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Characterization of Human Macrophage Antigens Identified by Monoclonal Antibodies

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U937 is a cell line derived from a human histiocytic lymphoma (30). This cell has some of the properties of macrophages (1–3,7,8,16,19,25,29,31,33). It elaborates factors that effect normal human hematopoiesis. It can be activated by supernatants of mixed lymphocyte cells or by 12-tetradecanoylphorbol-13 acetate to exhibit several properties associated with macrophages: myeloid morphology, binding of the chemotactic peptide fMet-Leu-Phe, antibody-dependent cell cytotoxicity, and Fc receptor activity. The cells also have strong esterase activity, produce lysozyme and endogenous pyrogen, are phagocytic, and bear receptors for complement and IgG.

This cell line thus provides an *in vitro* system for studies of a variety of processes in human macrophage function, for example, endocytosis, cytolysis, antigen processing, induction of T cell proliferation, and secretion of biologically active molecules. A number of these processes appear to involve the function of molecules on the cell surface (34). In some cases, proteins of the cell surface have been correlated with specific activities of animal or human macrophages: those of Fc receptors (1,2,5,18,24,32) and of certain proteins associated with antibody-dependent phagocytosis (10).

We have used the U937 cell line for the analysis of human macrophage structure and function. Sixteen monoclonal antibodies which bind to the surface of U937 cells have been obtained. Nine proteins were identified by immunoprecipitation, and the distribution of the antigens among blood cells was determined using flow cytofluorimetric analysis. This chapter describes the initial characterization and identification of the antigenic targets of this panel of antibodies.

MATERIALS AND METHODS

Cell Lines and Media

The myeloma cell line, P3X63-Ag8-653 (15), obtained from Dr. M. Scharff, was propagated without antibiotics in Dulbecco's modified Eagle medium (DMEM)

supplemented with 10% (v/v) heat-inactivated fetal calf serum and 20 μg/ml of 8-azaguanine. The human cell line, U937 (25,31), obtained from Dr. G. Rovera, was maintained as a suspension culture without antibiotics in DMEM supplemented with 10% (v/v) heat-inactivated fetal calf serum. The cell lines, Daudi and CCRF-CEM, were propagated as described (27). Hybrid cells were grown without antibiotics in DMEM supplemented with 20% (v/v) heat-inactivated Hyclone-fetal calf serum.

Immunization

U937 cells for immunization were collected by centrifugation at $800 \times g$ for 5 min and washed five times in serum-free DMEM. A female Sprague-Dawley rat was injected subcutaneously and intraperitoneally with a divided dose of 1×10^7 U937 cells in complete Freund's adjuvant. The animal was boosted on days 15 and 28 with a subcutaneous injection of 5×10^6 U937 cells in incomplete Freund's adjuvant. On day 49, the rat was injected intraperitoneally with 3×10^7 cells in phosphate-buffered saline.

Cell Fusion and Selection of Hybridomas

Three days later, spleen cells of this rat were fused with the murine myeloma cell line P3X63-Ag8-653 using a mixture of 45% (w/v) polyethylene glycol 1000 and 10% (v/v) dimethylsulfoxide, pH 7.2, according to procedures described previously (6). Cells were seeded into 100 wells of tissue culture plates at 10^6 spleen cells/well or into 300 wells containing a mouse peritoneal macrophage feeder layer at 2×10^5 spleen cells/well. Cells were cultured in hypoxanthine-aminopterin-thymidine (HAT) selective medium (20). After 14 days, cells were adapted to DMEM with 20% (v/v) calf serum by passage in medium containing hypoxanthine and thymidine but lacking *aminopterin*.

Supernatant fluids obtained from 400 primary hybridoma cultures were assayed for antibody binding to 50 μg of U937 crude cell homogenate using the indirect, solid phase antibody binding assay (12). Cultures were further screened by immunoprecipitation and those 12 which were positive by either criterion were cloned in soft agar.

Antibodies

Goat anti-rat IgG was purified and labeled with ^{125}I as described (12). Hybridoma culture supernatants were used as the source of monoclonal antibodies. Antibody isotypes were determined by Ouchterlony double diffusion analysis using class-specific antisera (Miles Laboratories).

Radiolabeling and Immunoprecipitation of U937 Proteins

U937 proteins were prepared in four different ways for analysis by immunoprecipitation:

(a) Glycoproteins present in 55 mg of U937 extract, prepared as described (30), were purified by affinity chromatography on a 1 ml column (Pharmacia) of con-

canavalin-A–Sepharose which had been cross-linked with glutaraldehyde (17). The column was washed and the protein applied in 20 mM Tris-HCl, pH 7.6, 100 mM, 0.2% (w/v) Triton X-100. Bound proteins were eluted with 0.3 M α-methyl-D-mannoside in the same buffer. After dialysis and vacuum concentration in 20 mM Tris-HCl, pH 7.6, 1mM EDTA, 100 mM NaCl, 0.2% (w/v) Triton X-100, the proteins were iodinated using chloramine-T as described by Hunter (14).

(b) Cell surface proteins were labeled by lactoperoxidase-catalyzed iodination (11) of intact U937 cells as modified by Pink and Ziegler (28).

