CHAPTER 4

Antibodies Specific for the Mac-1, LFA-1, p150,95 Glycoproteins or Their Family, or for Other Granulocyte Proteins, in the 2nd International Workshop on Human Leukocyte Differentiation Antigens

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Introduction

A family of functionally important leukocyte surface glycoproteins which share a common β subunit of M_r = 95,000 has recently been defined in humans and mice (1,2). These glycoproteins, the lymphocyte function-associated 1 (LFA-1), macrophage 1 (Mac-1), and p150,95 molecules each contain a different α subunit noncovalently associated with the common β subunit in an $\alpha_1\beta_1$ structure. Monoclonal antibodies specific for the LFA-1 and Mac-1 molecules have allowed definition of their cell distributions and functions.

The LFA-1 molecule is expressed on B and T lymphocytes, NK cells, monocytes, and granulocytes. mAbs to LFA-1 block cytolytic T lymphocyte-mediated killing, natural killing, and T helper cell responses (3,4). Anti-LFA-1 blocks the adhesion of cytolytic T lymphocytes to target cells, and it has been proposed that LFA-1 is an adhesion protein of wide importance in interactions of leukocytes with other cells (5,6).

The Mac-1 molecule, identical to OKMI and Mol, is expressed on granulocytes, monocytes, and natural killer cells, and is absent on lymphocytes (reviewed in Ref. 1). In response to chemo-attractants and secretagogues, Mac-1 surface expression on granulocytes is increased or "up-regulated" 5-fold (7)]. Mac-1 is identical to the complement receptor type 3 on myeloid cells, which mediates adherence to and phagocytosis of particles bearing iC3b, a cleaved, hemolytically inactive fragment of the third component of complement (8–10).

A genetic deficiency of this glycoprotein family has been found in certain patients with recurring bacterial infections (7). Deficiency is inherited as an autosomal, recessive trait. Over 19 patients have been identified worldwide within the last year (reviewed in Ref. 11). All patients lack the α subunits of Mac-1 and LFA-1 and the common β subunit on leukocyte surfaces. A latent, intracellular pool of Mac-1 is also deficient. Biosynthesis experiments have suggested that β subunit synthesis is defective in patient cells, and that normal α chain precursors are present, but are not transported to the cell surface, due to a lack of association with the β subunit (7).

Monoclonal antibodies specific for LFA-1, specific for Mac-1, or cross-reacting between LFA-1, Mac-1, and p150,95 have previously been obtained. Studies on antigen preparations in which the α and β subunits had been dissociated by brief exposure to high pH showed that these mAbs were specific for the LFA-1 α (αL), the Mac-1 α (αM), or the common β subunit, respectively (1). Two-dimensional isoelectric focusing SDS–PAGE and peptide mapping have confirmed that these molecules contain different α subunits and identical β subunits (1,12–14).

The third member of the family, p150,95, named after the $M_r \times 10^{-3}$ of its subunits, has been defined biochemically after isolation from myeloid cells with anti-β mAb (1). The p150,95 α subunit, designated αX, was found to be antigenically distinct from αM and αL, since it did not react with mAbs defining multiple epitopes on the latter subunits. It was predicted that it should be possible to obtain mAbs to unique epitopes of αX, which would be useful for characterization of the structure and function of p150,95.

In this study, the 118 mAbs submitted in the myeloid panel of the Second International Conference on Human Leukocyte Differentiation Antigens were tested for reactivity with members of the Mac-1, LFA-1, p150,95 family. It was of interest to compare mAbs from different labs, which have been the subject of several publications (1,3,4,7,11,15–22). Furthermore, the following questions were addressed: (1) Which mAbs reacted with specific members of the family and which cross-reacted with all three members? Did any show unusual specificities, such as cross-reaction between only two members of the family? (2) Could mAbs specific for the p150,95 molecule be identified in the panel? (3) Which members of the family were up-regulated on myeloid cell surfaces by chemo-attractants? (4) Which mAbs in the myeloid panel were negative on Mac-1-, LFA-1-deficient patients? How specific was the deficiency to the Mac-1, LFA-1, p150,95 glycoprotein family?

In the course of these studies, information was also obtained on molecules distinct from Mac-1, LFA-1, and p150,95. This is presented as an appendix.

Methods

Lactoperoxidase-catalyzed ^{125}I-labeling of granulocytes, immunoprecipitation and SDS–PAGE, labeling of cells with mAbs and FITC anti–mouse IgG followed by analysis on an Epics V flow cytometer, and Mac-1-, LFA-1-deficient patients have been previously described (1,7,11).

