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p150,95, THE THIRD MEMBER OF THE MAC-1, LFA-1 HUMAN LEUKOCYTE ADHESION GLYCOPROTEIN FAMILY[1]

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Monoclonal antibodies specific for p150,95, a third member of the Mac-1 and lymphocyte function-associated antigen-1 (LFA-1) leukocyte adhesion protein family, have been identified and used to study the biochemistry and cellular expression of p150,95. p150,95 is a noncovalently associated heterodimer containing αX and β subunits of M_r = 150,000 and 95,000 respectively. Findings suggest that the p150,95 αXβ complex shares a common β subunit with the αLβ LFA-1 and αMβ Mac-1 complexes. Co-precipitation experiments demonstrated identity between the p150,95 molecule precipitated by anti-β MAb and by p150,95-specific MAb. Patients with a previously demonstrated genetic deficiency in Mac-1 and LFA-1 fail to express p150,95. Deficiency of the Mac-1, LFA-1, and p150,95 αβ complexes on the surface of patient cells appears due to a defect in the common β subunit. The lack of cross-reaction of p150,95-specific MAb with LFA-1 and Mac-1, which appear to utilize identical β subunits, suggests that the determinant is specified by the αX rather than the β subunit of p150,95. The data suggest that αX is yet a third member of a family of α subunit proteins that associate with a common β subunit, are differentially regulated in leukocyte differentiation, and function in adhesion reactions. p150,95 is normally expressed on blood monocytes and granulocytes. Chemoattractants such as f-Met-Leu-Phe stimulate a rapid, fivefold increase in surface expression that is not dependent on protein synthesis and appears to reflect mobilization of an intracellular latent pool. The intimate relation between the lack of chemoattractant-stimulated upregulation of p150,95 and Mac-1 by patient granulocytes and their failure to upregulate adhesiveness to these same stimuli in vitro, or to diapedese and migrate into inflammatory sites in vivo, is discussed.

Several leukocyte cell surface molecules that share a common β subunit of M_r = 95,000 and function in adhesion reactions have been characterized both in mice and humans (1–3). The lymphocyte function-associated antigen-1 (LFA-1)[2] participates in T lymphocyte-mediated killing and other leukocyte cell-cell adhesion reactions (3). The macrophage 1 (Mac-1) molecule appears identical to the complement receptor type 3 on monocytes and granulocytes and also functions in adhesion of these cells to protein-coated surfaces (4, 5). Human Mac-1 is identical to the Mo1 and OKM1 antigens (6, 7). LFA-1 and Mac-1 each contain a different α subunit (αL = 180,000 M_r and αM = 165,000 M_r respectively) noncovalently associated with a common β subunit of M_r = 95,000 in an $\alpha_1\beta_1$ structure. Both α and β subunits are glycosylated and appear to be membrane glycoproteins with surface exposure. The αL and αM subunits have 35% N-terminal sequence homology, suggesting that they are products of a family of distinct but evolutionarily related genes (8). Heritable deficiency of the common β subunit in humans leads to an absence of surface expression of the αL and αM subunits as well (9). Mac-1, LFA-1 deficiency manifests itself clinically in recurrent, life-threatening bacterial infections, and in vitro in multiple leukocyte adhesion defects (4).

A putative third type of α subunit of M_r = 150,000 has been biochemically detected after precipitation with a monoclonal antibody (MAb) specific for a determinant on the common β subunit (2). This subunit was designated αX. There was concern that αX might be a breakdown product of the higher m.w. αL or αM subunits; however, no evidence for a proteolytic derivation could be found. Cross-linking experiments showed that the αX subunit was noncovalently associated with the β subunit in an $\alpha_1\beta_1$ complex. The αXβ complex was denoted p150,95. If αX truly represented a third, distinct member of the α subunit family, it should be possible to obtain anti-αX MAb that would be specific for p150,95. Such MAb should be useful for characterizing the biochemistry, cellular expression, and function of this molecule. Here, we report on such MAb. We show that the p150,95 molecule defined by specific MAb is a member of the Mac-1, LFA-1 protein family, because it reacts with a MAb to the shared β subunit. Furthermore, patients with a genetic deficiency in the expression of Mac-1 and LFA-1, apparently due to a defect in the common β subunit, also lack expression of p150,95. We also demonstrate that p150,95 is stored in a latent pool that can be mobilized to the cell surface in response to inflammatory stimuli.