(c) U937 cells were metabolically labeled with ^{35}S-methionine as follows: 5×10^6 cells at a density of 3.6×10^5 cells/ml were resuspended in 20 ml of Eagle's minimal essential medium lacking methionine and containing 10 mM *N*-Z-hydroxyethyl-piperazine-*N'*-2 ethane sulfonic acid (HEPES), pH 7.5 at 37°C for 2 hr. The cells were then incubated for 6 or 18 hr in 10 ml of the same medium containing 0.25 mCi ^{35}S-methionine/ml and 5% (v/v) dialyzed fetal calf serum. Labeled proteins were extracted as before.

(d) An unfractionated Triton X-100 extract of U937 cells was iodinated using chloramine-T.

Immunoprecipitations were performed essentially as described (12,13). The reaction mixture contained 3×10^6 cpm of acid insoluble ^{35}S-methionine-labeled protein or $1–2 \times 10^6$ cpm of acid insoluble, ^{125}I-labeled protein, 2 mg/ml gelatin and 200 λ of hybridoma tissue culture supernatant or 0.5 λ of immune rat serum. After a 1 hr incubation at 4°C, 10 μl of goat antiserum against rat IgG was added and the incubation was continued overnight. Immunoprecipitates were washed by centrifugation twice with 2 ml of 20 mM Tris-HCl, pH 7.6, 1 mM EDTA, 100 mM NaCl, 2.5 M KCl, 0.5% (w/v) NP 40, and once with 2 ml of 20 mM Tris-HCl, pH 7.6. Samples were analyzed by gel electrophoresis (4) and autoradiography or fluorography using En^{3}Hance (New England Nuclear).

Molecular weight standards included cytochrome C, 11,700; chymotrypsinogen, 25,500; ovalbumin, 43,000; bovine serum albumin, 68,000; phosphorylase a, 97,400; and myosin heavy chain, 200,000.

Cell Binding Assay

Antibody binding to intact cells was determined as described (12,13) using intact U937 cells.

Two-dimensional Gel Electrophoresis

Total extracts of iodinated glycoproteins or iodinated surface proteins were dissolved in 50 μl O'Farrell Buffer A [9.5 M urea, 2% (w/v) nonidet-P-40, 2% (v/v) ampholines, and 5% (v/v) 2-mercaptoethanol] for analysis by two-dimensional gel electrophoresis (26). Immunoprecipitated proteins were likewise analyzed.

Chemical Characterization of Antigens (Lipid Extraction)

Total lipid extracts were obtained from U937 cells as described (22). Briefly, U937 cells (0.2 g wet weight) were homogenized in 0.6 ml of H_2O at 4°C. The

homogenate was added to 2.0 ml of methanol, and 1.0 ml of chloroform was then added with constant stirring at room temperature for 30 min. The extract was centrifuged at 15,000 × *g* for 10 min. The pellet was homogenized in 0.4 ml of H_2O and extracted with 1.5 ml of chloroform and methanol (1:2 by volume). Supernatant fractions were pooled and rotoevaporated. The lipid residue was dissolved in H_2O and ethanol (1:1 by volume), and the protein pellet was resuspended in H_2O by homogenization. Equivalent amounts of the lipid and protein fractions were compared to 50 μg of the total U937 extract using the solid phase indirect antibody binding assay.

Protease Sensitivity

U937 cells were solubilized in extraction buffer, [5 mM Tris-HCl, pH 9.2, 1 mM EDTA, 400 mM KCl, 1% (w/v) Triton X-100], without protease inhibitors. After 30 min on ice, the extract was centrifuged at 10,000 × *g* for 10 min. The supernatant was dialyzed against 20 mM Tris-HCl, pH 7.6, 1 mM EDTA, 100 mM NaCl, 0.2% (w/v) Triton X-100 and then centrifuged at 100,000 × *g* for 1 hr. Protein concentration was adjusted to 1 mg/ml. Samples were incubated with or without 20 μg/ml Pronase (Calbiochem) for 18 hr at 37°C. Proteolysis was quenched by the addition of 2 mM phenylmethylsulfonyl fluoride (PMSF) and 5 mg/ml bovine serum albumin (BSA). Antigen was measured using the indirect solid phase antibody binding assay.

Pronase sensitivity of proteins isolated by chloroform:methanol extraction was determined by incubating the equivalent of 4 mg/ml total U937 protein with 80 μg/ml pronase for 18 hr at 37°C. The reaction was quenched as before and results were analyzed by the indirect, solid phase antibody binding assay.

Flow Cytofluorimetric Analysis

Flow cytofluorimetry was kindly performed by Dr. Giovanni Rovera as described (27). An Ortho-cytofluorograph system H50, connected to a Data General MP/200 microprocessor (Ortho Instruments, Westwood, Mass.) was used. Target cells were washed twice in phosphate-buffered saline (PBS) before use and resuspended at 1.2×10^7/ml in PBS containing 0.1% gelatin and 0.1% NaN_3 (washing buffer). Twenty-five microliters of cell suspension were incubated with 20 μl of hybridoma culture supernatant, or the appropriate dilution of rat polyclonal serum against U937 cells, in round-bottom microtiter plates for 30 min at room temperature. The cells were then washed three times in washing buffer. Cells were incubated with the appropriate dilution of a fluorescein-conjugated anti-rat globulin (Huntingdon Research Center) for 30 min at 4°C. The plates were washed as before. Monocytes, granulocytes, and lymphocytes were distinguished on the basis of their right- and forward-angle light scatter, and fluorescence intensity was determined simultaneously for the three different cell types, as described (9).