Results and Discussion

Identification of mAbs to Mac-1, LFA-1, and p150,95

Granulocytes were used to screen for mAbs to Mac-1, LFA-1, or p150,95, since they express all three molecules (1). Granulocytes were stimulated with f-Met-Leu-Phe to increase Mac-1 and p150,95 expression, labeled with ^{125}I, and subjected to immunoprecipitation with each workshop mAb. Immunoprecipitates of positive mAbs were then compared side-by-side in further experiments with the previously characterized TS1/22 anti-αL, OKM1 (or OKM10) anti-αM, and TS1/18 anti-β MAb (1) (Fig. 4.1). The LFA-1 αL and β subunits of M_r = 177,000 and 95,000, respectively, were precipitated by TS1/22,* MHM24, 25.3.1, and CIMT (Fig. 4.1, lanes 1, 2, 11, and 13). The Mac-1 αM and β subunits of 165,000 and 95,000 M_r, respectively, were precipitated by VIM12, CC1.7, OKM10, D12, M3D11, Ki-M5, and 44 (Fig. 4.1, lanes 14–22). The workshop mAb 60.1 appeared to precipitate Mac-1 and, more weakly, other members of the family (Fig. 4.1, lane 5), but further testing with authentic 60.1 showed precipitation of only Mac-1 (not shown). The MHM23, TS1/18, 60.3 (Fig. 4.1, lanes 3, 6–8, and 12) and CLB-54 mAb (not shown) precipitated the αL, αM, and αX subunits and the common β subunit.

Interestingly, the SHCL3 and Ki-M1 mAb appeared to specifically precipitate the αX and β subunits of p150,95 [Fig. 4.1, lanes 9 and 10, and see below, Fig. 4.5(A), lane 1]. The αX and β subunits precipitated by SHCL3 were identical to those precipitated by the TS1/18 anti-β mAb in isoelectric focusing and in coprecipitation experiments (T. Springer and L. Miller, unpublished). Thus, SHCL3 clearly is specific for p150,95, the third member of the glycoprotein family which had previously been detected by precipitation with anti-β mAb (1). Because of the identity of the β subunits among Mac-1, LFA-1, and p150,95, the specificity of SHCL3 suggests reactivity with the p150,95 αX subunit; however, this remains to be experimentally verified.

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* Since M72, the Workshop TS1/22, appeared contaminated with an anti-β MAb (Fig. 4.1, lane 4), authentic TS1/22 was used in these studies.

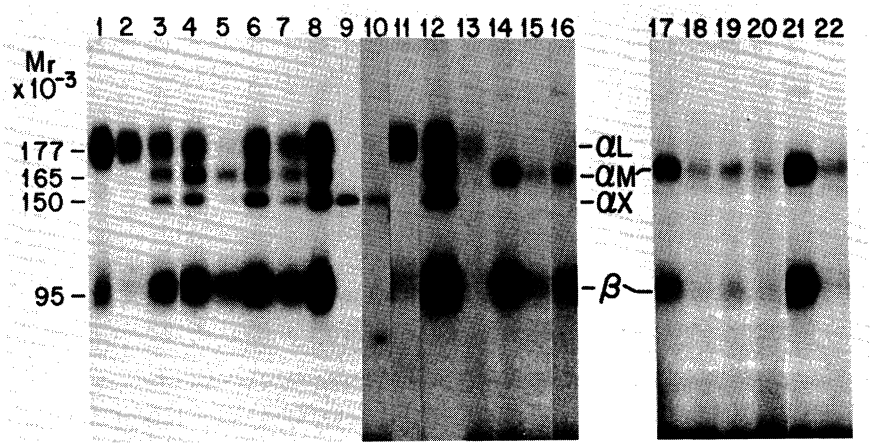

Fig. 4.1. Precipitation from ^{125}I-labeled granulocytes by mAbs specific for Mac-1, LFA-1, p150,95, or their family. ^{125}I-labeled f-Met-Leu-Phe-stimulated granulocyte lysates were immunoprecipitated with TS1/22–Sepharose CL-4B (lane 1); M56, MHM24 (lane 2); M55, MHM23 (lane 3); M72, TS1/22 (lane 4); M76, 60.1 (lane 5); M89, TS1/18.11 (lane 6); TS1/18.11 (lane 7); TS1/18.11–Sepharose CL-4B (lane 8); M46, SHCL3 (lane 9); M93, Ki-M1 (lane 10); 25.3.1 (lane 11); M75, 60.3 (lane 12); M107, CIMT (lane 13); M26, VIM12 (lane 14); M41, CC1.7 (lane 15); OKM10 (lane 16); M26, VIM12 (lane 17); M41, CC1.7 (lane 18); M42, D12 (lane 19); M78, M3D11 (lane 20); M88, Ki-M5 (lane 21); M104, 44 (lane 22). Immunoprecipitates were subjected to SDS–7.5% PAGE and autoradiography for 4 days (lanes 1–9, 17–22) or 20 days (lanes 10–16). Precipitates in lanes 2–7 and 9–16 were formed with 2–4-μl Workshop mAb, 60 μl rabbit anti–mouse IgG, and 50 μl *S. aureus* bacteria; those in lanes 17–22 were formed with 1-μl Workshop mAb and a mixture of rabbit anti–mouse IgG (60 μl) and sheep anti–mouse MOPC 104E IgM myeloma (5 μl) and no *S. aureus*.

