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Chapter 11

Functional and Structural Interrelationships among the Mac-1, LFA-1 Family of Leukocyte Adhesion Glycoproteins, and their Deficiency in a Novel Heritable Disease

TIMOTHY A. SPRINGER and DONALD C. ANDERSON

1. Introduction

Cell surface adherence reactions are of central importance in the immune functions of lymphocytes, monocytes, and granulocytes. Lymphocytes adhere to antigen-presenting macrophages or dendritic cells in the induction of T-lymphocyte immune responses, and to target cells in cell-mediated killing. Adhesive interactions are fundamental to a wide sprectrum of functions of granulocytes, monocytes, and macrophages. Specific recognition of opsonized microorganisms is facilitated by membrane receptors for IgG and for the third component of complement (C3), which mediate microbe–cell adhesion prior to the triggering of cytoskeletal events leading to endocytosis. Adhesion mediated by IgG (Fc) receptors can also trigger antibody-dependent killing of target cells, independently of endocytosis. In the absence of opsonins, some microorganisms/particles may adhere to granulocytes/monocytes without undergoing ingestion or may be phagocytized inefficiently, depending on the physical properties of the microorganisms.[1]

Cell translocation *in vitro* and *in vivo* has also been shown to be influenced by the precise nature of cell–substrate adherence interactions.[1–3] Studies employing time-lapse photography or photomicrography have shown that granulocytes adhere preferentially to vascular endothelium adjacent to a site of inflammation.[4] Evidence exists that physical properties of endothelial cells or

TIMOTHY A. SPRINGER • Laboratory of Membrane Immunochemistry, Dana-Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115. DONALD C. ANDERSON • Baylor College of Medicine, Department of Pediatrics, Houston, Texas 77050.

experimental substrates (glass, plastic, albumin, fibrinogen, fibronectin) influence adherence, and secondarily the extent and direction of cell migration.[3,5] Substrates that promote irreversible adhesion or those that significantly diminish or prevent adherence effectively impair cell translocation. Directed migration (chemotaxis) appears to require intermittent adhesion that is sufficiently strong to allow attachment to a substrate but sufficiently localized temporally to allow selective detachment.[6]

A family of high-molecular-weight glycoproteins with identical β subunits has recently been characterized on leukocyte surfaces that is important in many of the above adhesion reactions. Monoclonal antibodies have been instrumental in elucidating the structural interrelationships and functions of these three glycoproteins, macrophage antigen 1 (Mac-1), lymphocyte function-associated antigen 1 (LFA-1), and p150,95. Furthermore, this research has led to the definition, with monoclonal antibodies, of a novel heritable disease that manifests itself in defects in leukocyte adherence and motility.

Using this glycoprotein family as an example, this chapter emphasizes advances in research on the structure and function of cell surface proteins and their abnormalities in disease that have uniquely been made possible by the monoclonal antibody technique of Köhler and Milstein.

2. Probing Cell Surface Functions with Monoclonal Antibodies

The lymphoid immune system has been extensively studied as a model of cell adhesion, both because its cells circulate and can be readily obtained in suspension, and because adhesive interactions between T cells and other cells are extremely important in the development, regulation, and expression of specific immunity. The interaction between cytolytic T lymphocytes (CTL) and target cells has proved particularly amenable to study. CTLs are readily elicited to virus-infected cells or to histoincompatible foreign cells. CTLs adhere to and lyse target cells bearing specific antigen. Prior to the introduction of monoclonal technology, CTL-mediated killing had been shown to consist of a Mg^{2+}-dependent adhesion step and a Ca^{2+}-dependent lethal hit delivery step[8]; however, at the molecular level, CTL-mediated killing was essentially a "black box." With monoclonal antibody probes, the structure and function of a number of molecules participating in the cytolytic T lymphocyte–target cell interaction have now been elucidated (reviewed by Krensky *et al.*, this volume, Chapter 35). These include the T-lymphocyte receptor for specific antigen and a molecule with a more general role in leukocyte adhesion reactions, lymphocyte function-associated antigen (LFA-1).

The use of highly specific "functional screening" strategies has allowed MAbs to cell interaction molecules on CTLs to be readily identified, despite the use of whole cells to elicit the antibodies[9] (Fig. 1). In studies on mouse CTL-mediated killing, rats were immunized to mouse CTLs and 1000 rat × mouse hybridoma cultures were established per spleen. Culture supernatants containing secreted antibodies were harvested from the hybridomas 2 weeks

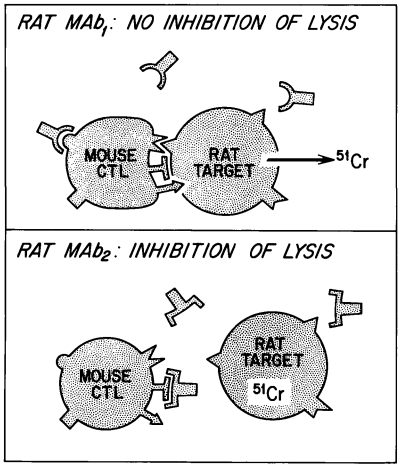

Figure 1. Screening for monoclonal antibodies blocking CTL effector cell function. Rat anti-mouse CTL MAbs are tested for blocking of mouse CTLs with specificity for rat lymphoma target cells. Antibodies that might block by binding to the target are avoided, since the target cell is syngeneic with the rat antibody donor in the system shown.[9] Screening for mouse MAbs to human anti-human histocompatibility antigen CTLs has also led to the identification of function-associated antigens on the CTL effector, and additionally, to the identification of antigens on the target cell important in CTL recognition.[49]

after fusion and incubated with CTLs. Then ^{51}Cr-labeled target cells were added and lysis of target cells was assessed by ^{51}Cr release (Fig. 1). This assay has allowed rapid screening of MAbs that inhibited CTL-mediated killing, and the identification of LFA-1 in mouse[9,10] and human.[11]