MATERIALS AND METHODS

Monoclonal antibodies (MAb). TS1/22 anti-αL MAb and TS1/18 anti-β MAb have been described (2, 10). OKM1 and OKM10 anti-αM

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[2] Abbreviations used in this paper: CR1, complement receptor type 1; LFA-1, lymphocyte function-associated antigen-1; MAb, monoclonal antibody.

MAb (2) were the kind gift of Dr. G. Goldstein, Ortho Pharmaceuticals, Raritan, NJ. 44D anti-complement receptor type 1 (CR1) MAb (11) was the kind gift of Dr. V. Nussenzweig. A panel of 118 anti-myeloid MAb were studied in the Second International Leukocyte Workshop, and two MAb were found to be p150,95 specific (12). Decoding in September 1984 revealed that these were SHCL3 (13), contributed by Dr. R. Schwarting, Institut fur Pathologie, Berlin, W. Germany, and Ki-M1 (14), contributed by Dr. H. Radzun, University of Kiel, Kiel, W. Germany. SHCL3 may be obtained as Leu-M5 from Becton-Dickinson, Mountain View, CA.

Other methods. Granulocytes and mononuclear cells were isolated on Ficoll-Hypaque layers of d = 1.106 and 1.08, respectively (9). f-Met-Leu-Phe-stimulated granulocytes were labeled with ^{125}I by using lactoperoxidase (9). Immunoprecipitates were formed from Triton X-100 lysates with purified TS1/18 or TS1/22 coupled to Sepharose CL-4B, or for other MAb with *Staphylococcus aureus* bacteria (9). Immunoprecipitates reduced with 5% 2-mercaptoethanol were subjected to Laemmli SDS-PAGE. For immunofluorescence, leukocytes were incubated with or without 10^{-8} M f-Met-Leu-Phe for 30 min at 37°C or were held for 30 min at 4°C, were labeled at 4°C with saturating concentrations of specific MAb or control MAb (P3X63, IgG1), were washed, were labeled with fluorescein isothiocyanate-conjugated, affinity-purified goat anti-mouse IgG, were subjected to immunofluorescence flow cytometry on an Epics V, and were quantitated for fluorescence intensity as described (4, 9). Mac-1, LFA-1-deficient patients were cases No. 1 and 2 (severely deficient) and No. 6, 7, and 8 (moderately deficient) (4).

RESULTS

Antibodies submitted to the myeloid panel of the Second International Workshop on Human Leukocyte Differentiation Antigens were tested for precipitation of p150,95 from lysates of granulocytes labeled with ^{125}I by using lactoperoxidase. Two MAb out of 118 tested, SHCL3 and Ki-M1, precipitated subunits of 150,000 and 95,000 M_r (Fig. 1*A*, lane 1; results are for the most strongly precipitating MAb, SHCL3). The 150,000 and 95,000 M_r subunits precipitated by SHCL3 were not linked by disulfide bonds, as determined by SDS-PAGE under non-reducing conditions (not shown). The 150,000 and 95,000 M_r subunits co-migrated with the αX and β subunits, respectively, precipitated by an anti-β MAb (Fig. 1*A*, lane 3). The anti-β MAb additionally precipitated the $\alpha L\beta$ and $\alpha M\beta$ complexes, as shown by the presence in the precipitate of the αL and αM subunits respectively (Fig. 1*A*, lane 3). Antibodies to the subunits αM and αL precipitated the Mac-1 $\alpha M\beta$ and the LFA-1 $\alpha L\beta$ complexes respectively (Fig. 1*A*, lanes 2 and 4), and showed no cross-reaction with αX, as reported (2).