The distribution of these antigens was examined by immunofluorescent flow cytometry. The staining pattern on mononuclear cells was characteristic for each type of antibody specificity. Representative immunofluorescence histograms are shown for Mac-1, LFA-1, p150,95-cross-reactive mAbs [Fig. 4.2(a)–(c)], anti-Mac-1 mAbs [Fig. 4.2(d)–(f)], anti-LFA-1 mAbs [Fig. 4.2(g)–(i)], and anti-p150,95 mAbs [Fig. 4.2(j) and (k)]. Anti-β mAbs [Fig. 4.2(a)–(c)] and anti-LFA-1 mAbs [Fig. 4.2, (g)–(i)] stained all mononuclear cells, and revealed differences among subpopulations in the quantity of antigen expressed, as previously described (3). Weaker staining by CIMT than other anti-LFA-1 mAbs (Fig. 2i) was due to its lower titer, as revealed by staining at different CIMT mAb concentrations (not shown). Anti-Mac-1 and anti-p150,95 mAbs stained a subpopulation of mononuclear cells [Fig. 4.2(d)–(f), (j) and (k)]. Scatter gating showed this

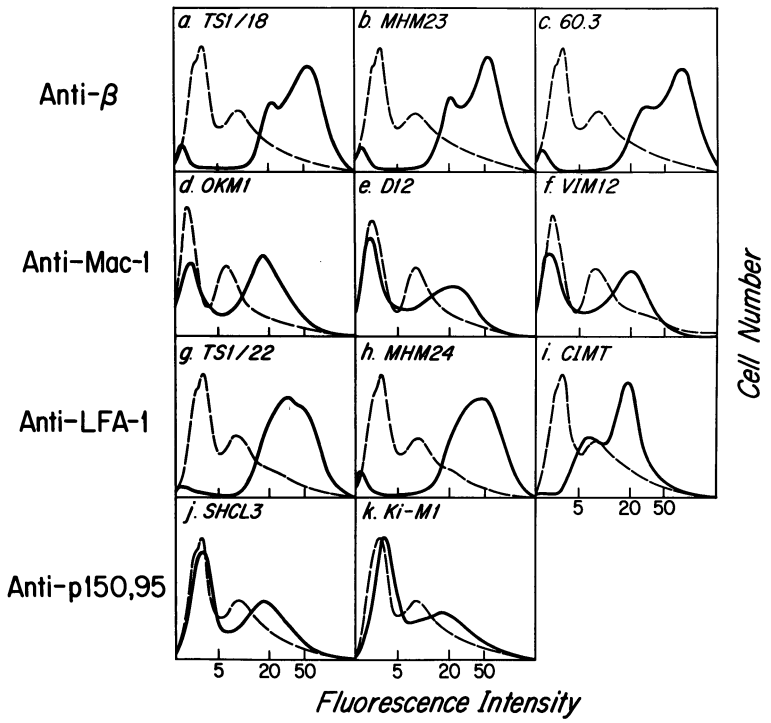

Fig. 4.2. Fluorescent staining of mononuclear cells by antibodies specific for Mac-1, LFA-1, p150,95, and their family. Mononuclear cells prepared by Ficoll–Hypaque centrifugation (2.5×10^7/ml in 50 μl) were labeled with an equal volume of 1/200 Workshop mAb (solid curves) or P3X63 IgG1 control mAb (dashed curves), then with FITC anti–mouse IgG, and subjected to immunofluorescence flow cytometry. Fluorescent unit = 1/20 the intensity of 2% bright beads (Coulter).

positive subpopulation corresponded to monocytes, and the vast majority of lymphocytes were negative (data not shown, and see below, Fig. 4.3). The intensity of staining of granulocytes was also characteristic for each antibody specificity; staining by anti-Mac-1 and anti-family mAbs was stronger than staining by anti-LFA-1 and anti-p150,95 mAbs (Table 4.1).

The mAbs to the Mac-1, LFA-1, p150,95 glycoproteins could be placed into four distinct groups based on specificity for one of the three members of the family or reactivity with the entire family (Table 4.1). The specificities suggest reaction with individual α subunits or the common β subunit. However, these subunit specificities remain speculative unless confirmed by reaction with isolated subunits. Subunit specificity of these antibodies has thus far been directly demonstrated only for the OKM1 anti-Mac-1 α mAb, the TS1/22 anti-LFA-1 α mAb, and the TS1/18 anti-β mAb (1).