The paradigm of inhibiting (or stimulating; see Kaye, this volume, Chapter 32) specific cellular functions with MAbs appears to be generally applicable to the identification and study of cell surface receptors. The concentrations of MAbs secreted into tissue culture medium (20–200 μg/ml)[12] are high enough to give saturation binding to cells for most MAbs. The antibodies we have selected inhibit cellular function at concentrations of 1–5 μg/ml (Refs. 11 and 13 and T. A. Springer and D. C. Anderson, unpublished results). Most effective blocking is obtained when excess antibody is present throughout the functional assay. This is particularly important for receptors for which increased surface expression can be functionally triggered (see below). Possible artifactual effects on function due to binding to antigens with a high number of sites/cell or due to cell agglutination, toxicity, or binding to Fc receptors must always be considered. Such artifacts have been ruled out for anti-LFA-1,[13] but one example of an apparently artifactual inhibition of CTL-mediated killing has been found. An IgM anti-Thy-1 MAb was obtained that agglutinated CTLs and gave variable but sometimes strong inhibition of killing.[10]

3. Functional Properties of LFA-1

LFA-1 is expressed on the surface of T and B lymphocytes, monocytes, and granulocytes, but is absent from nonhematopoietic cells. Functional studies in the mouse and human have given concordant results. Anti-LFA-1 MAbs inhibit the first step in T-cell-mediated killing, i.e., the adherence of the killer cell to the target cell.[13,14] Non-antigen-specific "natural" killing[11,15] and polymorphonuclear leukocyte antibody-dependent cellular cytotoxicity[16] are also inhibited.

The broad distribution of LFA-1 on leukocytes, and the inhibition of granulocyte-mediated, antibody-dependent cellular cytotoxicity as well as T-cell functions by anti-LFA-1 MAb, suggested that LFA-1 might be of general importance in leukocyte cell interactions. Further support for this idea has come from experiments showing that LFA-1 can mediate adhesion between lymphocytes in the apparent absence of any other ligand–receptor interactions. LFA-1 MAbs inhibit spontaneous and phorbol ester-induced self-aggregation of B-cell lymphoblastoid lines.[17] In common with the adhesion step in CTL-mediated killing, this self-aggregation is divalent cation-dependent. Overall, the findings suggest that LFA-1 acts as a cell adhesion molecule, which can synergize with antigen-specific receptors, Fc receptors, or other receptors to increase the efficiency of cell–cell interactions.

4. Functional Properties of Mac-1, a Molecule Related to LFA-1

The macrophage 1 (Mac-1) molecule of mouse and human has a β subunit identical to that of LFA-1, but contains a different α subunit.[18,19] In contrast to LFA-1, Mac-1 is absent from B and T lymphocytes. It is present on monocytes, granulocytes, and large granular lymphocytes.[20]

A MAb to Mac-1 was found to inhibit the complement receptor type 3 (CR3) on mouse and human cells.[21] The CR3 mediates adherence and phagocytosis of particles opsonized with iC3b, a cleaved, hemolytically inactive form of the third component of complement. MAbs to the Mac-1, OKM10, and in some cases the OKM1 determinants on the human Mac-1 molecule also inhibit CR3 function.[19,22,23] MAbs that bind to other determinants on Mac-1 but do not inhibit CR3 activity have also been obtained.[23,24] Such MAb–Mac-1 antigen complexes bound to *Staphylococcus aureus* bacteria specifically agglutinate iC3b opsonized erythrocytes,[23] positively demonstrating that the Mac-1 molecule has CR3 activity. In common with the adherence reactions in which LFA-1 is implicated, CR3-mediated adherence is dependent on divalent cations.[23]

5. Glycoprotein Family Interrelationships

Serology has for many years been a sensitive method for detecting similarities and differences between molecules. However, pure antigen preparations were required either for immunization or absorption. This was because it was important to distinguish between (1) a true cross-reaction between a subpopulation of antibodies to antigen 1 with related antigen 2, or (2) immunization with an antigen 1 preparation contaminated with a small amount of antigen 2, resulting in an antiserum with independent populations of antibodies to unrelated antigens 1 and 2. Since at the forefront of research on newly discovered molecules pure antigen preparations are almost never at

hand, it is a tremendous advantage to be able to obtain monospecific, cloned hybridoma lines after immunization with impure antigen. Using this technique, highly specific MAbs, as well as those recognizing potential cross-reactions, may be obtained.

Both types of MAb were obtained to the Mac-1, LFA-1 glycoprotein family, allowing the interrelationships of its glycoprotein subunits to be elucidated by immunochemical techniques. The cross-reaction of monoclonal and conventional antibodies between LFA-1 and Mac-1 was one of the first clues that these surface molecules were related.[18,25] Three types of MAb were obtained: those specific for Mac-1 (Fig. 2A, lane 2), those specific for LFA-1 (Fig. 2A, lane 4), and those cross-reacting between Mac-1 and LFA-1 (Fig. 2A, lane 3).[19,24] Both molecules contain α and β subunits noncovalently associated in $\alpha_1\beta_1$ complexes, as shown by cross-linking experiments.[24,26] The α subunits of Mac-1 and LFA-1 are M_r 170,000 and 180,000, respectively; the β subunits are each M_r 95,000. Western blotting of murine Mac-1 and LFA-1 showed that the cross-reactive MAb reacted with the β subunits of both antigens.[24] None of five MAbs specific for LFA-1 or four MAbs specific for Mac-1 reacted in Western blotting. The subunit reactivity of two of the MAbs specific for LFA-1 was elucidated during elution of LFA-1 from MAb–Sepharose columns with increasing pH. The β subunit was eluted in early fractions, followed by the α subunit in later fractions. Thus, disruption of the noncovalent association between the α and the β subunits preceded dissociation of the α subunit from the MAb. These results showed that two determinants specific to LFA-1 are present on its α subunit. Polyclonal rat antisera, but none of seven rat anti-mouse MAbs tested, were reactive with the Mac-1 or LFA-1 α subunits after pH-induced dissociation.

