to have different carbohydrate structures on the different molecular forms (Brown & Williams, 1982). No antibody raised against L-CA has yet been established to be anti-carbohydrate even though the carbohydrate determinants I and i have been identified on some forms of L-CA with antibodies against these specificities (Childs & Feizi, 1981).

If the antigenicity of L-CA is protein based then different L-CA forms must show differences in their protein sequence. This is being tested by sequencing cloned complementary DNA from thymocytes and B-lymphocytes.

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The LFA-1, Mac-1 leucocyte adhesion glycoprotein family and its deficiency in a heritable human disease

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Cell-surface adherence reactions are of central importance in the immune functions of lymphocytes, monocytes and granulocytes. Lymphocytes adhere to antigen-representing macrophages or dendritic cells in the induction of T-lymphocyte immune responses, and to target cells in cell-mediated killing. Monocytes/macrophages and granulocytes can adhere to micro-organisms opsonized with antibody or complement, and can also adhere in the absence of opsonins to some micro-organisms, the extracellular matrix, and artificial substrates such as glass and plastic. Adherence is not only important in triggering effector functions such as phagocytosis and degranulation, but also in allowing cell locomotion during chemotaxis. A family of high-M_r glycoproteins with identical β-subunits has recently been characterized on leucocyte surfaces which is functionally important in a wide variety of these adhesion reactions. The identification and structural characterization of this LFA-1, Mac-1 glycoprotein family has only been possible by the application of the Kohler & Milstein (1975) mAb technique. Furthermore, to the best of our knowledge, we describe, for the first time, the definition with mAbs of an inherited disease.

Abbreviations used: mAb, monoclonal antibody; f-Met-Leu-Phe, formyl-L-methionyl-L-leucyl-L-phenylalanine; PHA, phytohaemagglutin; IgG, immunoglobulin G; SDS, sodium dodecyl sulphate.

Probing cell-surface functions with mAbs

By 1977, T-lymphocyte-mediated killing had been shown to consist of a Mg^{2+}-dependent adhesion step and a Ca^{2+}-dependent lethal hit-delivery step (Martz, 1977); however, at the molecular level, the killing process was essentially a 'black box.' Further progress in this research area has been totally dependent on the mAb methodology, which allows antibodies monospecific for individual cell-surface proteins to be obtained after immunization with whole cells. mAbs can be added to complex cellular assays to probe *in situ* for molecules functioning on the surfaces of interacting cells, and can also be used to isolate molecules from detergent lysates of the same cells (Springer *et al.*, 1982). Thus, they are powerful reagents which can link function with structure. The assumption in these studies is that binding of a mAb to functionally important surface molecules will result in steric hindrance and inhibit function, or alternatively stimulate function, whereas binding of a mAb to other surface molecules will have no effect. This assumption appears correct, as long as effects due to antibody density on the surface, agglutination, toxicity and Fc interactions are ruled out (Springer *et al.*, 1982). mAbs usually inhibit cellular assays at a concentration of 1 μg/ml. Since antibodies are secreted into tissue culture medium at concentrations of 20–200 μg/ml, this allows inhibition of function to be used to directly screen primary, uncloned hybridoma cultures (Sanchez-Madrid *et al.*, 1982). It has been shown with mAbs that six molecules, LFA-1, LFA-2, T3, T4, T8, and the antigen receptor, play an important role in the interaction between T-lymphocytes and target cells (Krensky *et al.*, 1983; Martz *et al.*, 1983). Of these, LFA-1 appears to have the broadest role in leucocyte adhesion reactions.

Functional properties of LFA-1 and Mac-1

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 (Davignon *et al.*, 1981; Krensky *et al.*, 1984). Non-antigen-specific 'natural' killing (Hildreth *et al.*, 1983; Krensky *et al.*, 1983) and antibody-dependent cellular cytotoxicity (Kohl *et al.*, 1984) are also inhibited. The importance of LFA-1 in leucocyte adhesion is not limited to immune interactions. Anti-LFA-1 mAbs inhibit spontaneous and phorbol-ester-induced homotypic aggregation of B-cell lymphoblastoid lines (Springer *et al.*, 1984*a*). The findings suggest that LFA-1 acts as a cell-adhesion molecule which can synergize with antigen-specific, Fc or other receptors to increase the efficiency of cell–cell interactions.