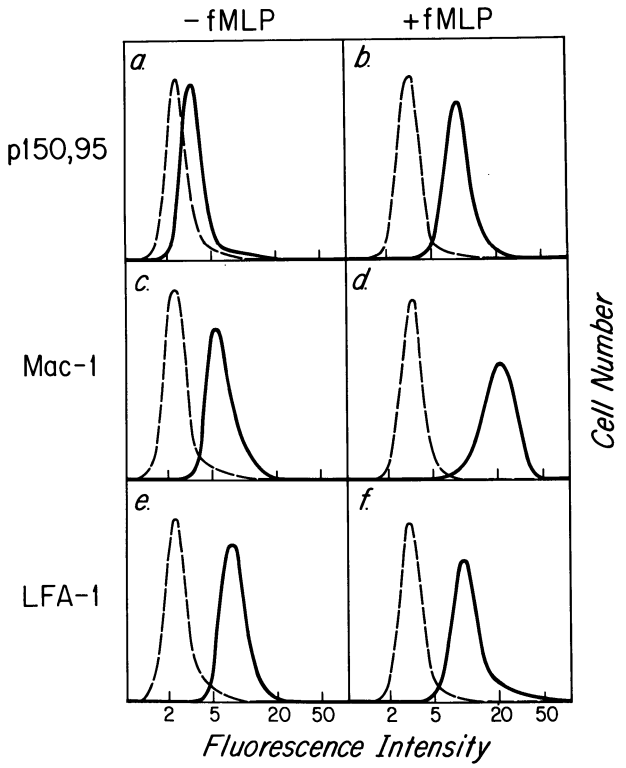

Fig. 4.3. Chemo-attractant stimulates increased expression of p150,95 and Mac-1 but not LFA-1 on monocytes. Mononuclear cells were incubated ½ hr at 37°C with 10^{-8} *M* f-Met-Leu-Phe, or held at 4°C as indicated. Cells were stained at 4°C with specific (solid curves) or control (dashed curves) mAb, followed by FITC anti-mouse IgG, and subjected to immunofluorescence flow cytometry. Fluorescence of monocytes was determined by gating on 90° and forward-angle light scatter to exclude lymphocytes.

Up-regulation of Mac-1 and p150,95 Surface Expression

Previous work has shown that Mac-1 but not LFA-1 surface expression is increased when granulocytes are stimulated with the chemo-attractant and secretagogue f-Met-Leu-Phe (7). All Workshop anti-Mac-1 mAbs detected such "up-regulation," with an average increase of 3.7-fold, while LFA-1 was not up-regulated. The β subunit showed an intermediate increase (data not shown).

It was further studied whether p150,95 was up-regulated on granulocytes, and whether the expression of these molecules on monocytes was altered by f-Met-Leu-Phe stimulation. p150,95 was strikingly increased an average of 4.4-fold when granulocytes were f-Met-Leu-Phe-stimulated, as shown with the two different anti-p150,95 mAbs. The granulocyte fluores-

Table 4.1. Myeloid workshop anti-Mac-1, LFA-1, p150,95 antibodies.

Specificity	Workshop no.	Local name	Precipitation	Lymph/monocytes[a]	Gran. fluor. inten.[b]	Deficient on patients[c]	Subclass
Mac-1	—	OKM1	Mac-1	−/+	130	+	γ2
Mac-1	M26	V1M12	Mac-1	−/+	104	+	γ1
Mac-1?[d]	M41	CC1.7	Mac-1	+/+	106	+	γ1
Mac-1	M42	D12	Mac-1	−/+	114	+	γ2a
Mac-1	M76	60.1	Mac-1	−/+	128	+	γ2a
Mac-1	M78	M3D11	Mac-1	−/+	60	+	μ
Mac-1	M88	Ki-M5	Mac-1	−/+	90	+	γ2a
Mac-1	M104	44	Mac-1	−/+	102	+	γ1
LFA-1	—	TS1/22	LFA-1	+/+	15	+	γ1
LFA-1	M56	MHM24	LFA-1	+/+	14	+	ND
LFA-1	M107	C1MT	LFA-1	+/+	4	+	γ1
LFA-1	—	25.3.1	LFA-1	ND/ND[e]	ND	ND	—
p150,95	M46	SHCL3	p150,95	−/+	13	+	γ2b
p150,95	M93	Ki-M1	p150,95	−/+	8	+	μ
Family	M55	MHM23	Family	+/+	122	+	γ1
Family	M73	CLB-54	Family	+/+	93	+	γ1
Family	M75	60.3	Family	+/+	172	+	γ2a
Family	M89	TS1/18	Family	+/+	102	+	γ1

[a] By immunofluorescence, as described in captions to Figs. 4.2 and 4.3.
[b] Fluorescence intensity (relative to TS1/18 anti-β = 100) on f-Met-Leu-Phe-stimulated granulocytes. Background fluorescence has been subtracted.
[c] By immunofluorescence, as described in caption to Fig. 4.4.
[d] This mAb was specific for Mac-1 by immunoprecipitation, but an unexpected reactivity with lymphocytes was found in immunofluorescence. Since this could be due to an accidental contamination, and no reactivity with LFA-1 was seen, this mAb has been designated a tentative anti-Mac-1.
[e] ND: Not done.

cence intensities shown in Table 4.1 are for f-Met-Leu-Phe-treated cells; on unstimulated granulocytes p150,95 is the most weakly expressed member of the family.

Stimulation of monocytes with f-Met-Leu-Phe resulted in dramatic 6.5-fold and 5.0-fold increases in surface expression of p150,95 and Mac-1, respectively [Fig. 4.3, (a)–(d)]. In contrast, LFA-1 expression on monocytes [Fig. 4.3(e) and (f)] and on lymphocytes (not shown) was unaffected. These large increases in surface expression occurred within ½ hr, suggesting a latent store of Mac-1 and p150,95 in granulocytes and monocytes. In contrast, LFA-1 was not up-regulated. Since the β subunits of these molecules are identical, the α chains appear to control subcellular targetting to the plasma membrane or to intracellular storage sites.