Since polyclonal antisera sample a wide variety of antigen epitopes, they are useful for obtaining more general conclusions about the immunochemical relationships between proteins. Mac-1 and LFA-1 were purified on MAb affinity columns. Antisera were then prepared to Mac-1 and to the LFA-1 α subunit, which was separated from the β subunit by an SDS–PAGE purification step. Antisera were tested with preparations of subunits dissociated at high pH. No cross-reactions between the α subunits were found at antibody concentrations 100- to 500-fold higher than required for reaction with the homologous α subunits. Thus, the α subunits are completely immunologically distinct. In contrast, cell lysates containing LFA-1 were as effective as those containing Mac-1 in absorbing antibodies to the Mac-1 β subunit present in anti-Mac-1 serum. Thus, the LFA-1 and Mac-1 β subunits are immunologically identical.[24]

Most mouse anti-human Mac-1 and LFA-1 MAbs were found to be immunoreactive with subunits dissociated by high pH. As in the mouse, MAbs specific for an individual member of the family were found to be α subunit-specific, while MAbs reacting with all types of αβ complex were found to react with the common β subunit.[19]

These results are in agreement with chemical characterization. The β subunits are identical by isoelectric focusing and peptide mapping; the α subunits

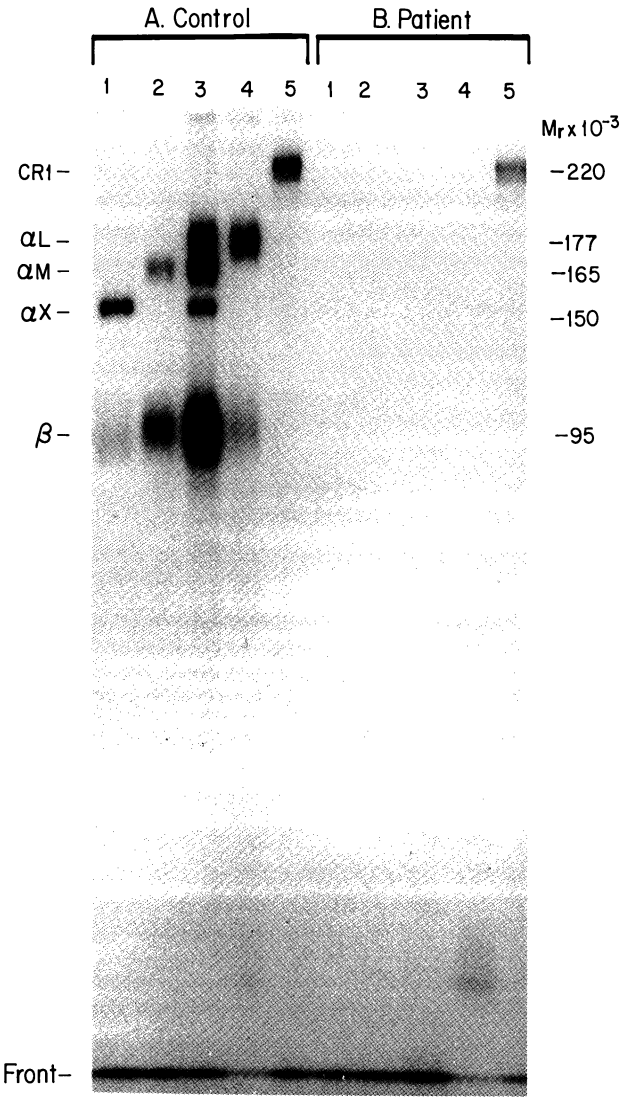

Figure 2. Immunoprecipitation of Mac-1, LFA-1, and p150,95 from healthy adults and deficient patient granulocytes. Granulocytes were surface-labeled with ^{125}I using lactoperoxidase. Triton X-100 lysates of (A) healthy control or (B) patient granulocytes were immunoprecipitated with anti-p150,95 MAb SHCL3 (lane 1), anti-Mac-1 α MAb OKM10 (lane 2), anti-β MAb TS1/18 (lane 3), anti-LFA-1 α MAb TS1/22 (lane 4), or anti-CR1 MAb 44D (lane 5). Precipitates were subjected to SDS 7% PAGE and autoradiography.

focus at distinct pI values and share at most one out of 17 tyrosyl tryptic peptides.[18,25,26] The NH_2-terminal amino acid sequences of the LFA-1 and Mac-1 α subunits show 33% homology (T. A. Springer, D. B. Teplow, and W. J. Dreyer, *Nature*, in press). This is highly significant homology, suggesting that the genes arose by a duplication event, but this degree of homology is generally too low to be immunochemically detected. As a rule, for globular proteins, 60% sequence homology is required for cross-reaction to occur.[27,28]

Polyclonal antiserum to Mac-1 has been useful in molecular biology experiments.[29,30] Polyclonal rabbit antiserum prepared to Mac-1 purified on MAb immunoaffinity columns[26] was found to immunoprecipitate Mac-1 translated in an *in vitro* reticulocyte lysate system, while none of several MAbs tested were effective. With polyclonal anti-Mac-1 IgG, Mac-1 mRNA preparations were purified approximately 1000-fold using the polysome purification procedure described by Kraus (this volume, Chapter 25). cDNA prepared to immunopurified mRNA, and oligonucleotide probes specified by the NH_2-terminal sequence, have been used to clone the Mac-1 α subunit gene (L. Sastre, T. Springer, T. Roberts, J. Roman, and W. Dreyer, unpublished results).

A third type of αβ complex has been identified in humans by precipitation with anti-β MAb.[19] (Fig. 2). This αβ complex has been termed p150,95 after its subunits of M_r 150,000 and 95,000. Recently, two MAbs specific for the p150,95 molecule were identified among MAbs in the myeloid panel of the second international workshop on human leukocyte differentiation[31] (see Fig. 2A, lane 1). Additionally, an IgM MAb to p150,95 has been obtained by immunizing mice with a mixture of human p150,95, Mac-1, and LFA-1. This antigen preparation was purified from granulocyte lysates using an anti-β subunit MAb coupled to Sepharose (L. Miller and T. A. Springer, unpublished). The p150,95 molecule is expressed on human monocytes and granulocytes but not on B or T lymphocytes.[31,32]

Surface expression of p150,95 and Mac-1 can be dramatically "upregulated" by physiological stimuli. The stimulatory agents include *f*-Met-Leu-Phe, an analogue of bacterial proteins, and C5a, an anaphylactic fragment of the complement component C5. These agents bind to specific receptors on myeloid cells and stimulate a broad spectrum of physiological responses, including increased adherence, chemotaxis, and granule secretion. *In vivo*, these agents stimulate circulating granulocytes and monocytes to aggregate, adhere to endothelial cells, and migrate into inflammatory sites.[4,6] When granulocytes[33] or monocytes (Fig. 3) are stimulated *in vitro* with *f*-Met-Leu-Phe (10^{-8} M), the amount of Mac-1 and p150,95 expressed on the cell surface is increased four- to sevenfold. In contrast, surface expression of LFA-1 is unaffected. These large increases in surface expression occur within ½ hr, suggesting a latent store of Mac-1 and p150,95 in granulocytes and monocytes. Secretion of components stored in secondary granules and perhaps in other secretory vesicles is stimulated under these conditions.[34] This suggests an intracellular location, perhaps in the membrane bilayer surrounding secretory organelles, for the latent store of Mac-1 and p150,95. The lack of such a store of LFA-1 suggests that