To determine whether the 150,000 and 95,000 M_r subunits precipitated by SHCL3 were identical to the αX and β subunits precipitated with the TS1/18 anti-β subunit MAb, co-precipitation experiments were carried out. Preclearing with SHCL3 greatly reduced the amount of αX precipitated by the anti-β MAb (Fig. 2; compare lanes 8 and 9). Conversely, preclearing with the anti-β MAb removed the subunits precipitated by SHCL3 (Fig. 2; compare lanes 10 and 11). Thus, the molecule precipitated by SHCL3 is identical to the p150,95 molecule precipitated by anti-β subunit MAb (2).

In some p150,95 immunoprecipitates, the αX subunit was more apparent than the β subunit (for example, Fig. 2, lanes 10 and 13). Because preclearing with anti-β MAb (Fig. 2, lane 11) demonstrated association between αX and β, this must be due to the lower ^{125}I labeling efficiency of the β subunit, its greater diffuseness in SDS-PAGE (Figs. 1 and 3), or some dissociation of β during precipitation by SHCL3 MAb.

Previously, antibodies specific for Mac-1, LFA-1, and which cross-reacted with Mac-1, LFA-1 and p150,95,

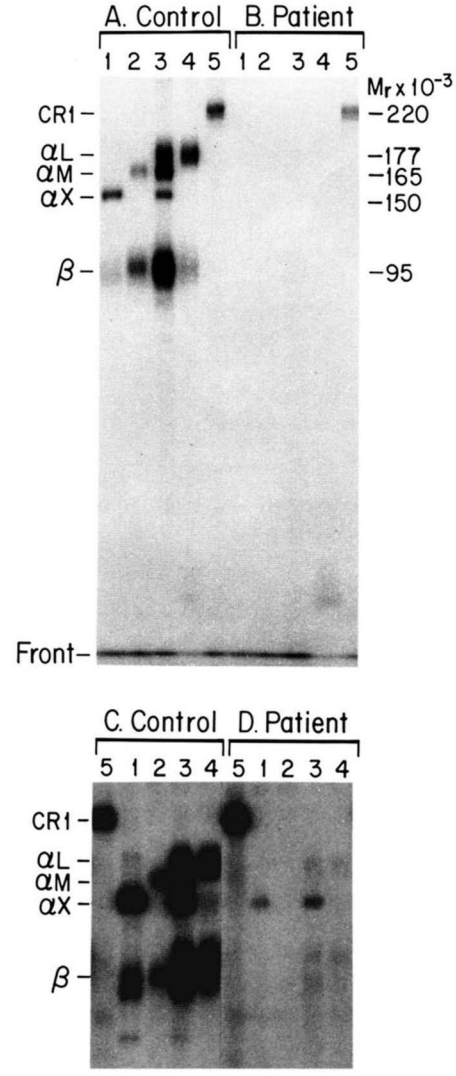

Figure 1. Immunoprecipitation from healthy control and Mac-1, LFA-1-deficient granulocytes. Granulocytes from a healthy control (*A*), the healthy heterozygote sister of patient 8 (*C*), severely deficient patient 1 (*B*), or moderately deficient patient 8 (*D*) were stimulated with 10^{-8} M f-Met-Leu-Phe, surface labeled with ^{125}I and lysed with Triton X-100. Lysates 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 as the positive control (*lane 5*). Immunoprecipitates were subjected to SDS-7% PAGE under reducing conditions and to normal (*A* and *B*) or prolonged (*C* and *D*) autoradiography.