Studies on Mac-1, LFA-1-Deficient Patients

As a second means of identifying antibodies to the Mac-1, LFA-1 family, each mAb in the myeloid panel was tested with granulocytes from a Mac-

1, LFA-1-deficient patient in an [^{125}I]anti–mouse IgG indirect binding assay (23). mAbs giving lower binding to patient than to normal cells were retested by immunofluorescent flow cytometry (Table 4.1; representative histograms are shown in Fig. 4.4). All eight mAbs to Mac-1, three mAbs to LFA-1, two mAbs to p150,95, and four mAbs to the family were negative on f-Met-Leu-Phe-stimulated patient granulocytes. The deficiency in patient cells was also demonstrated by precipitation with anti-Mac-1, anti-LFA-1, anti-p150,95, and anti-β subunit mAbs [Fig. 4.5(B) compared to 4.5(A)]. The results with the putative anti-p150,95 αX mAb extend previous findings that all three α subunits and the common β subunit are deficient on the cell surface (7). The primary defect in patient cells appears to be in the β subunit; α precursor is made but is neither processed nor transported to the cell surface or to latent stores in the absence of association with the β subunit (7).

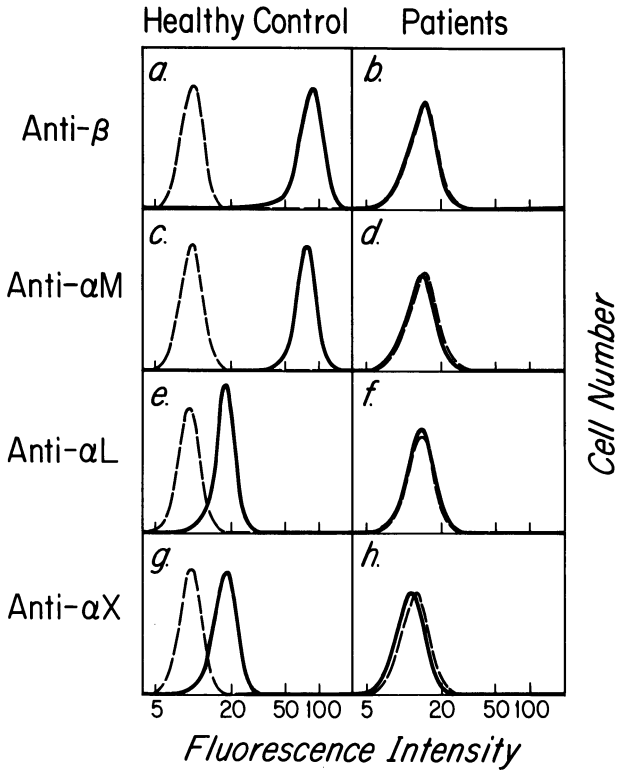

Fig. 4.4. Deficiency of α and β subunits on granulocytes of a patient with recurring infections. Granulocytes of a patient with severe deficiency [patient 1 (11)] or of a healthy adult control were incubated ½ hr at 37°C with 10^{-8} *M* f-Met-Leu-Phe, then stained with specific mAb (solid curves) or control mAb (dashed curves) and subjected to immunofluorescence flow cytometry as described for Fig. 4.2.

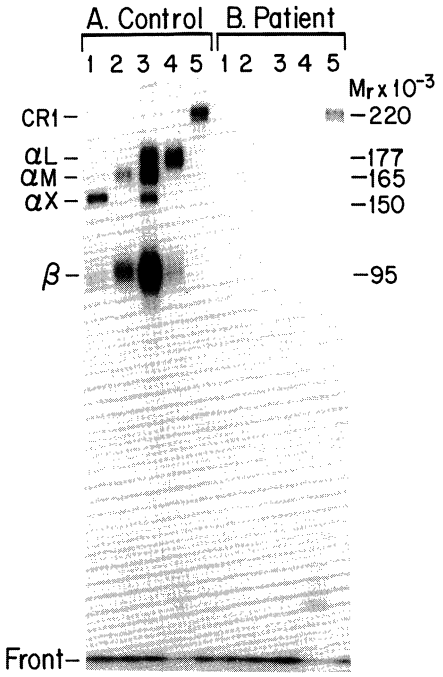

Fig. 4.5. Immunoprecipitation of p150,95, Mac-1, and LFA-1 from normal and deficient granulocytes. Triton X-100 lysates of ^{125}I-labeled healthy adult control (A) or severely deficient patient (B) f-Met-Leu-Phe-stimulated granulocytes were immunoprecipitated with anti-p150,95 SHCL3 mAb (lane 1); anti-Mac-1 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 positive control (lane 5). Immunoprecipitates were subjected to SDS–7% PAGE and autoradiography.