RESULTS AND DISCUSSION

Preparation and Selection of Antibodies

Twelve cultures with antibodies that immunoprecipitated most of the major surface proteins of U937 cells have been prepared, as described under Materials and Methods, and cloned in soft agar (Table 1). In addition, a few other clones that produced antibodies with strong binding to the cell extract were also retained. The isotypes of the monoclonal antibodies were IgG_{2a},7; IgM,6; and IgG_1,3.

Cell Surface Localization of the Antigens

Each of the antigens recognized by this panel of monoclonal antibodies are putative cell surface components, indicated by antibody binding to intact cells as compared to cell extracts. In some cases, cell surface localization of the antigen was also indicated by vectorial iodination with the lactoperoxidase procedure, as shown below.

Binding of antibodies to the surface of U937 was measured by an indirect cell binding assay (Fig. 1A and B). All of the antibodies bound to cells in an antigen-dependent manner. These results were compared to the binding of antibodies to equivalent amounts of a crude cell extract, also measured by a solid phase indirect binding assay (Fig. 2A and B). Only two antibodies, 4-10a/8/4 and 2-4/7/5, showed

TABLE 1. *Monoclonal antibodies to human macrophage cell surface proteins*

Antibody	Ig Subclass	Antigen (kDa)*
1-3/7/1	IgG_1	270/145
1-14/6/10	IgG_{2a}	70/20
1-18/8	IgM	—
2–4/7/5	IgG_{2a}	—
2-16/6/1	IgG_{2a}	280/160
4-10/6/3	IgG_{2a}	47/11
4-10a/8/4	IgG_{2a}	150
4-12/18	IgM	40
4-13/3/7	IgG_1	47 11
4-19/4	IgM	68
5-8/9	IgM	45/30/25
6-18/3	IgG_1	40/11
16-8/9/4	IgG_{2a}	19
17-20/20	IgM	—
22-6/17	IgM	—
22-19/19	IgG_{2a}	55,50/18

*Molecular weights were determined by immunoprecipitation and polyacrylamide gel electrophoresis under reducing conditions or by fractionation studies (23). See text for additional details.