Anti-Mac-1 mAbs inhibit the complement receptor type 3 (CR3) on mouse and human cells (Beller *et al.*, 1982). The CR3 mediates adherence and phagocytosis of particles opsonized with iC3b, a cleaved, haemolytically inactive form of the third component of complement. mAbs which bind to other determinants on Mac-1 but do not inhibit CR3 activity have been obtained (Sanchez-Madrid *et al.*, 1983*a*,*b*; Wright *et al.*, 1983). Such mAb–Mac-1 antigen complexes bound to *Staphylococcus aureus* bacteria specifically agglutinate iC3b opsonized erythrocytes, positively demonstrating that the Mac-1 molecule has CR3 activity (Wright *et al.*, 1983).

Inter-relationships of a glycoprotein family

The members of the LFA-1, Mac-1 glycoprotein family contain identical β-subunits non-covalently associated with different α-subunits (Fig. 1). Studies of subunits dissociated by brief exposure to high pH have clarified mAb specificity and confirmed subunit inter-relationships (Sanchez-Madrid *et al.*, 1983*a*,*b*). Antibodies specific for LFA-1 react with the αL-subunit; those specific for Mac-1 react with the αM-subunit. Antibodies to the shared β-subunit react with LFA-1, Mac-1, and a third member of the family, p150,95. mAbs to the shared β-subunit were the key to defining this protein family. The α-subunits are also related, the since *N*-terminal sequences of the αL- and αM-subunits show 33% homology (T. A. Springer, D. Teplow & W. J. Dreyer, unpublished work).

Deficiency of an entire surface glycoprotein family

Mac-1 has been found to be deficient in patients with recurring bacterial or fungal infections (Dana *et al.*, 1984). Further experiments have shown that not only Mac-1 but also LFA-1 and p150,95 are deficient in this disease (Fig. 2). mAbs specific for the αL-subunit of LFA-1, the αM-subunit of Mac-1 and the shared β-subunit precipitate the associated α- and β-subunits of these molecules from granulocytes surface-labelled with ^{125}I. However, granulocyte from a patient yielded no precipitate, although normal and parental cells were positive (Fig. 2*a–c*, lanes 1–4). In contrast, other surface molecules such as the complement receptor type 1 (CR1) are present in normal amounts on patient cells (Fig. 2*a–c*, lane 5). Quantitative immunofluorescent flow cytometry on granulocytes has detected low (0.5–1% of normal) surface expression in this and a second patient, and higher levels (7–9% of normal) in a third patient (Springer *et al.*, 1984*b*). Small amounts of LFA-1 are detected in prolonged autoradiogram exposures of immunoprecipitates from the latter but not the former two patients. Incubation of normal granulocytes with 10^{-8} M-f-Met-Leu-Phe resulted in about 1.3-fold, 5.5-fold and 3.7-fold 'up-regulation' in the amount of surface accessible αL-, αM-, and β-subunit, respectively. However, expression on cells of two patients showed little if any increase. This suggests deficiency of a latent, intracellular pool as well as at the cell surface.

Mothers and fathers of three patients have 53–63% of normal amounts of the subunits on the cell surface, as shown by flow cytometry (Springer *et al.*, 1984*b*). A single, uniform population of granulocytes was observed in the mothers, demonstrating no mosaicism due to X-chromosome inactivation. This finding, together with those from a fourth family with an affected father, son, and daughter, demonstrates somatic recessive inheritance.

Since the Mac-1, LFA-1 and p150,95 molecules utilize identical β-subunits, the lack of this entire glycoprotein family suggested that the primary defect might be in the β-subunit. Biosynthesis experiments in normal cells have shown that the α- and β-subunits are derived from separate precursors denoted α' and β', and that association into an $\alpha'\beta'$ complex precedes processing to the mature $\alpha\beta$-form and expression on the cell surface (Fig. 1).