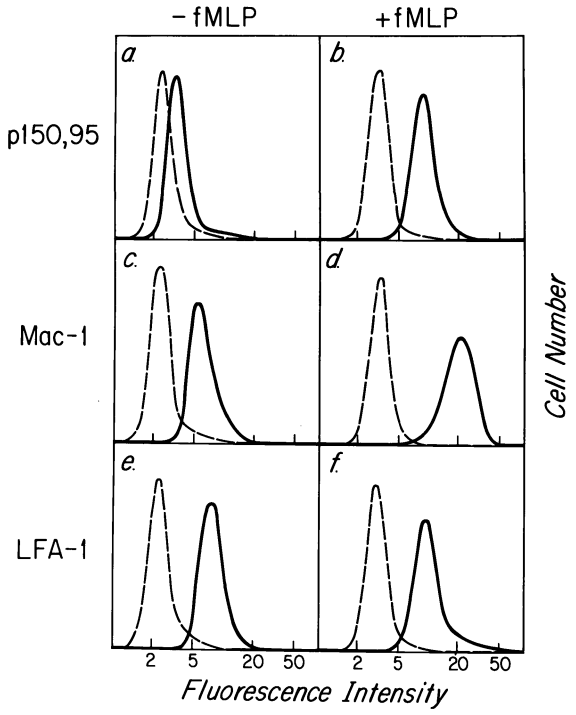

Figure 3. *f*-Met-Leu-Phe selectively stimulates increased Mac-1 and p150,95 expression on monocytes. Mononuclear cells were incubated with 10^{-8} M *f*-Met-Leu-Phe for 30 min at 37°C or were held at 4° to prevent upregulation stimulated by cell preparation,[43] labeled with MAb and FITC anti-mouse IgG at 4°C, and subjected to immunofluorescent flow cytometry on an Epics V.[19] Cells were stained with specific MAbs (solid curves) or P3X63 control IgG1 (dashed curves).

after biosynthesis, targetting of these molecules to the plasma membrane or secretory organelles is influenced by their α subunits.

6. Mac-1, LFA-1 Deficiency Disease

With the use of MAbs, a novel disease has been recognized in which the Mac-1, LFA-1 and p150,95 glycoproteins are deficient. Recurrent bacterial infection, progressive periodontitis, persistent leukocytosis, and/or delayed umbilical cord separation has been described in patients whose neutrophils demonstrated depressed phagocytic function and deficient adherence and chemotaxis.[35–39] When total, surface-labeled granulocyte proteins from these patients were subjected to SDS–PAGE, (a) glycoprotein(s) variously estimated to be M_r 110,000,[35] 180,000,[36] 150,000,[37] or 138,000[38] was found to be lacking. Deficiency of a specific surface molecule in one of these patients was pinpointed using MAbs to the α and β subunits of Mac-1.[38,40,41]

Deficiency on the cell surface of both the α and β subunits of Mac-1 might be secondary to a lack of the α or β subunit. If the primary deficiency were of the β subunit, a lack of LFA-1 and p150,95 would also be predicted. This hypothesis has been tested with MAbs specific for the LFA-1 αL and Mac-1 αM subunits and the common β subunit,[33] and more recently with p150,95-specific MAbs[31,39] which presumably react with the p150,95 αX subunit.

Expression on the surface of patient cells was quantitated by immunofluorescent flow cytometry. Patient granulocytes and mononuclear cells were markedly deficient in the entire glycoprotein family, as shown with MAbs specific for each α subunit and the common β subunit. An interesting quantitative difference was seen between patients. Patients 1 and 2 expressed between 0.2 and 0.5% of the normal amounts of Mac-1, LFA-1, and p150,95, values that are marginally distinguishable from complete absence. In contrast, patients 4–8 expressed clearly higher but subnormal amounts of 3–9% of these molecules. These levels of expression have been termed severe and moderate deficiency, respectively.[39] Incubation of patient granulocytes with 10^{-8} M *f*-Met-Leu-Phe resulted in a small absolute increase in the amount of surface-accessible Mac-1, p150,95, or the common β subunit in moderately deficient patients, but the percent of normal expression remained unchanged in comparison to similarly treated healthy cells.

To examine the deficiency at a molecular level, patients' granulocytes were labeled with ^{125}I and subjected to immunoprecipitation. A representative experiment with patient 1 is shown in Fig. 2. From control cells, the anti-αL MAb, anti-αM MAb, and anti-p150,95 precipitated the respective individual α subunits noncovalently associated with the common β subunit (Fig. 2A, lanes 1, 2, and 4). The anti-β MAb precipitated the β subunit and the three types of α subunits noncovalently associated with it: αL, αM, and the αX subunit of the p150,95 molecule (Fig. 2A, lane 3). In contrast, cells from patients were strikingly deficient in all these molecules (Fig. 2B, lanes 1–4). The normal parents and siblings of the patients were positive for immunoprecipitation.[33] The CR1 was present in similar amounts in normal and patient cells (Figs. 2A and 2B, lane 5).

To test for low levels of expression in patient cells, autoradiograms were subjected to prolonged exposure.[33,39] Precipitation of small amounts of LFA-1, Mac-1, and p150,95 was detected in moderately deficient but not in severely deficient patients. Notably, anti-αL and anti-β MAbs each precipitated both α and β subunits. This shows that when surface expression can be detected on patient cells, it is due to the presence of the normal αβ complex, rather than to the presence of free α or β subunits.

The deficiency appears to be limited to the Mac-1, LFA-1 glycoprotein family. The CR1, the neutrophil Fc receptor, and HLA antigen glycoproteins were present in normal amounts and were of normal mobility in SDS–PAGE. Furthermore, out of 118 antimyeloid MAbs screened in the second international leukocyte workshop, the only negative MAbs were 16 directed to the LFA-1, Mac-1 family or its members.[31] Normal numbers of *f*-Met-Leu-Phe receptors and normal granule secretion triggered by receptors for *f*-Met-Leu-Phe and C5a have previously been described on these patients' cells.[38,42] Furthermore, latent CR1[43] but not Mac-1 or p150,95 was present in patients' granulocytes, which could be expressed on the surface in response to *f*-Met-Leu-Phe or C5a.