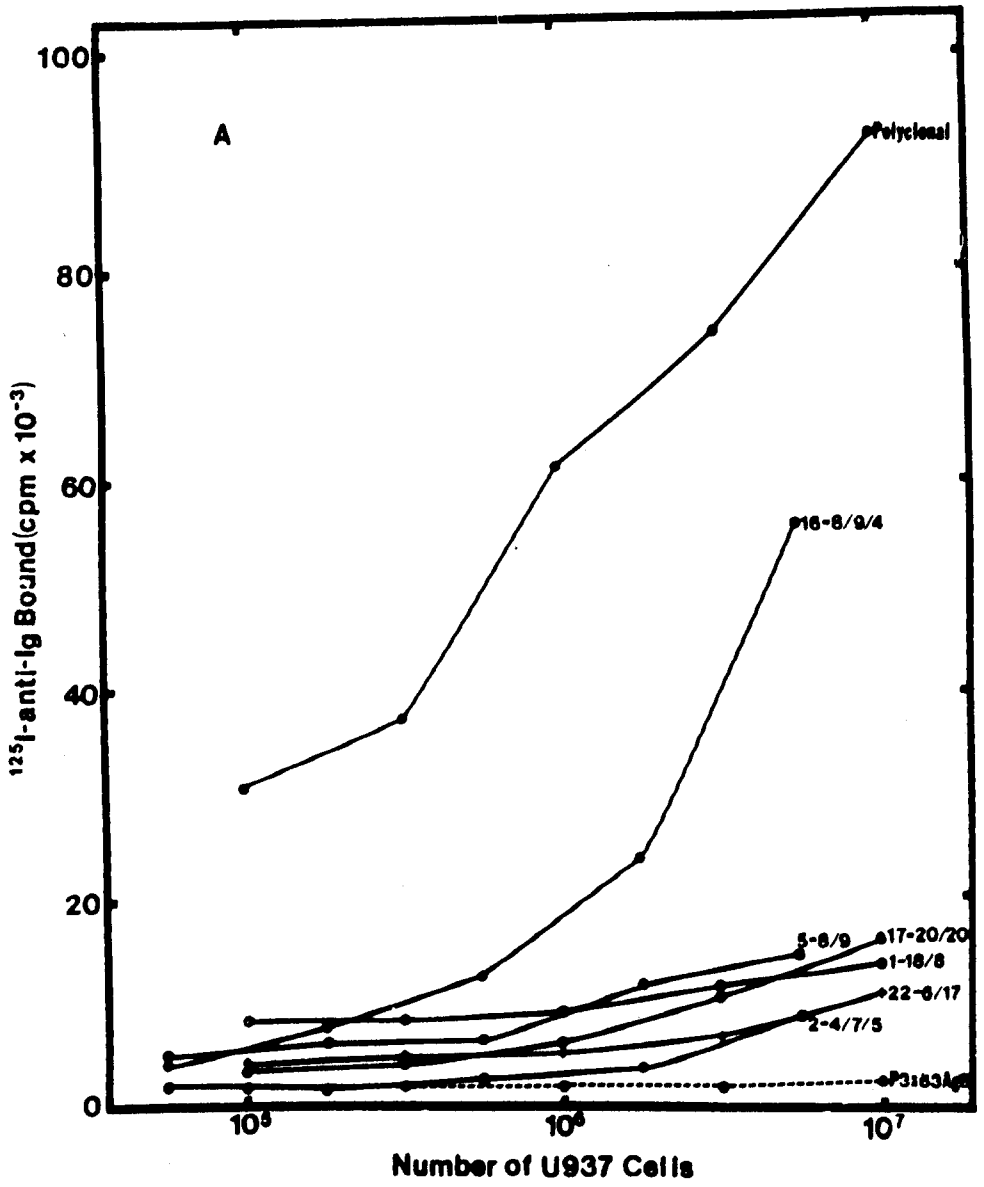


FIG. 1. Antibody binding to intact U937 cells was measured by the cell binding assay as described (12) using the indicated numbers of intact U937 cells with 50 μl of hybridoma culture supernatant or 50 μl of a 10^{-3} dilution of polyclonal rat serum against U937 cells, and 50 μl of ^{125}I-labeled goat anti-rat IgG secondary antibody (0.25 μg/ml, 2.5×10^4 cpm/ng). A: Binding of antibodies which did not immunoprecipitate proteins.

greater binding to the total cell extract. The corresponding antigens may have additional intracellular pools.

Chemical Composition of the Antigens

The majority of the antibodies of this collection appeared to act on proteins. This was indicated by the immunoprecipitation of proteins and by studies of protease

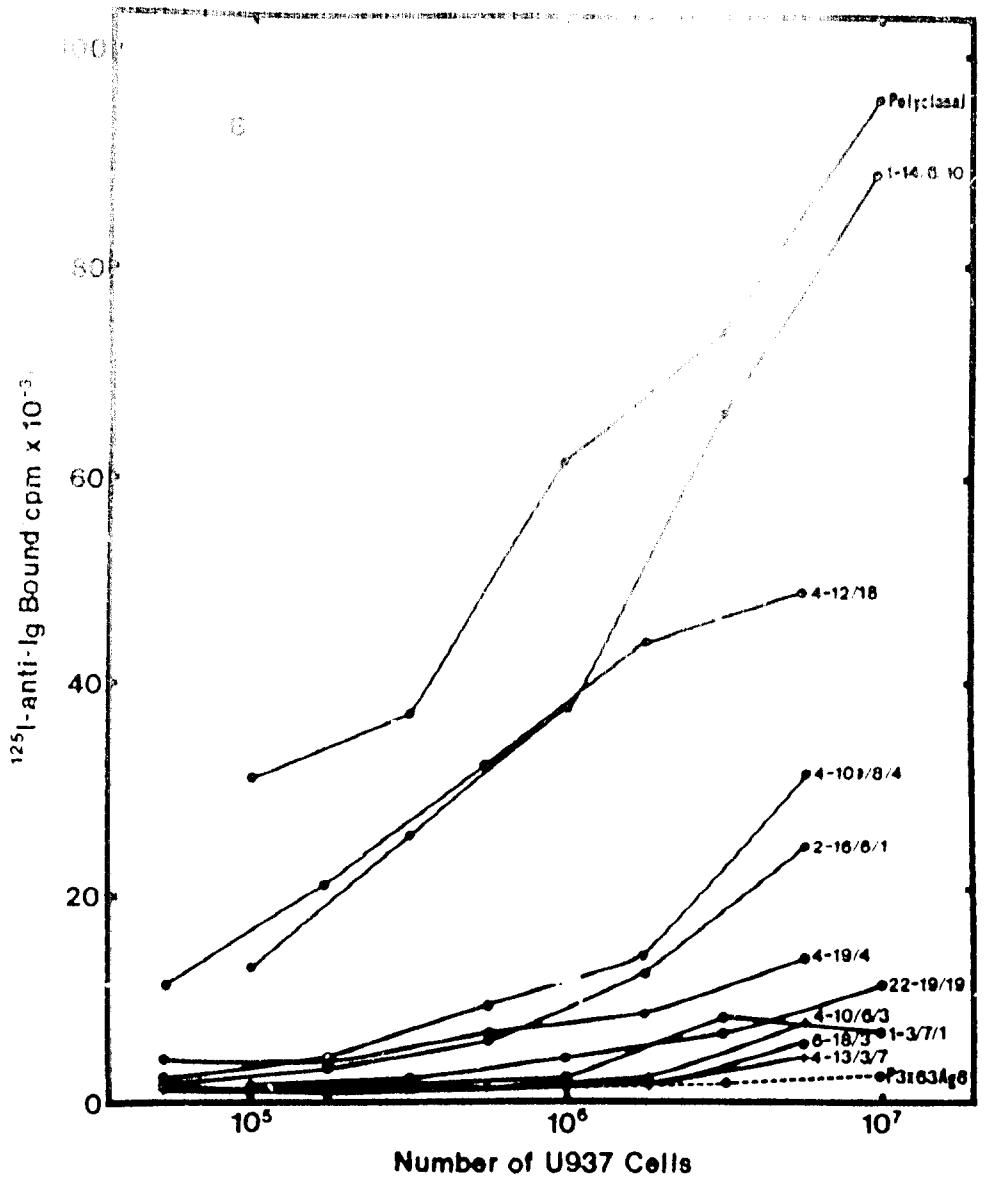


Fig. 1. *(continued)* **B:** Binding of antibodies which immunoprecipitated proteins.