were shown after dissociation by high pH of the $\alpha\beta$ complexes to be specific for the αM, αL, and β subunits respectively. Similar experiments were carried out with p150,95 both to examine the stability of the $\alpha X\beta$ complex and to attempt to localize the SHCL3 determinant. Triton X-100 lysates from ^{125}I-labeled granulocytes were adjusted to 3.0 or pH 11.5 for 4 hr, were neutralized, and were subjected to immunoprecipitation (Fig. 3). After pH 3 treatment, a small amount of β subunit, with no remaining associated α subunits, was precipitable with anti-β MAb (Fig. 3A, lane 1). There was no SHCL3 immunoprecipitate (Fig. 3*A*, lane 2). After pH 11.5 treatment, αX but not αL or αM remained associated with the β subunit, as shown with the anti-β MAb (Fig. 3; compare lanes *B*1 and *C*1). The $\alpha X\beta$ complex remained precipitable with SHCL3 MAb (Fig. 3*B*, lane 2). This shows that the $\alpha X\beta$ complex is more resistant to high pH-induced dissociation than $\alpha L\beta$ or $\alpha M\beta$. However, we were unable

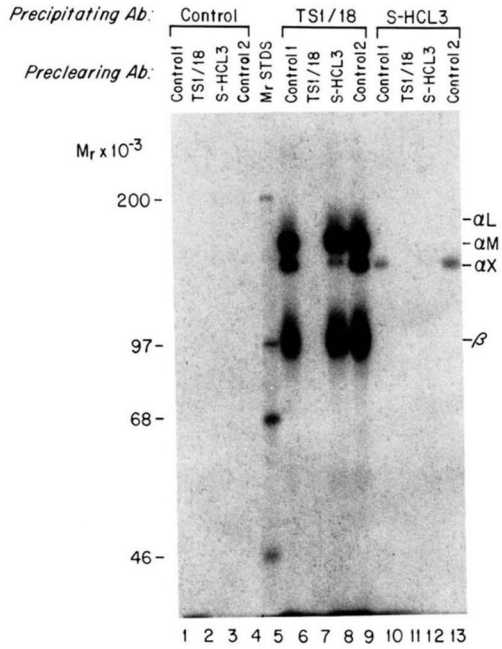

Figure 2. Preclearing between anti-p105,95 and anti-β MAb. ^{125}I-human granulocyte lysate (1.8×10^6 cpm) was precleared twice with CNBr-activated, quenched Sepharose CL-4B (*control 1*), TS1/18.1.2 anti-β MAb-Sepharose, SHCL3 anti-p150,95 MAb and *S. aureus* bacteria, or normal rabbit serum and *S. aureus* bacteria (*control 2*). The lysates were then immunoprecipitated with control 1, TS1/18.1.2 MAb-Sepharose, SHCL3 MAb and *S. aureus* bacteria, or control 2. Immunoprecipitates were subjected to SDS-7% PAGE and autoradiography for 4 days.

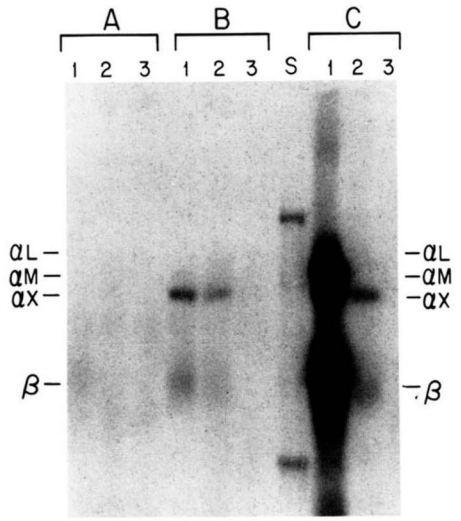

Figure 3. Effect of low and high pH on subunit association. Granulocyte Triton X-100 lysates (10^6 cpm) were dialyzed against 0.1 M glycine-HCl, 0.15 M NaCl, pH 3.0 (*A*); 0.1 M triethylamine, 0.15 M NaCl, pH 11.5 (*B*); or 0.1 M Tris-HCl, 0.15 M NaCl, pH 8.0 (*C*) for 4 hr at 4°C. Lysates were neutralized with 1/10 vol 1 M Tris-HCl, pH 8.0. Immunoprecipitates with TS1/18 MAb-Sepharose (*lane 1*), SHCL3 MAb and *S. aureus* bacteria (*lane 2*), or normal rabbit serum and *S. aureus bacteria* (*lane 3*) were subjected to reduction and SDS-7% PAGE and autoradiography. M_r standards: lane S.