A number of lines of evidence show that Mac-1, LFA-1, p150,95 deficiency is a heritable autosomal recessive disease[33,39]: (1) Mothers and fathers were found to express approximately half the normal amount of αL, αM, and β sub-

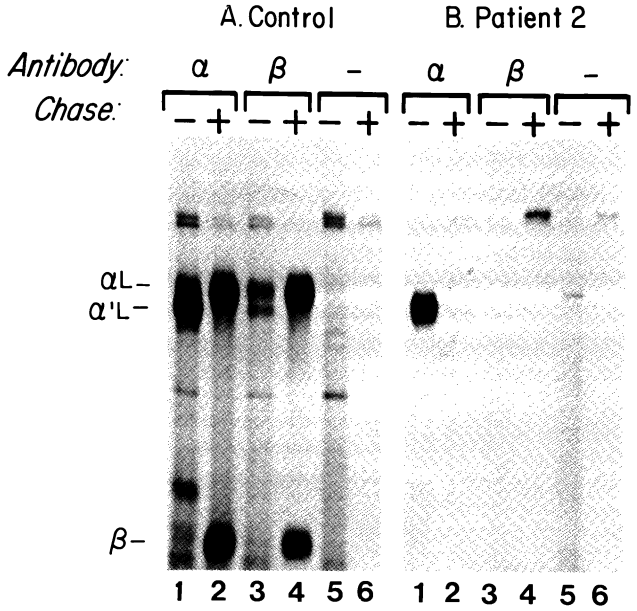

Figure 4. LFA-1 biosynthesis in patient and normal lymphocytes. Lymphocytes of patient 2 were blast-transformed with phytohemagglutinin (identical results were obtained with lymphocytes of patients 1 and 4).[33] Cultured blasts were then labeled with [^{35}S]methionine for 2 hr, and either harvested immediately or chased for 22 hr with unlabeled methionine as indicated. Triton X-100 and sodium deoxycholate-solubilized cell lysates were immunoprecipitated with Sepharose-coupled TS1/22 anti-LFA-1 α MAb, TS1/18 anti-β MAb, or activated, quenched Sepharose as a negative control. Precipitates were subjected to SDS–PAGE and fluorography.

units, both on unstimulated and on *f*-Met-Leu-Phe-stimulated granulocytes. (2) In the case of X-linked inheritance, two populations (normal and defective) of maternal granulocytes would be expected as a result of X-chromosome inactivation, as is observed in the X-linked form of chronic granulomatous disease. However, all four mothers had a single population of granulocytes expressing intermediate levels of each glycoprotein subunit. (3) One recently identified family contains a cluster of an affected father, son, and daughter.[39] (4) Equivalent numbers of male and female patients have been identified. (5) One patient (#3) was the product of a consanguinous marriage.

The molecular pathogenesis of this deficiency was investigated in biosynthesis experiments. Biosynthesis experiments with normal cells have shown that the α and β subunits are derived from separate precursors denoted as α′ and β′, and that association into an α′β′ complex precedes processing to the mature αβ form and expression on the cell surface.[19,44] In patients' cells, the α′L precursor was synthesized in normal amounts and had normal stability; however, α′L never matured to αL (Fig. 4B, lanes 1 and 2), and lack of precipitation of α′L by anti-β MAbs showed that it did not become β-associated (Fig. 4B, lanes 3 and 4). Two lines of evidence strongly suggest that the primary

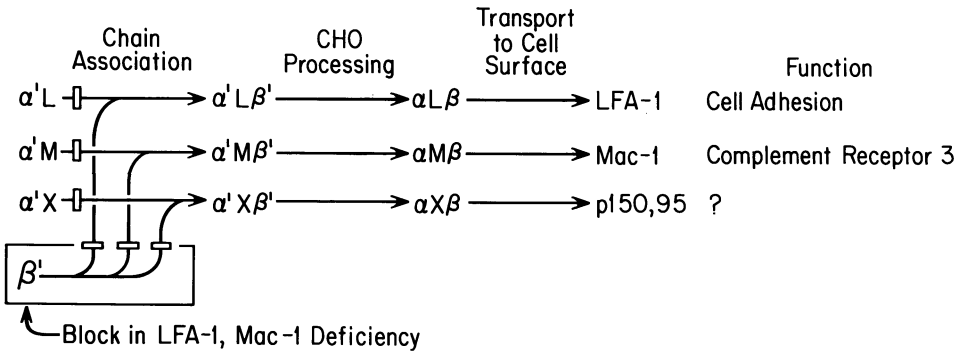

Figure 5. Biosynthesis of the Mac-1, LFA-1 glycoprotein family. The biosynthetic pathway in normal cells is as described in Ref. 19. The evidence for a primary block in β-subunit synthesis, a secondary block in α'L biosynthesis due to lack of β-subunit association, and hypothetically similar blocks in α'M and α'X biosynthesis is discussed in Ref. 33 and the text. Lack of α-subunit processing and transport to the cell surface or to latent pools appears to be due to a requirement for association with the β subunit.

defect is in the β subunit: (1) the presence of normal α'L but absence of any form of αLβ complex either at the cell surface or as an intracellular precursor that would have been detectable by precipitation of αL or α'L by anti-β MAbs, and (2) the lack of three different surface proteins, which have as their only common structural entity the β subunit.

The effect of β deficiency on biosynthesis of the Mac-1, LFA-1 family is summarized in Fig. 5. The presence of α'L precursor but no αL inside patients' cells and the absence of cell surface αL show that association with the β subunit is required for processing and transport to the surface. Biosynthesis experiments have been done thus far with cells that express only LFA-1, but since the biosynthetic pathways of Mac-1, p150,95, and LFA-1 are similar in normal cells,[19] it is proposed that α'M and α'X intracellular precursors are blocked at the same point in their biosynthetic pathway in patient myeloid cells, as shown in Fig. 5.

The lack of three α subunits on the cell surface as the result of β-subunit deficiency represents an interesting example of the effects of gene complementation on the phenotypic expression of an inherited defect. The lack of a latent pool of Mac-1 and p150,95 in patients' cell suggests that subunit association is also required for entry into this pool, which is presumably intracellular. Studies with endoglycosidase H digestion of Mac-1 have shown that the maturation of α'M to αM is due to the conversion of high-mannose to complex-type carbohydrates (T. Kishimoto, L. Sastre, and T. A. Springer, unpublished results). This type of carbohydrate processing is known to occur in the Golgi, suggesting that lack of association with β in patients' cells blocks transport to the Golgi. To further understanding of the nature of the mutation at the mRNA and DNA level, DNA probes specific for the α subunits (see above) and β subunits are being prepared.