digestion and lipid extraction (Table 2). The peptide composition of the antigens was analyzed by the effect of pronase on the Triton X-100 extract of the U937 cells and the lipid content of the antigen was analyzed by extraction into the organic phase of a chloroform:methanol (1:2) mixture. The antigenic activities associated with 12 of the 16 monoclonal antibodies were sensitive to incubation with 20 μg/ml pronase for 18 hr at 37°C. Four of the antigens (1-14/6/10, 1-18/8, 4-10/6/3, and 4-19/4) were resistant to pronase. These four did not appear to be glycolipids because they were present in the protein phase after extraction with chloro-

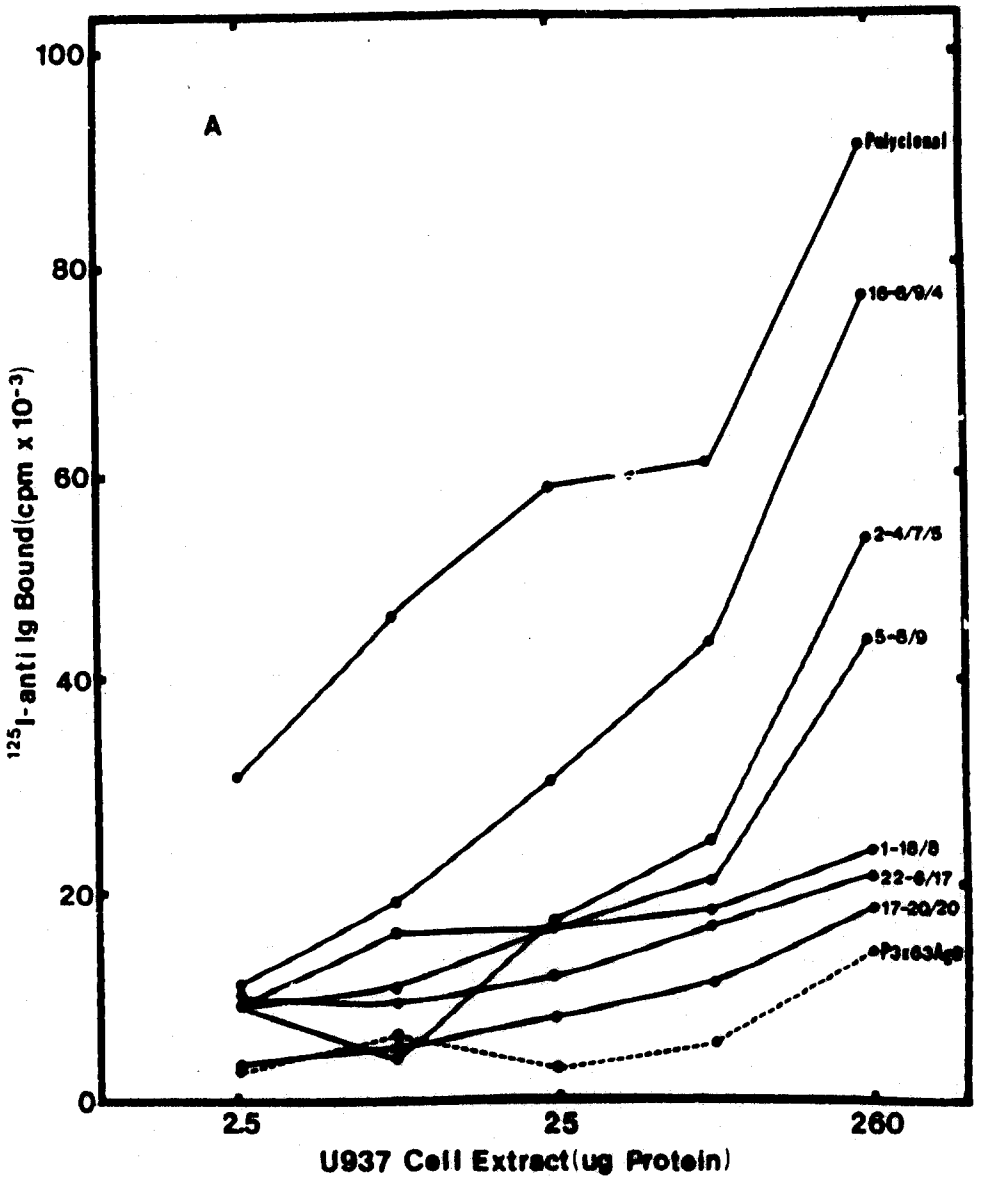


FIG. 2. Antibody binding procedure described in Fig. 1 legend. Cell extract was prepared by homogenizing U937 cells at 2 × 10^6/ml in phosphate buffered saline, 1 mM phenyl methylsulfonyl fluoride on ice. Protein was measured by the method of Lowry et al. (21); 260 μg of protein is the equivalent of 10^7 cells. A: Binding of antibodies which did not immunoprecipitate proteins.