to find conditions of extreme pH, urea, or guanidine-HCl denaturation under which the αX subunit was dissociated from β and the SHCL3 MAb reactivity was retained. The lack of cross-reaction of SHCL3 with the Mac-1 and LFA-1 molecules strongly suggests that its determinant is specified by the αX subunit rather than by the common β subunit. Whether the determinant is on the αX subunit or is due to the interaction between the αX and β subunits remains to be determined.

By using SHCL3 MAb and immunofluorescent flow cytometry, it was found that p150,95 is weakly expressed on peripheral blood granulocytes (Fig. 4*a*) and monocytes (Fig. 5*a*). The vast majority of peripheral blood lymphocytes are negative (12). It was previously found that brief exposure of granulocytes to chemoattractants resulted in markedly increased surface expression of Mac-1 but not LFA-1 (9). The availability of MAb specific for p150,95 allowed its modulation by chemoattractants to also be studied. After incubation with 10^{-8} M f-Met-Leu-Phe for 30 min at 37°C, surface expression on granulocytes of p150,95 was strikingly increased an average of fourfold to sevenfold, as shown with the two different anti-p150,95 MAb (Fig. 4*b* and Table I). In the experiment in

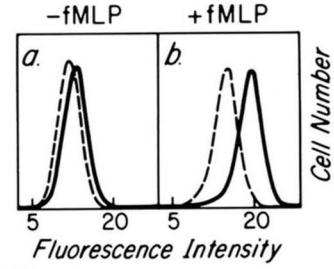

Figure 4. p150,95 surface expression on granulocytes is increased by f-Met-Leu-Phe stimulation. Granulocytes were (*a*) held at 4°C or (*b*) incubated 30 min at 37°C with 10^{-8} M f-Met-Leu-Phe. Cells were stained with SHCL3 anti-p150,95 MAb (*solid curves*) or control MAb (*dashed curves*), followed by fluorescein isothiocyanate-anti-mouse IgG, and were subjected to immunofluorescence flow cytometry.

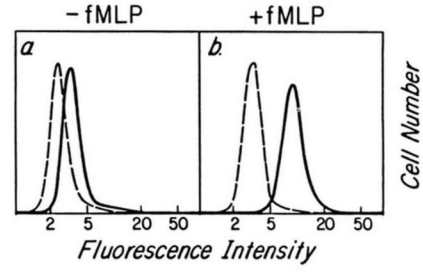

Figure 5. Chemoattractant stimulates increased expression of p150,95 on monocytes. Mononuclear cells were incubated for 30 min at 37°C with 10^{-8} M f-Met-Leu-Phe, or were held at 4°C as indicated. Cells were stained at 4°C with anti-p150,95 (*solid curves*) or control (*dashed curves*) MAb, followed by fluorescein isothiocyanate-anti-mouse IgG, and were subjected to immunofluorescence flow cytometry. Fluorescence of monocytes was determined by gating on 90° and forward angle light scatter to exclude lymphocytes.

TABLE I

f-Met-Leu-Phe-stimulated p150,95 upregulation on granulocytes[a]

	p150,95-Specific Fluorescence Intensity (% of baseline)
Experiment 1	
4°C	100
37°C	140
37°C + fMLP	440
Experiment 2	
4°C	100
37°C	100
37°C + fMLP	685
37°C + cycloheximide + fMLP	640

[a] Granulocytes, with or without preincubation with 10 μg/ml cycloheximide for 10 min, were held at 4°C, 37°C, or 37°C with 10^{-8}M f-Met-Leu-Phe for 30 min. Cells in experiment 1 were Ficoll-Hypaque purified before stimulation; those in experiment 2 were stimulated after dextran sedimentation, and then were Ficoll-Hypaque purified on a single layer of d = 1.106 and resolved from mononuclear cells by gating on both forward angle and 90° light scatter. Cells were washed at 4°C and labeled with anti-p150,95, and control MAb at 4°C, as described in *Materials and Methods*. Linear fluorescence intensity was calculated, control fluorescence was subtracted, and specific fluorescence intensity was normalized to baseline expression at 4°C.