To examine biosynthesis, patient and normal lymphoblasts were pulsed with [^{35}S]methionine to label precursors, and then either examined immediately or chased with unlabelled methionine to follow processing and maturation (Fig. 2*d*,*e*). In normal cells, α'L was synthesized (Fig. 2*d*, lane 1), and after the chase, was converted to the higher M_r αL-form (Fig. 2*d*, lanes 2 and 4), which was associated with β (Fig. 2*d*, lanes 2 and 4). A β'-precursor was not identified, suggesting it had weak reactivity with the mAb and/or ran in the same position as a background band. In patients' cells, the α'L-precursor was found but it never matured (Fig. 2*e*, lanes 1 and 2), and lack of precipitation of α'L by anti-β

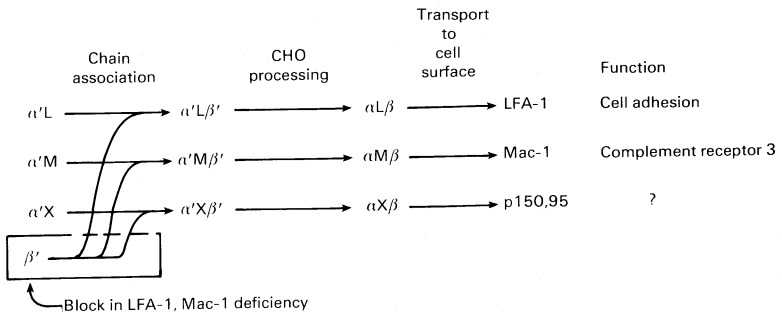

Fig. 1. *LFA-1, Mac-1 glycoprotein family and its biosynthesis (Sanchez-Madrid et al., 1983a; Springer et al., 1984b)*

The α-subunits, $\alpha L = 177000 M_r$, $\alpha M = 165000 M_r$ and $\alpha X = 150000 M_r$, are different. The β-subunits ($= 95000 M_r$) are identical. The subunits are assembled into non-covalently associated $\alpha_1\beta_1$ complexes. In deficient patients a primary block in β-subunit synthesis leads to a secondary block in α-subunit carbohydrate synthesis and transport to the cell surface.

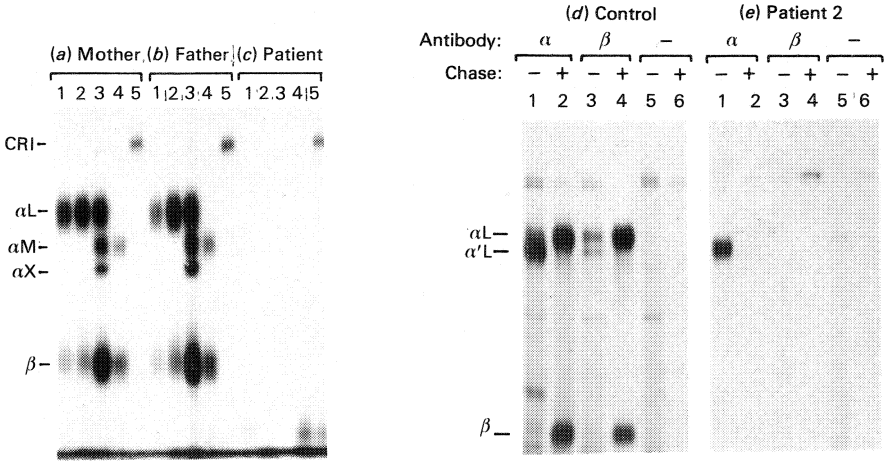

Fig. 2. *Immunoprecipitation from healthy adult and patient surface ^{125}I-labelled granulocytes and [^{35}S]methionine pulse-labelled lymphoblasts*