form:methanol. Even after this organic phase extraction to remove noncovalently associated hydrophobic molecules, these proteins remained pronase-resistant (data not shown). It is also noteworthy that the antigen reacting with antibody 16-8/9/4, although very pronase-sensitive, was totally extracted into an organic phase, indicating a high degree of hydrophobicity for this component. Several of the antigens

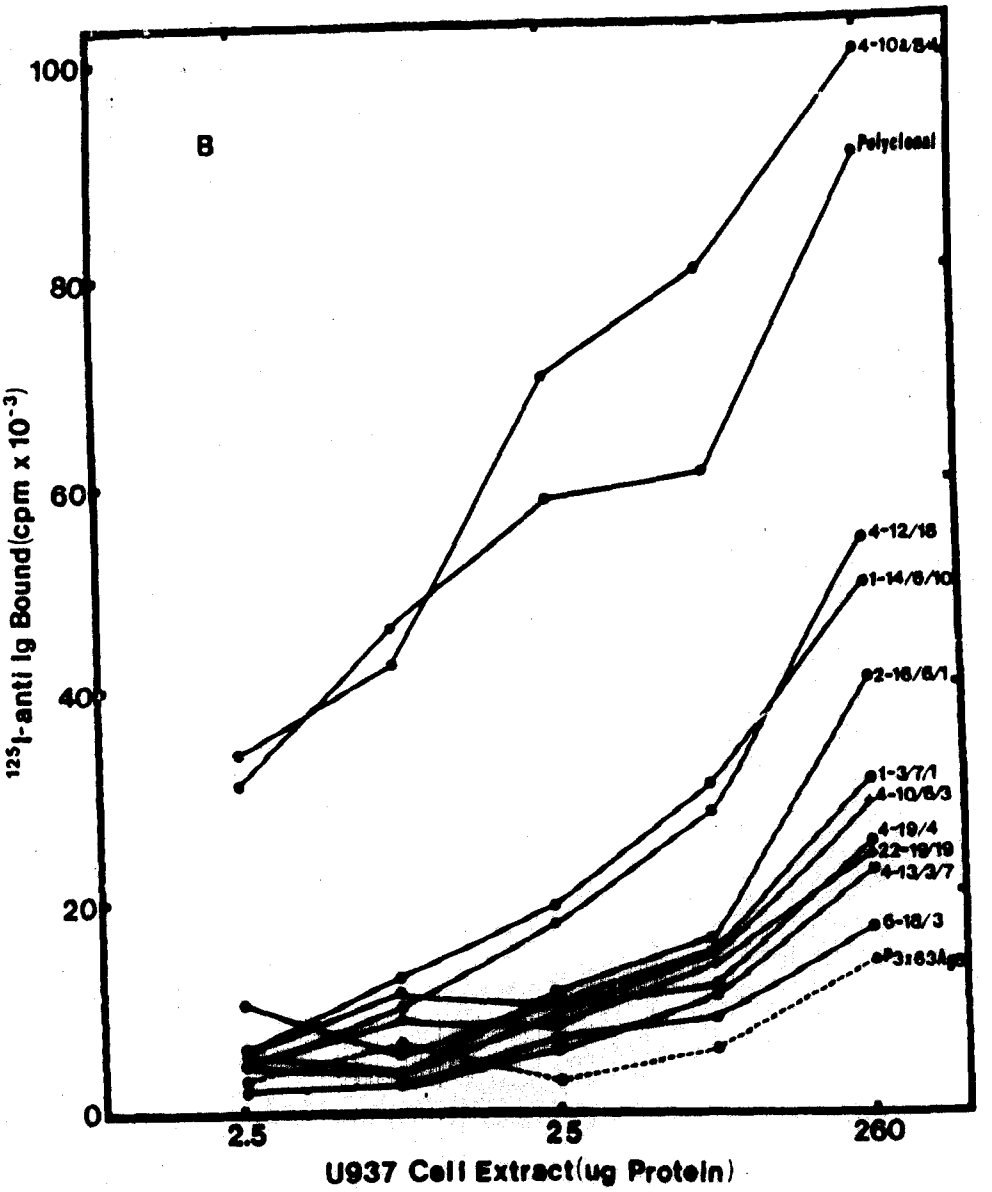


Fig. 2. *(continued)* **B:** Binding of antibodies which immunoprecipitated proteins.

show partial solubility in the organic phase, suggesting an amphipathic structure or substitution with lipid soluble constituents.

Cell Distribution of Antigens

Antigen concentration on the surface of intact cells was studied by flow cytofluorimetric analysis, comparing U937 with a cultured T cell (CEM), a cultured B cell (Daudi) and human peripheral granulocytes, monocytes, and lymphocytes

TABLE 2. *Chemical characterization of antigens*

Antibody	Antigenic activity		
	Pronase sensitivity[a]	$CHCl_3$:CH_3OH Extract[b]	
		Organic	Protein
Polyclonal	49	97	72
1-3/7/1	88	0	44
1-14/6/10	16	9	31
1-18/8	0	44	89
2-4/7/5	100	0	21
2-16/6/1	100	0	11
4-10/6/3	18	2	30
4-10a/8/4	68	4	62
4-12/18	100	0	18
4-13/3/7	100	51	44
4-19/4	0	26	126
5-8/9	100	22	65
6-18/3	100	0	24
16-8/9/4	100	108	3
17-20/20	99	25	89
22-6/17	89	24	69
22-19/19	83	14	15

[a]Percent reduction of antigenic activity.
[b]Percent of antigenic activity recovered in the protein and organic phases.