Figure 4, control cells were held at 4°C to avoid upregulation triggered by cell purification procedures (15). Other experiments in which purified granulocytes were held either at 4°C or at 37°C in the absence of f-Met-Leu-Phe, or at 37°C in the presence of f-Met-Leu-Phe, demonstrated that the increase due to temperature alone was 1.4-fold, and the increase at 37°C due to f-Met-Leu-Phe addition alone was 3.1-fold (Table I, experiment 1). When granulocytes were stimulated with f-Met-Leu-Phe before purification, there was no temperature-dependent upregulation, and upregulation due to f-Met-Leu-Phe was sixfold (Table I, experiment 2). Upregulation was maximal within 8 min at 37°C, and was unaffected by the inhibitor of protein synthesis cycloheximide (Table I). p150,95 surface expression on granulocytes was also "upregulated" by phorbol myristate acetate and C5a.

Similar studies were carried out on monocytes. p150,95 surface expression on monocytes was dramatically increased by f-Met-Leu-Phe (Fig. 5*b*). Upregulation was consistently found in all experiments, and ranged from fourfold to sixfold.

We next examined whether patients with a genetic deficiency of the Mac-1 and LFA-1 molecules were also deficient in p150,95. Granulocytes from a severely deficient patient were studied after stimulation with f-Met-Leu-Phe (Fig. 6). Patient cells not only lacked surface expression of the β, αM, and αL subunits (Fig. 6*b*, *d*, and *f*) as reported (9), but were also deficient in the $\alpha X\beta$ complex as shown with the anti-p150,95 MAb (Fig. 6*h*). Studies conducted on both unstimulated (not shown) and on f-Met-Leu-Phe-stimulated granulocytes show that patient cells lack basal surface expression as well as a latent, upregulatable pool of p150,95. Deficiency was also studied by immunoprecipitation from lysates of surface ^{125}I-labeled, f-Met-Leu-Phe-stimulated granulocytes (Fig. 1). No immunoprecipitation of p150,95 or other members of the Mac-1, LFA-1 glycoprotein family could be detected from granulocytes of severely deficient patients (a representative patient is shown in Fig. 1*B*), even after prolonged autoradiogram exposure (not shown). In a representative moderately deficient patient, p150,95 was detectable (Fig. 1*D*, lane 1), but in greatly diminished amounts compared with granulocytes from his healthy, heterozygote sister (Fig. 1*C*, lane 1). Cells from three affected relatives of this male patient (father, sister, and third cousin) exhibited identically reduced amounts of p150,95. The p150,95 αX subunit was also detected in moderately deficient cells after precipitation with anti-β MAb (Fig. 1*C*, lane 3), suggesting that it is present on the surface of these cells as an $\alpha X\beta$ complex. As a positive control, the CR1 was precipitable in similar amounts from control and patient cells (compare Fig. 1*A* and *B*, lane 5, and Fig. 1*C* and *D*, lane 5).