(*a–c*) Granulocytes were surface-labelled with ^{125}I by using lactoperoxidase. Triton X-100 lysates were immunoprecipitated with a mixture of five different anti-LFA-1 α mAbs (lane 1), anti-LFA-1 α TS1/22 mAb–Sepharose CL-4B (lane 2), anti-β TS1/18 mAb–Sepharose (lane 3), anti-αM OKM1 mAb–Sepharose (lane 4) or anti-CR1 mAb (kindly provided by Dr. V. Nussenzweig) (lane 5). Immunoprecipitates for lanes 1, 4, and 5 were formed with anti-mouse IgG and *S. aureus*. Precipitates were subjected to 7% SDS/PAGE and autoradiography. (*d,e*) PHA-stimulated lymphoblasts were labelled with [^{35}S]methionine for 2h, and either harvested immediately or chased for 22h with unlabelled methionine as indicated. Lysates with Triton X-100 and sodium deoxycholate were immunoprecipitated with purified antibodies coupled to Sepharose CL-4B: TS1/22 anti-LFA-1 α, TS1/18 anti-β, or activated, quenched Sepharose as negative control. Precipitates were subjected to SDS/polyacrylamide-gel electrophoresis and fluorography.

mAb showed it did not become β-associated (Fig. 2*e*, lanes 3 and 4). Patient and control α′L were present in similar amounts after the 2h pulse, suggesting they have equal stability. As expected, the α′L-precursor was degraded in patients' cells after the 22h chase, showing that intracellular, unassociated α′L-subunits have a shorter half-life than extracellular, associated αL-subunits (Fig. 2*e*, lane 2 compared with Fig. 2*d*, lane 2).

The findings on the molecular basis of the defect in patients' cells are summarized in Fig. 1. The presence of normal α′L-precursor, together with surface deficiency of three molecules which have as their only common structural entity the β-subunit, suggests the primary defect in patients' cells is in the β-subunit. The presence of α′L-precursor but no αL inside patients' cells, and the absence of cell-surface αL, shows that subunit association is required for processing and transport to the surface. Biosynthesis experiments have been done thus far in cells which express only LFA-1, but the similar biosynthetic pathways of Mac-1 and p150,95 in normal cells suggests that this finding can be generalized as indicated in Fig. 1. The lack of three α-subunits on the cell surface as the result of β-subunit deficiency is an interesting example of gene complementation in the phenotypic expression of an inherited defect.

This experiment of nature has allowed a second, independent means of testing the functional importance of these surface molecules. Flow cytometry has shown that all leucocyte lineages are affected. LFA-1 deficiency in lymphocytes is reflected in proliferative responses to PHA which are 10–25% of control values at low PHA doses and somewhat less affected at normal PHA doses, and much lower cytolytic T-lymphocyte and natural killing activity (Beatty *et al.*, 1984; Springer *et al.*, 1984). Interestingly, the low PHA responses and cytotoxic activity of patients' cells can be reduced even further with anti-LFA-1 mAbs, suggesting that the small quantities of LFA-1 present on their cells can contribute to functional responses. Patients' granulocytes are deficient in the complement receptor type 3, as measured by iC3b rosetting. In contrast receptors for C3b, f-Met-Leu-Phe, Fc and C5a are normal (Anderson *et al.*, 1984). Genetic deficiency of LFA-1 and Mac-1 and incubation of normal cells with antibodies to these molecules cause very similar functional defects.

Patients' granulocytes are also strikingly deficient in a wide variety of other adherence reactions: the Rebuck skin window test, baseline and stimulated adherence to glass, plastic and fibronectin-coated gelatin, spreading on surfaces, and aggregation in response to f-Met-Leu-Phe. Opsonized zymosan phagocytosis and stimulation of the oxidative burst are deficient. Granulocyte antibody-dependent cellular cytotoxicity is low. Orientation and motility in chemotactic gradients are affected, probably secondary to lack of normal adhesion. Granulocyte cytoskeletal proteins, membrane fluidity and non-adhesive responses including cell bipolarization to soluble stimuli are normal (Anderson *et al.*, 1984). Since normal granulocytes express Mac-1, LFA-1 and p150,95, all may play a role in these deficiencies. These findings emphasize the general importance of this glycoprotein family in leucocyte adhesion.