Other patients appear to resemble the eight we have studied in lacking multiple members of the Mac-1, LFA-1 glycoprotein family. Two patients in the eastern U.S.,[40,41,45] one in the western U.S.,[46] three in England,[47] and one in France[48] have a similar deficiency of β and of multiple α subunits. Although none of these patients have been characterized by biosynthetic labeling of precursors, the findings in each case are suggestive of a similar β-subunit defect.

Heterogeneity among these patients in their symptoms heretofore hindered their classification into a single clinical entity. A common molecular basis for their disease has now been demonstrated: inherited Mac-1, LFA-1 deficiency. Severe and moderate deficiency variants of the disease have been recognized; heterogeneity with respect to the severity of clinical illness and/or abnormalities of leukocyte functions observed among recognized examples of this disease appears causally related to quantitative differences among the patients in surface expression of this critical glycoprotein family.[39] Granulocytes of patients 4–8 have about 5% of normal amounts on the cell surfaces, whereas patients 1–3 have a clearly more severe, almost complete deficiency. Patients 2 and 3 died before the age of 2; patient 1 is 5 years old. Among our patient population, patients 4–8 appear to have a relatively less severe clinical disease as compared to patients 1–3 and have survived longer (mean age 19 years). The presence of identical molecular deficiencies among these patients has confirmed that they represent examples of the same genetic disease. However, the possibility exists that disease among different recognized kindreds results from independent mutations within or affecting the same gene. This is suggested by the quantitative variations in Mac-1, LFA-1, p150,95 surface expression and the different ethnic backgrounds among the patients, including Anglo-Saxon, Iranian, and Hispanic.

Since a deficiency of the Mac-1, LFA-1 family has been clearly documented on multiple types of leukocytes, including granulocytes, monocytes, and T and B lymphocytes, the observed functional abnormalities of both phagocytic and lymphoid cells of these patients[39,42] are not unexpected. Recurrent soft tissue infections secondary to bacterial microorganisms is unquestionably related to granulocyte dysfunction in this disorder. Patient granulocytes are deficient in multiple adhesion-dependent functions, including attachment and spreading, aggregation, chemotaxis, antibody-dependent cytotoxicity, and CR3-mediated adherence and phagocytosis.[35,38,42,46] Deficiency of CR3 function as measured with iC3b-opsonized particles is in good agreement with the previous identification of Mac-1 as the CR3[21,23] of human myeloid cells. The wide spectrum of adhesion-dependent abnormalities may be related to all three members of this glycoprotein family, since all are expressed on normal granulocytes and monocytes. Treatment with combinations of anti-αL, -αM, and -β MAbs reproduces abnormalities in adhesion-related functions in normal granulocytes,[16,38,42,46] while no blockade of adherence-independent functions, including shape change and specific binding of chemotactic factors, is observed. Interestingly, granulocyte adhesion, in common with other functions in which Mac-1 and LFA-1 are implicated, is divalent-cation dependent.[3]

The failure of patients' granulocytes to hyperadhere in response to chemoattractants *in vitro* may be related to deficient upregulation of Mac-1 and p150,95.[38] Since granulocyte adherence to endothelial cells *in vivo* and to a variety of substrates *in vitro* represents the prerequisite for mobilization to inflammatory sites and for chemotaxis, respectively, the adherence abnormalities in this disorder provide a plausible explanation for clinical features of impaired pus formation, occurrence of necrotic infections of skin and mucous membranes, and impaired wound healing. Lack in patients of granulocyte or monocyte accumulation at abraded skin sites (Rebuck skin window test) is also notable in this respect.[39]

Although lymphocytes express only one member of the family, LFA-1, some evidence supports the clinical pathological importance of lymphoid cell dysfunction in this disorder. Of importance, the death of patient 3 was related to a fulminant and progressive viral (pico RNA virus) respiratory infection. Impaired proliferative responses of patient cells to PHA (10–25% of normal values) and diminished cytolytic T-lymphocyte and natural killing activity have been documented in studies on some of our patients[16,17] as well as in a patient reported elsewhere.[46] Interestingly, the PHA responses and cytotoxic killing by lymphocytes of patients 1, 2, and 4 are even further decreased by anti-LFA-1 MAb, suggesting that even the small amounts of LFA-1 present on patient cells can contribute to their functional responsiveness.[17] The functional defects of LFA-1-deficient lymphocytes appear identical to the defects observed in normal lymphocytes treated with anti-LFA-1 MAb. The excellent agreement between studies on MAb-treated normal cells and on genetically deficient cells, both for LFA-1 and Mac-1, is an important confirmation of the validity of using MAb probes for the study of cell surface function.

Previously, immunodeficiency diseases have been described in which specific cell lineages are absent or abnormally developed, as in severe combined immunodeficiency; secreted components are lacking, as in complement deficiencies; or intracellular enzymes are lacking, as in chronic granulomatous disease. To the best of our knowledge, Mac-1, LFA-1 deficiency is the first reported instance of an immunodeficiency disease related to the lack of specific cell surface proteins. The combined tools of monoclonal antibodies and an inherited deficiency syndrome should allow rapid progress in understanding the important functions of the Mac-1, LFA-1 glycoprotein family in leukocyte adhesion reactions.