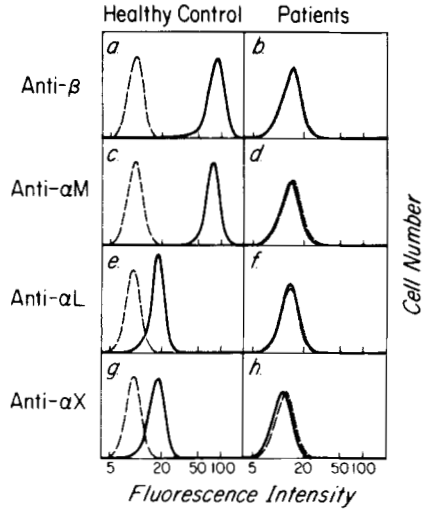

Figure 6. Deficiency of p150,95 on granulocytes of a severely Mac-1, LFA-1-deficient patient. Granulocytes of severely deficient patient no. 1 (4) or of a healthy control were incubated for 30 min at 37°C with 10^{-8} M f-Met-Leu-Phe, were then stained with specific MAb (*solid curves*) or control MAb (*dashed curves*), and were subjected to immunofluorescence flow cytometry.

DISCUSSION

A third member of the Mac-1, LFA-1 leukocyte adhesion protein family, p150,95, has been defined in this report. Previously, the p150,95 αX subunit had only been detected with anti-β subunit MAb in immunoprecipitates from monocytes and granulocytes (2). It was distinguished from the αL subunit of LFA-1 and the αM subunit of Mac-1 by its relative molecular mass in SDS-PAGE. Cross-linking experiments demonstrated that all three types of $\alpha\beta$ complexes precipitated by anti-β MAb, $\alpha L\beta$, $\alpha M\beta$, and $\alpha X\beta$, were present after solubilization with nonionic detergent as $\alpha_1\beta_1$ quaternary structures. The $\alpha X\beta$ complex was not precipitated by MAb specific for the αL and αM subunits of LFA-1 and Mac-1 respectively, suggesting that αX was immunochemically distinct. Indeed, MAb specific for p150,95 are reported here. Two different MAb reacted with p150,95 but not with Mac-1 or LFA-1. Co-precipitation experiments demonstrated that the p150,95 molecule defined by SHCL3 is identical to the p150,95 molecule previously defined by anti-β MAb. The $\alpha X\beta$ complex is more resistant than $\alpha M\beta$ and $\alpha L\beta$ to dissociation by high pH. The identity of the p150,95, Mac-1, and LFA-1 β subunits in two-dimensional isoelectric focusing (2), and the previously demonstrated reactivity with α subunits of MAb specific for the Mac-1 and LFA-1 molecules in both mouse and human systems (1, 2), suggest that the p150,95-specific MAb recognize determinants specified by the αX subunit.

The presence of unique antigenic determinants on the p150,95 molecule strengthens previous evidence (2) that the αX subunit is a distinct member of the α subunit protein family and is not derived from αL or αM by proteolysis or processing. Previous studies on a cell line biosynthesizing all three types of α subunits demonstrated that αX, αL, and αM had distinct precursors that appeared with similar kinetics (2). Sequencing of murine αL and αM demonstrated that they are products of distinct and homologous genes (8). Sharing of the same β subunit by αX, and its distinctness from αM and αL, suggest that it is encoded by a third distinct and homologous gene. It will be important to test this prediction by amino acid or nucleic acid sequencing of αX.

The p150,95 molecule was found here to be expressed on monocytes and granulocytes and to be absent from the majority of peripheral blood lymphocytes. These findings are in agreement with recent extensive studies by Schwarting et al. (13) on the tissue distribution of this antigen. Tissue macrophages stain much more strongly for p150,95 than for Mac-1 (OKM1/Mo1), whereas the opposite is true of neutrophils (13). We found that like neutrophils, blood monocytes also express more Mac-1

(OKM1) than p150,95 (12), suggesting that after monocytes enter tissues and differentiate into macrophages, the relative expression of Mac-1 and p150,95 is reversed. αX expression appears to be independently regulatable from αL and αM, because αX is present in low quantities on some B cell lines that fail to express αM, and αL^+ myelomonocytic cell lines become αX^+ after phorbol ester stimulation (L. Miller, unpublished).