Mac-1 mRNA purification and translation

Mac-1 complementary DNA clones would be invaluable for analysing the genetic basis of the defect in patients' cells, the complete amino acid sequence of the α- and β-subunits, the genetic organization of the protein family, and the regulation of gene expression in differentiation. As an important step towards this goal, Mac-1 mRNA has been isolated and its translation products *in vitro* identified. Preliminary experiments showed that mAbs were ineffective

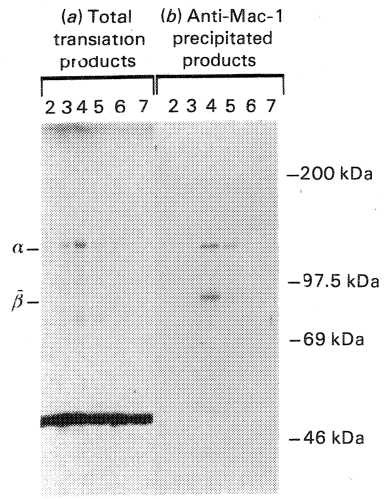

Fig. 3. *Translation products of immunoselected Mac-1 polysomes*

$P388D_1$ polysomes were incubated with rabbit anti-Mac-1 IgG and specific Mac-1 polysomes were bound to a protein A–Sepharose column as described by Shapiro & Young (1981). The mRNA from the immunoselected polysomes was eluted with 20mM-EDTA; 400μl fractions were collected and ethanol precipitated. One-fourth of the mRNA in each fraction was collected by centrifugation and translated *in vitro* by a rabbit reticulocyte system. The products of translation were analysed by 7.5% SDS/polyacrylamide-gel electrophoresis and fluorography. (*a*) shows the polypeptides present in 1μl of total translation products *in vitro* from eluted fractions 2–7. (*b*) shows the polypeptides that were immunoprecipitated from 24μl of translation products with rabbit anti-Mac-1 serum by the method of Anderson & Blobel (1983). The positions of the α- and β-precursors synthesized *in vitro* from total $P388D_1$ mRNA and identified by immunoprecipitation are indicated.

in precipitating translation products. Therefore, a rabbit antiserum was prepared against mouse Mac-1 antigen purified on mAb immuno-affinity columns (Kurzinger & Springer, 1982). Polysomes reacted with anti-Mac-1 IgG were isolated by passage through protein A–Sepharose (Shapiro & Young, 1981). Fractions were translated by using a rabbit reticulocyte lysate system in the presence of [^{35}S]-methionine and subjected to SDS/polyacrylamide-gel electrophoresis. The major translation product was the Mac-1 α-subunit (Fig. 3*a*). Its identity was confirmed by immunoprecipitation with anti-Mac-1 serum, which also detected the β-subunit (Fig. 3*b*). Since the Mac-1 molecule represents only 0.1% of $P388D_1$ cellular protein (Kurzinger & Springer, 1982), polysome immunoselection achieved a remarkable purification of specific mRNA.

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Molecular analysis of the human T-cell-antigen receptor

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T-lymphocytes, unlike B-lymphocytes, predominantly recognize antigen when it is associated with membrane-bound products of the MHC (Schlossman, 1972; Zinkernagel & Doherty, 1975; Benacerraf & McDevitt, 1979; Corradin & Chiller, 1979). This 'dual' recognition is important for activation of both cytotoxic effector T-cells and immunoregulatory T-cells. T-cells of the former category lyse specific target cells, including cells infected with virus that display viral antigens (Cerottini, 1980; Doherty, 1980; Hunig & Bevan, 1981), whereas immunoregulatory T-cells act as inducers or suppressors for interactions between T-cells, B-cells, macrophages and other cells (Quinnan *et al.*, 1982; Wallace *et al.*, 1982; Meuer *et al.*, 1983*d*; Gershon, 1974; Cantor & Boyse, 1976).

The specificity of T-cells is determined by their cell-surface receptors, and therefore knowledge of these receptors is particularly useful in understanding the cellular interactions that underlie the different activities of T-cells. Because T-lymphocytes recognize antigen in a precise

Abbreviation used: MHC, major histocompatibility complex.