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References

1. Dawson, P., and Mandell, O., 1980, Phagocyte strategy vs. microbial tactics, *Rev. Inf. Dis.* **2**:817–836.

2. Carter, S. B., 1967, Haptotaxis and the mechanism of cell motility, *Nature* **213**:256–260.
3. Smith, C. W., Hollers, J. C., Patrick, R. A., and Hassett, C., 1979, Motility and adhesiveness in human neutrophils: Effects of chemotactic factors, *J. Clin. Invest.* **63**:221–229.
4. Atherton, A., and Born, G. V. R., 1972, Quantitative investigations of the adhesiveness of circulating polymorphonuclear leucocytes to blood vessel walls, *J. Physiol.* **222**:447–474.
5. Keller, H. U., Barandum, S., Kistler, P., and Ploem, J., 1979, Locomotion and adhesion of neutrophil granulocytes: Effects of albumin, fibrinogen and gammaglobulins studied by reflexion contrast microscopy, *Exp. Cell Res.* **122**:351–362.
6. Wilkinson, P. C., and Lackie, J. M., 1979, The adhesion, migration and chemotaxis of leukocytes in inflammation, *Curr. Top. Path.* **68**:47–88.
7. Köhler, G., and Milstein, C., 1975, Continuous cultures of fused cells secreting antibody of predefined specificity, *Nature* **256**:495–497.
8. Martz, E., 1977, Mechanism of specific tumor cell lysis by alloimmune T-lymphocytes: Resolution and characterization of discrete steps in the cellular interaction, *Contemp. Top. Immunobiol.* **7**:301–361.
9. Davignon, D., Martz, E., Reynolds, T., Kürzinger, K., and Springer, T., A., 1981, Lymphocyte function-associated antigen 1 (LFA-1): A surface antigen distinct from Lyt-2,3 that participates in T lymphocyte–mediated killing, *Proc. Natl. Acad. Sci. USA* **78**:4535–4539.
10. Sanchez-Madrid, F., Davignon, D., Martz, E., and Springer, T. A., 1982, Antigens involved in mouse cytolytic T-lymphocyte (CTL)-mediated killing: Functional screening and topographic relationship, *Cell. Immunol.* **73**:1–11.
11. Krensky, A. M., Sanchez-Madrid, F., Robbins, E., Nagy, J., Springer, T. A., and Burakoff, S. J., 1983, The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: Cell surface antigens associated with CTL-target interactions, *J. Immunol.* **131**:611–616.
12. Springer, T. A., 1982, Quantitation of hybridoma immunoglobulins and selection of myeloma or specific light chain loss variants, *Meth. Enzymol.* **92**:147–160.
13. Davignon, D., Martz, E., Reynolds, T., Kürzinger, K., and Springer, T. A., 1981, Monoclonal antibody to a novel lymphocyte function-associated antigen (LFA-1): Mechanism of blocking of T lymphocyte-mediated killing and effects on other T and B lymphocyte functions, *J. Immunol.* **127**:590–595.
14. Krensky, A. M., Robbins, E., Springer, T. A., and Burakoff, S. J., 1984, LFA-1, LFA-2 and LFA-3 antigens are involved in CTL-target conjugation, *J. Immunol.* **132**:2180–2182.
15. Hildreth, J. E. K., Gotch, M. F., Hildreth, P. D. K., and McMichael, A. J., 1983, A human lymphocyte-associated antigen involved in cell-mediated lympholysis, *Eur. J. Immunol.* **13**:202–208.
16. Kohl, S., Springer, T. A., Schmalstieg, F. C., Loo, L. S., and Anderson, D. C., 1984, Defective natural killer cytotoxicity and polymorphonuclear leukocyte antibody-dependent cellular cytotoxicity in patients with LFA-1/OKM-1 deficiency, *J. Immunol.* **133**:2972–2978.
17. Springer, T. A., Rothlein, R., Anderson, D. C., Burakoff, S. J., and Krensky, A. M., 1985, The function of LFA-1 in cell-mediated killing and adhesion: Studies on heritable LFA-1, Mac-1 deficiency and on lymphoid cell self-aggregation, in: *Mechanisms of Cell-Mediated Cytotoxicity II*, Plenum Press, New York, In press.
18. Kürzinger, K., Ho, M. K., and Springer, T. A., 1982, Structural homology of a macrophage differentiation antigen and an antigen involved in T-cell-mediated killing, *Nature* **296**:668–670.
19. Sanchez-Madrid, F., Nagy, J., Robbins, E., Simon, P., and Springer, T. A., 1983, A human leukocyte differentiation antigen family with distinct alpha subunits and a common beta subunit: The lymphocyte-function associated antigen (LFA-1), the C3bi complement receptor (OKM1-Mac-1), and the p150,95 molecule, *J. Exp. Med.* **158**:1785–1803.
20. Unkeless, J. C., and Springer, T. A., 1984, Macrophages, in: *Handbook of Experimental Immunology*, 4th ed., Volume IV: *Applications of Immunological Methods in Biomedical Sciences* (D. M. Weir, C. C. Blackwell, L. A. Herzenberg, and L. A. Herzenberg, eds.), Blackwell, Oxford, In press.
21. Beller, D. I., Springer, T. A., and Schreiber, R. D., 1982, Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor, *J. Exp. Med.* **156**:1000–1009.