None of the antigens defined by the 102 non-Mac-1, LFA-1 mAbs were deficient. M103 antigen showed lower than normal expression on patients' cells, but was clearly detectable by immunoprecipitation (not shown). The complement receptor type 1 (CR1) was also normally expressed on patient cells [Fig. 4.5(A) and (B), lane 5]. Thus, the deficiency is highly specific to the Mac-1, LFA-1 glycoprotein family.

The idea that LFA-1, Mac-1, and p150,95 function as leukocyte adhesion molecules has received support from functional studies on deficient patient cells. Patient cells show defects in natural, cytolytic T-lymphocyte-mediated, and antibody-dependent cell-mediated killing (6,20,22), and in the complement receptor type 3 (19,21). This is consistent with blocking of these functions on normal cells by anti-LFA-1 and anti-Mac-1 mAbs, respectively. Furthermore, patient granulocytes and monocytes show multiple adhesion-dependent defects, including adherence to surfaces, spreading, chemotaxis, and aggregation (11,21). The finding here

that the entire Mac-1, LFA-1 family, including p150,95, is specifically deficient on patient cells, is of great importance in understanding these multiple defects in adhesion-dependent functions. Stimulated adherence, a phenomenon in which granulocytes and monocytes exhibit increased adherence and aggregate in response to chemo-attractants such as f-Met-Leu-Phe, is also defective in the patients (21). Since other surface receptors are up-regulated normally upon stimulation of patient cells (7), these functional deficiencies may be related to a lack of Mac-1 and p150,95 up-regulation. These deficiencies *in vitro* appear to explain the inability of patient neutrophils *in vivo* to extravasate and migrate into inflammatory sites (11).

Appendix

Non-Mac-1, LFA-1 mAbs

Data were also obtained on mAbs which immunoprecipitated non-LFA-1, Mac-1 antigens. Thirteen mAbs precipitated a spectrum of proteins at M_r = 220,000, 180,000, 155,000, 130,000, and 98,000 (Fig. 4.6, lanes 1–10, 13–15, and Table 4.2). These mAbs are specific for Galβ1→4(Fuc-α1→3)GlcNAcβ1→R (X-hapten) (this volume, Chapters 2 and 3), which thus appears to be linked to the above polypeptide chains. The X-hapten is also found on glycolipids, which would not be labeled with ^{125}I and hence would not be visualized in these experiments.

The M103 mAb precipitated two chains with mobility identical to the M_r = 220,000 and 130,000 chains precipitated by the anti-hapten X mAbs

Table 4.2. Molecular properties of non-LFA-1, Mac-1 antigens on granulocytes.

Workshop no.	Polypeptide (M.W. × 10^{-3})	Fluorescence intensity[a] −fMLP	Fluorescence intensity[a] +fMLP	Up-regulation (-fold)
M5, M13, M19, M25, M33, M52, M61, M77, M80, M91, M110, M111, M117	220, 180, 155, 130, 98	442	653	1.5
M103	220, 130	ND[b]	10	
M87	68	80	158	2.0
M115	65	1.5	1.4	—
M22, M57, M59, M82	62, 54 (diffuse)	270	384	1.4
M3	50, 25	ND	ND	
M102	27	ND	ND	

[a] Relative to TS1/18 anti-β = 100 on f-Met-Leu-Phe-stimulated granulocytes. Cells were treated with 10^{-8} *M* f-Met-Leu-Phe for ½ hr at 37°C or held at 4°C as described for Fig. 4.3. Fluorescence data are for the underlined MAb.
[b] ND: Not done.