TABLE 3. *Flow cytofluorimetric analysis of monoclonal antibody binding*

Antibody	Cell[a] (%)					
	U937	CEM T cell	Daudi B cell	Granulocyte	Monocyte	Lymphocyte
Control monoclonal Ig	<1	2	8	<1	4	1
Rat polyclonal Ig	97	75	95	84	97	93
1-3/7/1	17	4	3	<1	11	3
1-14/6/10	97	99	12	10	24	2
1-18/8	2	5	18	2	19	3
4-10a/8/4	27	4	19	1	3	1
4-12/18	97	93	27	29	63	5
5-8/9	38	32	7	<1	8	12
22-19/19	33	4	—	67	52	2

[a]Percent positive cells as detected by flow cytofluorimetric analysis.

(Table 3). Seven of the 16 monoclonal antibodies showed relatively strong fluorescence with various populations of cells. Two of the antibodies (1-14/6/10, 4-12/18), including those reacting most strongly, targeted antigens present on T cells and granulocytes as well as monocytes and the U937 cells; there was little activity, however, with the cultured B cell or lymphocytes. Four (1-3/7/1, 1-18/8, 4-10a/8/

4, 22-19/19) were relatively specific for myelomonocytic cells. One, 5-8/9, showed a reactivity with T cells and lymphocytes as well as U937.

Molecular Properties of Individual Antigens

The identity of many of the antigens has been determined by immunoprecipitation or fractionation studies.

270 kDa Glycoprotein

Antibody 1-3/7/1, IgG_1, precipitated a polypeptide of 270 kDa from iodinated U937 glycoproteins (Fig. 3). The mobility of p270 was the same in reducing and nonreducing gels, and the glycoprotein isoelectric point was 5.5 (Fig. 4). The glycoprotein was metabolically labeled in U937 cells with ^{35}S-methionine and was labeled on intact cells by lactoperoxidase-catalyzed iodination.

The antibody also immunoprecipitated a molecule of 145 kDa from the purified glycoprotein fraction. The properties of this polypeptide were otherwise similar to p270 except that it was not labeled with ^{35}S-methionine or by vectorial iodination. We suspect that the p145 is a cleavage product of p270 generated during the preparation of the glycoprotein fraction.

p270 can be immunoprecipitated from concentrated medium of cells incubated for 12 hr with ^{35}S-methionine. The protein thus appears to be secreted by the cells and possibly is a biologically active molecule.

70 and 20 kDa Cell Surface Polypeptides

Antibody 1-14/6/10, IgG_{2a}, immunoprecipitated two polypeptides of 70 and 20 kDa (Fig. 3). Two-dimensional electrophoresis showed that p70 was slightly acidic; the 20 kDa polypeptide was comprised of four isomorphic forms with isoelectric points of 6.4, 5.9, 5.5, and 5.3 (Fig. 4). These polypeptides were immunoprecipitated solely from surface-iodinated U937 cells; they were not detected in the glycoprotein fraction isolated by concanavalin A affinity chromatography, nor did they incorporate ^{35}S-methionine during either a 6 hr or 18 hr metabolic labeling of U937 cells.

The chemical composition of the antigen is possibly unusual. Antigenic activity of a Triton X-100 extract of U937 cells was resistant to trypsin and pronase (Table 2). Moreover, proteins which had been extracted by chloroform:methanol were only 50% pronase-sensitive when treated for 18 hr at 37°C.

The 70 and 20 kDa polypeptides were present in high concentration on the surface of U937 cells, as indicated by antibody binding to intact cells (Fig. 1B). Antibody binding gave strong fluorescence on the surface of cells, and the antigen was expressed by 97% of U937 cells and 99% of the CEM-cultured T cells, as measured by flow cytofluorimetric analysis (Table 3). Antibody also bound to subpopulations of monocytes and granulocytes, but not to peripheral blood lymphocytes.