22. Arnaout, M. A., Todd, R. F., III, Dana, N., Melamed, J., Schlossman, S. F., and Colten, H. R., 1983, Inhibition of phagocytosis of complement C3- or immunoglobulin G-coated particles and of C3bi binding by monoclonal antibodies to a monocyte-granulocyte membrane glycoprotein (Mol), *J. Clin. Invest.* **72:**171–179.
23. Wright, S. D., Rao, P. E., Van Voorhis, W. C., Craigmyle, L. S., Iida, K., Talle, M. A., Westberg, E. F., Goldstein, G., and Silverstein, S. C., 1983, Identification of the C3bi receptor of human monocytes and macrophages with monoclonal antibodies, *Proc. Natl. Acad. Sci. USA* **80:**5699–5703.
24. Sanchez-Madrid, F., Simon, P., Thompson, S., and Springer, T. A., 1983, Mapping of antigenic and functional epitopes on the alpha and beta subunits of two related glycoproteins involved in cell interactions, LFA-1 and Mac-1, *J. Exp. Med.* **158:**586–602.
25. Trowbridge, I. S., and Omary, M. B., 1981, Molecular complexity of leukocyte surface glycoproteins related to the macrophage differentiation antigen Mac-1, *J. Exp. Med.* **154:**1517–1524.
26. Kürzinger, K., and Springer, T. A., 1982, Purification and structural characterization of LFA-1, a lymphocyte function-associated antigen, and Mac-1, a related macrophage differentiation antigen, *J. Biol. Chem.* **257:**12412–12418.
27. Ibrahimi, I. M., Prager, E. M., White, T. J., and Wilson, A. C., 1979, Amino acid sequence of California quail lysozyme. Effect of evolutionary substitutions on the antigenic structure of lysozyme, *Biochemistry* **13:**2736–2744.
28. Prager, E. M., Wilson, A. C., Perin, J. P., and Jolles, P., 1978, Further studies of an anomalous cross-reaction involving worm and vertebrate lysozymes, *Immunochemistry* **15:**577–583.
29. Springer, T. A., Sastre, L., Schmalstieg, F. C., and Anderson, D. C., 1985, Inherited LFA-1, Mac-1 deficiency and its molecular biology, in: *Mononuclear Phagocytes and Inflammation* (R. van Furth and Z. A. Cohn, eds.), Martinus Nijhoff, in press.
30. Springer, T. A., Sastre, L., and Anderson, D. C., 1985, The LFA-1, Mac-1 Leukocyte adhesion glycoprotein family and its deficiency in a heritable human disease, *Biochem. Soc. Trans.* **13:**3–6.
31. Springer, T. A., and Anderson, D. C., 1985, Antibodies specific for the Mac-1, LFA-1, p150,95 glycoproteins or their family, or for other granulocyte proteins, in: *Leukocyte* Typing II (E. Reinherz, ed.), Springer-Verlag, Berlin, (in press).
32. Schwarting, R., Stein, H., and Wang, C. Y., 1984, The monoclonal antibodies anti S-HCL 1 (anti Leu 14) and anti S-HCL 3 (anti Leu M5) allow the diagnosis of hairy cell leukemia, *Blood* (in press).
33. Springer, T. A., Thompson, W. S., Miller, L. J., Schmalstieg, F. C., and Anderson, D. C., 1984, Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis, *J. Exp. Med.* **160:**1901–1918.
34. Gallin, J. I., Fletcher, M. P., Seligman, B. E., Hoffstein, S., Cehes, K., and Mounessa, N., 1982, Human neutrophil-specific granule deficiency: A model to assess the role of neutrophil-specific granules in the evolution of the inflammatory response, *Blood* **59:**1317–1329.
35. Crowley, C. A., Curnutte, J. T., Rosin, R. E., Andre-Schwartz, J., Gallin, J. I., Klempner, M., Snyderman, R., Southwick, F. S., Stossel, T. P., and Babior, B. M., 1980, An inherited abnormality of neutrophil adhesion: Its genetic transmission and its association with a missing protein, *N. Engl. J. Med.* **302:**1163–1168.
36. Buchanan, M. R., Crowley, C. A., Rosin, R. E., Gimbrone, M.A., and Babior, B. M., 1982, Studies on the interaction between GP-180-deficient neutrophils and vascular endothelium, *Blood* **60:**160–165.
37. Arnaout, M. A., Pitt, J., Cohen, H. J., Melamed, J., Rosen, F. S., and Colten, H. R., 1982, Deficiency of a granulocyte-membrane glycoprotein (gp150) in a boy with recurrent bacterial infections, *N. Engl. J. Med.* **306:**693–699.
38. Anderson, D. C., Schmalstieg, F. C., Kohl, S., Arnaout, M. A., Hughes, B. J., Tosi, M. F., Buffone, G. J., Brinkley, B. R., Dickey, W. D., Abramson, J. S., Springer, T. A., Boxer, L. A., Hollers, J. M., and Smith, C. W., 1984, Abnormalities of polymorphonuclear leukocyte function associated with a heritable deficiency of high molecular weight surface glycoproteins (GP138): Common relationship to diminished cell adherence, *J. Clin. Invest.* **74:**536–551.

39. Anderson, D. C., Schmalstieg, F. C., Finegold, M. J., Hughes, B. J., Rothlein, R., Miller, L. J., Kohl, S., Tosi, M. F., Jacobs, R. L., Waldrop, T. C., Goldman, A., Shearer, W. T., and Springer, T. A., 1985, The severe and moderate phenotypes of heritable Mac-1, LFA-1 deficiency: their quantitative definition and relation to leukocyte dysfunction and clinical features, *J. Inf. Dis.* (in press).
40. Springer, T. A., 1985, The LFA-1, Mac-1 glycoprotein family and its deficiency in an inherited disease: Introduction, *Fed. Proc.* (in press).
41. Dana, N., Todd, R. F., III, Pitt, J., Springer, T. A., and Arnaout, M. A., 1984, Deficiency of a surface membrane glycoprotein (Mol) in man, *J. Clin. Invest.* **73**:153–159.
42. Anderson, D. C., Schmalstieg, F. C., Shearer, W., Freeman, K., Kohl, S., Smith, C. W., and Springer, T., 1985, Abnormalities of PMN monocyte function and recurrent infection associated with a heritable deficiency of adhesive surface glycoproteins, *Fed. Proc.* (in press).
43. Fearon, D. T., and Collins, L. A., 1983, Increased expression of C3b receptors on polymorphonuclear leukocytes induced by chemotactic factors and by purification procedures, *J. Immunol.* **130**:370–375.
44. Ho, M. K., and Springer, T. A., 1982, Mac-1 antigen: Quantitative expression in macrophage populations and tissues, and immunofluorescent localization in spleen, *J. Immunol.* **128**:2281–2286.
45. Arnaout, M. A., Dana, N., Pitt, J., and Todd, R. F., III, 1985, Deficiency of two human leukocyte surface membrane glycoproteins (Mol and LFA-1), *Fed. Proc.* (in press).
46. Beatty, P. G., Harlan, J. M., Rosen, H., Hansen, J. A., Ochs, H. D., Price, T. D., Taylor, R. F., and Klebanoff, S. J., 1984, Absence of monoclonal-antibody-defined protein complex in boy with abnormal leucocyte function, *Lancet* **I**:535–537.
47. Ross, G. D., Thompson, R. A., Walport, M. J., Springer, T. A., Watson, J. V., Ward, R. H. R., Lida, J., Newman, S. L., Harrison, R. A., and Lachmann, P. J., 1984, Characterization of patients with an increased susceptibility to bacterial infections and a genetic deficiency of leukocyte membrane complement receptor type three (CR3) and the related membrane antigen LFA-1, *Blood*, manuscript submitted.
48. Fischer, A., Seger, R., Durandy, A., Grospierre, B., Virelizier, J. L., Griscelli, C., Fischer, E., Kazatchkine, M., Bohler, M. C., Descamps-Latscha, B., Trung, P. H., Springer, T. A., Olive, D., and Mawas, C., 1984, Deficiency of the adhesive protein complex LFA-1, C3bi complement receptor, p150,95 in a girl with recurrent bacterial infections, *Blood* (in press).
49. Sanchez-Madrid, F., Krensky, A. M., Ware, C. F., Robbins, E., Strominger, J. L., Burakoff, S. J., and Springer, T. A., 1982, Three distinct antigens associated with human T lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3, *Proc. Natl. Acad. Sci. USA* **79**:7489–7493.