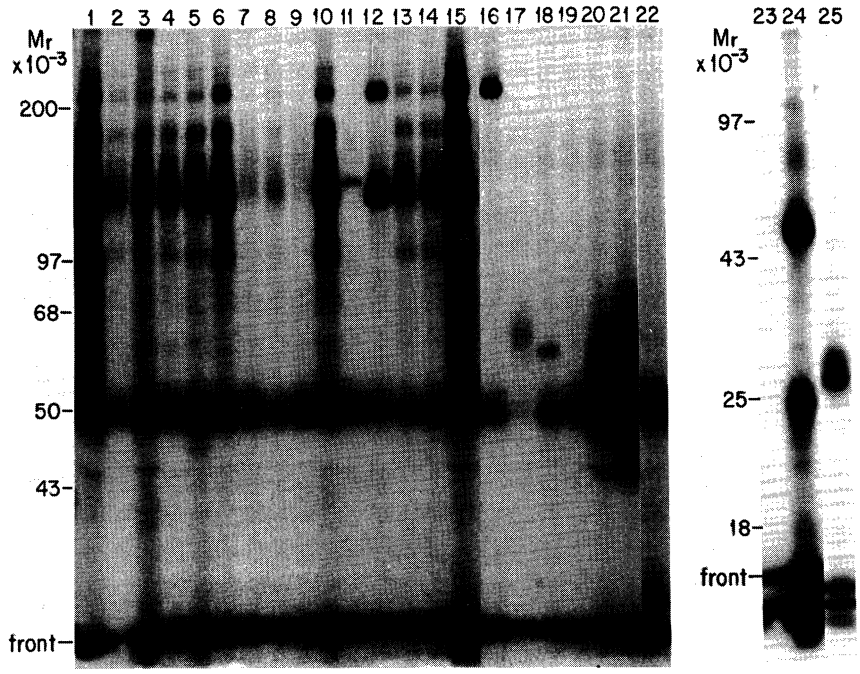

Fig. 4.6. Non-Mac-1, LFA-1 proteins precipitated from ^{125}I-labeled granulocytes. ^{125}I-labeled f-Met-Leu-Phe-stimulated granulocyte lysates were immunoprecipitated with myeloid Workshop antibodies M5, 92H5 (lane 1); M13, 1G10 (lane 2); M19, HLC5 (lane 3); M25, VIMC6 (lane 4); M33, G7C5 (lane 5); M52, HG-1 (lane 6); M61, 29 (lane 7); M77, G9F9 (lane 8); M80, CLB gran 7 (lane 9); M91, 28 (lane 10); M93, Ki-M1 (lane 11); M103, PMN-3 (lane 12); M110, LMA-1 (lane 13); M111, LMA-3 (lane 14); M117, HG-3 (lane 15); anti-CR1, 44D (lane 16); M87, CLB gran 5 (lane 17); M115, CAMAL-1 (lane 18); irrelevant control mAb (lane 19); M22, BW243/41 (lane 20); M57, CLB FcR gran 1 (lane 21); M102, CIKM5 (lane 22); M2, nonprecipitating mAb (lane 23); M3, 82H3 (lane 24); M102, CIKM5 (lane 25). Precipitates were electrophoresed on SDS gels of 7.5% polyacrylamide (lanes 1–22) or 11% polyacrylamide (lanes 23–25). The positions of marker proteins and the buffer front are marked.

(Fig. 4.6, lane 12). Other bands precipitated by anti-hapten X mAbs were not present, showing M103 has a different specificity. Furthermore, the fluorescence intensity (Table 4.2) shows there are many fewer M103 antigen than X-hapten sites on granulocytes. It is possible that mAb M103 defines a protein determinant on a subset of X-hapten-bearing polypeptide chains. Under nonreducing conditions, more of the 220,000 and less of the 130,000 M_r chains were seen (not shown). The 130,000-M_r chain may therefore be a monomer or a fragment of the 220,000-M_r chain.

The CR1 molecule (Fig. 4.6, lane 16) has a mobility similar to that of the M_r = 220,000 chains precipitated by anti–X-hapten and M103 mAb (cf.

Fig. 4.6, lanes 12 and 13). To test for identities between these molecules, advantage was taken of the previously described genetic polymorphism in the size of the CR1 (24). Both CR1 bands of 240,000 and 220,000 were precipitated by anti-CR1 from a heterozygote, but M103 and anti–X-hapten mAbs only precipitated a 220,000 M_r band (not shown). This suggests that the molecules defined by these mAbs are distinct.

Two mAbs, M87 and M115, precipitated bands of M_r = 68,000 and 65,000, respectively (Fig. 4.6, lanes 17 and 18). M115 gave very little labeling of intact cells (Table 4.2), suggesting it might define an intracellular or granule constituent which was released or accessible during ^{125}I-labeling.

The M22, M57, M59, and M82 mAbs precipitated diffuse bands of M_r = 62,000 and 54,000 (Fig. 4.6, lanes 20, 21) similar to the neutrophil FcR (25).

The M3 mAb precipitated two chains of 50,000 and 25,000 M_r, reminiscent of IgG (Fig. 4.6, lane 24). Under nonreducing conditions, the chains appeared to be associated together into a M_r = 150,000 complex. Precipitation of this antigen by M3 was not inhibited by human IgG. It is possible that M3 recognizes an Fc receptor associated with IgG.

Finally, the M102 mAb precipitated a chain of M_r = 27,000 (Fig. 4.6, lanes 22 and 25).

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References

1. Sanchez-Madrid, F., J. Nagy, E. Robbins, P. Simon, and T.A. Springer. 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.
2. Sanchez-Madrid, F., P. Simon, S. Thompson, and T.A. Springer. 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.
3. Krensky, A.M., F. Sanchez-Madrid, E. Robbins, J. Nagy, T.A. Springer, and S.J. Burakoff. 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.
4. Hildreth, J.E.K., F.M. Gotch, P.D.K. Hildreth, and A.J. McMichael. 1983. A human lymphocyte-associated antigen involved in cell-mediated lympholysis. *Eur. J. Immunol.* **13:**202.
5. Springer, T.A., D. Davignon, M.K. Ho, K. Kürzinger, E. Martz, and F. Sanchez-Madrid. 1982. LFA-1 and Lyt-2,3, molecules associated with T lym-

phocyte-mediated killing; and Mac-1, an LFA-1 homologue associated with complement receptor function. *Immunol. Rev.* **68:**111.

