscores the importance of the molecules in mediating immune cell adhesion.

Clinical Manifestations 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 [reviewed by Anderson and Springer, 1987]. The infectious microbes include a wide spectrum of fungi and bacteria, but most commonly staphylococcal or gram-negative enteric bacteria. Infections may progress locally or systemically. Typical small nonpustular skin lesions progress to form large ulcerative lesions. These lesions are slow to heal and largely devoid of granulocytes, despite chronic peripheral blood leukocytosis (5–20 × normal levels). The failure of leukocytes to mobilize is also observed in rebuck skin window assays.

Early biochemical analysis showed that granulocytes from these patients were missing a high molecular weight glycoprotein. This missing glycoprotein was subsequently shown by immunochemical analysis to be the LFA-1, Mac-1, and p150,95 family [reviewed in Anderson and Springer, 1987]. Heterogeneity in the defect causing this disease was first observed in the extent of the glycoprotein deficiency at the cell surface. Patients were classified as severely deficient (<0.5% normal levels of expression) and moderately deficient (3–10% normal levels of expression). The extent of the protein deficiency is also reflected in the severity of the clinical complica-

tions. Severely deficient patients usually die in childhood from overwhelming bacterial and fungal infections, despite aggressive anti-microbial therapy. Moderately deficient patients have a much better prognosis, although they too suffer from recurrent infections.

Insights from the Study of LAD into the Biology of Leukocyte Adhesion

Analysis of leukocyte adhesion deficiency disease has yielded a wealth of information about the role of the leukocyte adhesion proteins in vivo, particularly in regard to their role in leukocyte mobilization. In vitro, lymphocytes, monocytes, and granulocytes from these patients display profound defects in the same spectrum of adhesion-related functions that are inhibitable by MAb against the leukocye adhesion proteins [reviewed extensively in Anderson and Springer, 1987; Springer et al., 1987]. These observations demonstrate that LFA-1, Mac-1, and p150,95 are adhesion receptors, and rule out the trivial explanation of negative signaling induced by MAb-binding.

In vitro study of cells from LAD patients also led to the identification of a natural ligand for LFA-1. Phorbol ester-induced homotypic aggregation of immune cells is an LFA-1-dependent phenomenon. Rothlein and Springer [1986] showed that LFA-1$^-$ LAD cells do not form homotypic aggregates; however, LFA-1$^+$ control cells can form aggegates with LFA-1$^-$ LAD cells. These results suggested that LFA-1 binds a ligand distinct from itself, and that LAD cells should express the ligand. Rothlein et al. [1986] raised MAb against LFA-1$^-$ LAD cells 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 90,000-dalton molecule called ICAM-1, which fit these criteria. ICAM-1 is a widely distributed molecule, whose expression is upregulated by a variety of cytokines, including interleukin-1, tumor necrosis factor, and γ-interferon [Dustin et al., 1986]. ICAM-1 expression is prominent on activated endothelial cells during inflammation [Dustin et al., 1986], as would be expected if LFA-1 is important in mediating leukocyte mobilization. Marlin and Springer [1987] showed that purified ICAM-1 incorporated into planar lipid membranes can mediate binding of LFA-1$^+$ cells but not LFA-1$^-$ LAD cells. This binding is inhibitable by pretreating cells with LFA-1 MAb or pretreating the planar membrane with ICAM-1 MAb. ICAM-1, which has recently been cloned and sequenced [Simmons et al., 1988; Staunton et al., 1988], has been shown to be a member

Table I. Classification of LAD mutations

Class	Number of patients	Phenotype	β precursor	β mRNA levels	Reference
I	2	severe	none detectable	none detectable	Kishimoto et al., 1987a
	2	?	none detectable	?	Dimanche et al., 1987
II	1	moderate	little or none	low	Kishimoto et al., 1987a
III	4(related)	moderate	aberrantly small	normal	Kishimoto et al., 1987a
IV	1	severe	aberrantly large	normal	Kishimoto et al., 1987a
V	1	severe	normal-size	normal	Kishimoto et al., 1987a
	2	severe	normal-size	normal	Dana et al., 1987
	1	moderate	normal-size	normal	Kishimoto et al, 1987a
	2	moderate	normal-size	normal	Dana et al., 1987
	1	moderate	normal-size	normal	Wardlaw et al., unpublished
	1	?	normal-size	?	Dimanche et al., 1987

of the immunoglobulin superfamily with closest homology to the neural adhesion molecules NCAM and MAG.

Molecular Basis of Leukocyte Adhesion Deficiency

Early studies strongly suggested that a defect in the β subunit common to LFA-1, Mac-1, and p150,95 could account for LAD [Springer et al., 1984; Marlin et al., 1986]. To define the defects in the common β subunit, our laboratory has developed two molecular tools. The first is a rabbit antiserum directed against purified, denatured β subunit, which would allow us to study the β subunit precursor in classical bioynthesis studies. The second is the cDNA clone encoding the β subunit, which would allow us to study β subunit mRNA expression and genomic DNA organization. Using these two tools we provided the first evidence that mutations in the β subunit cause LAD, and that the mutations are heterogenous [Kishimoto et al., 1987a]. Ten LAD patients, 13 kindred, and 4 healthy controls were studied. Five phenotypes of β subunit expression and structure were identified (table I).

Two classes of mutations resulted in little or no mRNA or protein precursor. Southern analysis of genomic DNA from these patients showed no

gross deletions of the β subunit gene. One moderately deficient patient synthesized trace amounts of the β subunit precursor and low levels of mRNA message. Apparently, this low level of expression is sufficient to account for the moderate phenotype (i.e. 3–10% normal surface expression of the leukocyte integrins) and less severe clinical complications. Dimanche et al. [1987] studied 2 patients with no apparent β subunit precursor synthesis which 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. One patient synthesizes an aberrantly large β subunit precursor. However after endoglycosidase H digestion, the protein backbone appears about normal size. One hypothesis is that some point mutation causes an amino acid change which creates a novel consensus N-glycosylation site (Asn-X-Ser/Thr). Four moderately deficient patients, who are all related, synthesize an aberrantly small precursor, which is 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 glycosylation. The deletion in the protein is reflected in the mRNA message; S1 nuclease protection assay shows a deletion of 90 bp. Moreover, this deletion is due to an aberrant splicing event [Kishimoto et al., manuscript in preparation].

Finally 3 unrelated patients studied by us [Kishimoto et al., 1987a; Wardlaw et al., unpublished], a group of 4 patients studied by Dana et al. [1987], and one patient studied by Dimanche et al. [1987] synthesized both a normal size β subunit precursor and a normal size α subunit precursor. Neither subunit is 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 possibility of a mutation in the α subunit. This class of mutation includes both severely and moderately deficient patients; thus there must be at least two distinct mutations.

Therapy for LAD

Patients of the severe deficiency phenotype have a high incidence of death in early childhood due to overwhelming microbial infections. Bone marrow transplantation successfully restored leukocyte function in 4 severe-

ly deficient patients [Fischer et al., 1988]. The children, now 6, 4, 2.5, and 1 years after transplantation, are healthy and require no further treatments. Two other patients had successful engraftments, but one died of chronic graft-versus-host disease and another died in an accident.

The recognition that LAD is a monogenic disease makes it a good candidate for gene therapy. Once the technology for gene therapy becomes available, it should be relatively straightforward to introduce a normal β subunit gene into hematopoietic stem cells. Virtually all leukocytes normally express the β subunit. In addition, surface expression of the LFA-1, Mac-1, and p150,95 proteins would be regulated by the appropriate synthesis of the α subunits.

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