Schwarting et al. (13) found that SHCL3 precipitated a molecule with chains of 150,000 and 95,000 M_r from hairy leukemia cells, similar to the p150,95 molecule isolated here from granulocytes and previously with anti-β MAb from monocytes and U937 cells (2). However, Schwarting et al. (13) did not describe a molecular relationship to Mac-1 and LFA-1, as is reported here.

We found that chemoattractants and phorbol esters triggered a rapid increase in p150,95 expression on the granulocyte cell surface. This increase was not blocked by inhibitors of protein synthesis, suggesting that it was due to mobilization to the surface of a pre-existing intracellular pool. These agents induce similar increases in Mac-1 expression on granulocytes (6, 9). Neutrophil secondary granule secretion occurs under the same conditions, and secondary granules may be the site of the Mac-1 latent pool (6, 16). The site of the p150,95 latent pool remains to be determined.

None of the members of the Mac-1, LFA-1 family had previously been studied as to upregulation on monocytes. We found here that p150,95 expression was increased fourfold to sixfold after incubation of monocytes at 37°C with f-Met-Leu-Phe compared with incubation at 4°C alone. However, under the conditions of cell preparation used here, almost as much upregulation occurs when monocytes are incubated at 37°C alone (data not shown). More recently, we have minimized the number of centrifugation steps before monocytes are treated with f-Met-Leu-Phe, and fivefold upregulation compared with cells incubated at 37°C alone has been found. Mac-1 is also upregulated on monocytes (T. Springer, unpublished). Because monocytes have different types of cytoplasmic granules from neutrophils, and because monocyte upregulation of Mac-1 and p150,95 might be important in the inflammatory response, this phenomenon is under additional investigation.

A group of patients with recurring bacterial infections and a defect in mobilization of neutrophils and monocytes into inflammatory sites has been found to be genetically deficient in the expression of the LFA-1 and Mac-1 molecules (9, and reviewed in Reference 4). Neither the αL, αM, or β subunits were expressed on the surface of cells from these patients. The p150,95 molecule was not detected by precipitation with anti-β MAb (9). In this study we have found that p150,95 is also not detectable with two different p150,95-specific MAb. This provides additional evidence for the identity between the p150,95 molecule defined by anti-β- and p150,95-specific MAb, and for the deficiency of p150,95 in patient cells. All five patients thus far examined, of both severe and moderate phenotypes, are deficient in the expression of $\alpha X\beta$, $\alpha L\beta$, and $\alpha M\beta$ complexes. It appears that the primary defect in patient cells is in the β subunit, and that α-chains are biosynthesized but require β subunit association for normal carbohydrate processing and transport to the cell surface and latent pools (9). The absence of p150,95 expression in patient cells is also evidence for a close relationship with Mac-1 and LFA-1, and is consistent with the proposed defect in the common β subunit. The molecular deficiency in this disorder appears highly specific, because of 118 different anti-myeloid MAb tested, only those to Mac-1, LFA-1 or p150,95 were negative on patient cells (12).

Patient granulocytes show multiple defects in adhesion-related functions, including adherence and spreading on protein-coated surfaces, cell-cell aggregation, and chemotaxis (reviewed in References 4 and 5). The lack of chemoattractant-stimulated upregulation of p150,95 and Mac-1 by patient granulocytes appears intimately related to their inability to aggregate, hyperadhere, or chemotact to these stimuli, and to their in vivo defect in diapedesis and migration into inflammatory sites. Baseline and stimulated adherence by normal granulocytes is inhibited by MAb, with the order of potency anti-$\beta \geq$ anti-Mac-1 > anti-p150,95 > anti-LFA-1 (5). These and other observations about this glycoprotein family (3) suggest that it is of central importance in the regulation of leukocyte adherence reactions. The three members of this family can now be studied individually with specific MAb. This should greatly aid additional study of their individual functional contributions to different adherence reactions, regulation of their expression in leukocyte differentiation, and purification and biochemical characterization.

Note Added in Proof: The recent findings of Lanier et al. (17) are consistent with those presented here and by us at the Second International Workshop on Leukocyte Differentiation (12).

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