6. Springer, T.A., R. Rothlein, D.C. Anderson, S.J. Burakoff, and A.M. Krensky. 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, p. 311.
7. Springer, T.A., W.S. Thompson, L.J. Miller, F.C. Schmalstieg, and D.C. Anderson. 1984. Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. *J. Exp. Med.*, **160:**1901.
8. Beller, D.I., T.A. Springer, and R.D. Schreiber. 1982. Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor. *J. Exp. Med.* **156:**1000–1009.
9. Wright, S.D., P.E. Rao, W.C. Van Voorhis, L.S. Craigmyle, K. Iida, M.A. Talle, E.F. Westberg, G. Goldstein, and S.C. Silverstein. 1983. Identification of the C3bi receptor of human monocytes and macrophages with monoclonal antibodies. *Proc. Natl. Acad. Sci. U.S.A.* **80:**5699.
10. Arnaout, M.A., R.F. Todd III, N. Dana, J. Melamed, S.F. Schlossman, and H.R. Colten. 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.
11. Anderson, D.C., F.C. Schmalstieg, M.J. Finegold, B.J. Hughes, R. Rothlein, L.J. Miller, S. Kohl, M.F. Tosi, R.L. Jacobs, A. Goldman, W.T. Shearer, and T.A. Springer. 1984. Inherited deficiency of the leukocyte Mac-1, LFA-1, glycoprotein family: relationship of clinical expression to severity of molecular and functional abnormalities. *J. Inf. Dis.*, in press.
12. Kürzinger, K., and T.A. Springer. 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.
13. Kürzinger, K., M.K. Ho, and T.A. Springer. 1982. Structural homology of a macrophage differentiation antigen and an antigen involved in T-cell-mediated killing. *Nature* **296:**668.
14. Trowbridge, I.S., and M.B. Omary. 1981. Molecular complexity of leukocyte surface glycoproteins related to the macrophage differentiation antigen Mac-1. *J. Exp. Med.* **154:**1517.
15. Sanchez-Madrid, F., A.M. Krensky, C.F. Ware, E. Robbins, J.L. Strominger, S.J. Burakoff, and T.A. Springer. 1982. Three distinct antigens associated with human T lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. *Proc. Natl. Acad. Sci. U.S.A.* **79:**7489.
16. Ware, C.F., F. Sanchez-Madrid, A.M. Krensky, S.J. Burakoff, J.L. Strominger, and T.A. Springer. 1983. Human lymphocyte function associated antigen-1 (LFA-1): identification of multiple antigenic epitopes and their relationship to CTL-mediated cytotoxicity. *J. Immunol.* **131:**1182.
17. Krensky, A.M., E. Robbins, T.A. Springer, and S.J. Burakoff. 1984. LFA-1, LFA-2 and LFA-3 antigens are involved in CTL–target conjugation. *J. Immunol.* **132:**2180.
18. Beatty, P.G., J.A. Ledbetter, P.J. Martin, T.H. Price, and J.A. Hansen. 1983.

Definition of a common leukocyte cell-surface antigen (Lp95-150) associated with diverse cell-mediated immune functions. *J. Immunol.* **131:**2913.

19. Dana, N., R.F. Todd III, J. Pitt, T.A. Springer, and M.A. Arnaout. 1984. Deficiency of a surface membrane glycoprotein (Mol) in man. *J. Clin. Invest.* **73:**153.
20. Beatty, P.G., J.M. Harlan, H. Rosen, J.A. Hansen, H.D. Ochs, T.D. Price, R.F. Taylor, and S.J. Klebanoff. 1984. Absence of monoclonal-antibody-defined protein complex in boy with abnormal leucocyte function. *Lancet* **1984(1):**535.
21. Anderson, D.C., F.C. Schmalstieg, S. Kohl, M.A. Arnaout, B.J. Hughes, M.F. Tosi, G.J. Buffone, B.R. Brinkley, W.D. Dickey, J.S. Abramson, T.A. Springer, L.A. Boxer, J.M. Hollers, and C.W. Smith. 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.
22. Kohl, S., T.A. Springer, F.C. Schmalstieg, L.S. Loo, and D.C. Anderson. 1984. Defective natural killer cytotoxicity and polymorphonuclear leukocyte antibody-dependent cellular cytotoxicity in patients with LFA-1/OKM-1 deficiency. *J. Immunol.*, **133:**2972.
23. Springer, T.A., A. Bhattacharya, J.T. Cardoza, and F. Sanchez-Madrid, 1982. Monoclonal antibodies specific for rat IgG1, IgG2a, and IgG2b subclasses, and kappa chain monotypic and allotypic determinants: Reagents for use with rat monoclonal antibodies. *Hybridoma* **1:**257.
24. Fearon, D.T., and W.W. Wong. 1983. Complement ligand–receptor interactions that mediate biological responses. *Annu. Rev. Immunol.* **1:**243.
25. Fleit, H.B., S.D. Wright, and J.C. Unkeless. 1982. Human neutrophil Fc-gamma receptor distribution and structure. *Proc. Natl. Acad. Sci. U.S.A.* **79:**3275.