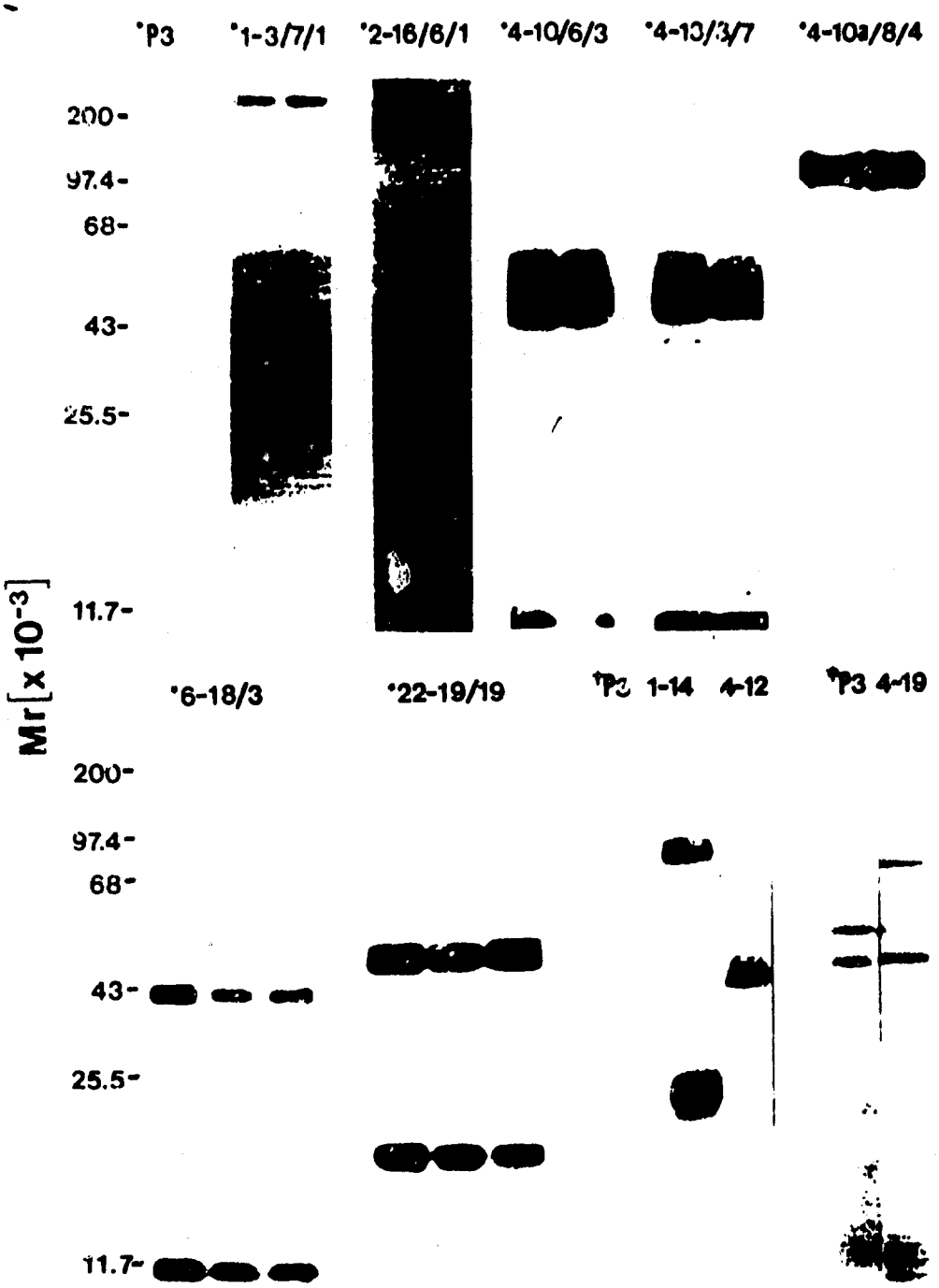


FIG. 3. Autoradiograms of proteins immunoprecipitated by hybridoma antibodies. U937 glycoproteins (*) were isolated by affinity chromatography on concanavalin A-Sepharose and were iodinated using chloramine-T. Proteins were precipitated using supernatants from different agar colonies generated during the cloning process. P3 indicates P3-X63Ag8 supernatant. U937 cell surface proteins (†) were labeled using lactoperoxidase-catalyzed iodination and were immunoprecipitated using the indicated antibodies. A Triton X-100 extract of total U937 cellular proteins (‡) was prepared and iodinated using chloramine-T. The indicated antibodies were used for immunoprecipitation. The molecular weights correspond to the positions of the following marker proteins: myosin heavy chain (200,000), phosphorylase a (97,400), bovine serum albumin (68,000), ovalbumin (43,000), chymotrypsinogen (25,500), and cytochrome c (11,700).

280 kDa Glycop[illegible]

Antibody 2-16/6/1, IgG_{2a}, precipitated gl[illegible] [illegible] kDa from an iodinated glycoprotein fraction of U937 [illegible]. The mobility of this doublet was unchanged in a nonreducing gel. There [illegible] acidic proteins (Fig. 4). The 280 kDa component, but not the 160 kDa, was labeled with ^{35}S-methionine and at the cell surface with lactoperoxidase and ^{125}I. Possibly, the 160 kDa component is a proteolytic cleavage fragment generated during glycoprotein purification.

47 and 11 kDa Glycoprotein Complexes

Antibodies 4-10/6/3 and 4-13/3/7, IgG_{2a} and IgG_1, respectively, immunoprecipitated the same set of polypeptides from iodinated U937 glycoproteins: two major bands of 47 and 11 kDa and a minor band of 65 kDa (Fig. 3). The 47 and 11 kDa species were the major iodinated glycoproteins recognized by rat polyclonal serum against U937 cells and were the major iodinated components of the glycoproteins isolated by concanavalin A affinity chromatography. Under nonreducing conditions, the immunoprecipitate migrated as a single 65 kDa species. Two-dimensional gel electrophoresis of the immunoprecipitated polypeptides showed that the 47 kDa polypeptide is composed of a series of at least seven isomorphic fractions between pI 7.3 and 6.4, and an additional component with pI 5.0. The 11 kDa polypeptide focused as 3 components at pI 7.6, 7.5, and 4.7 (Fig. 4).

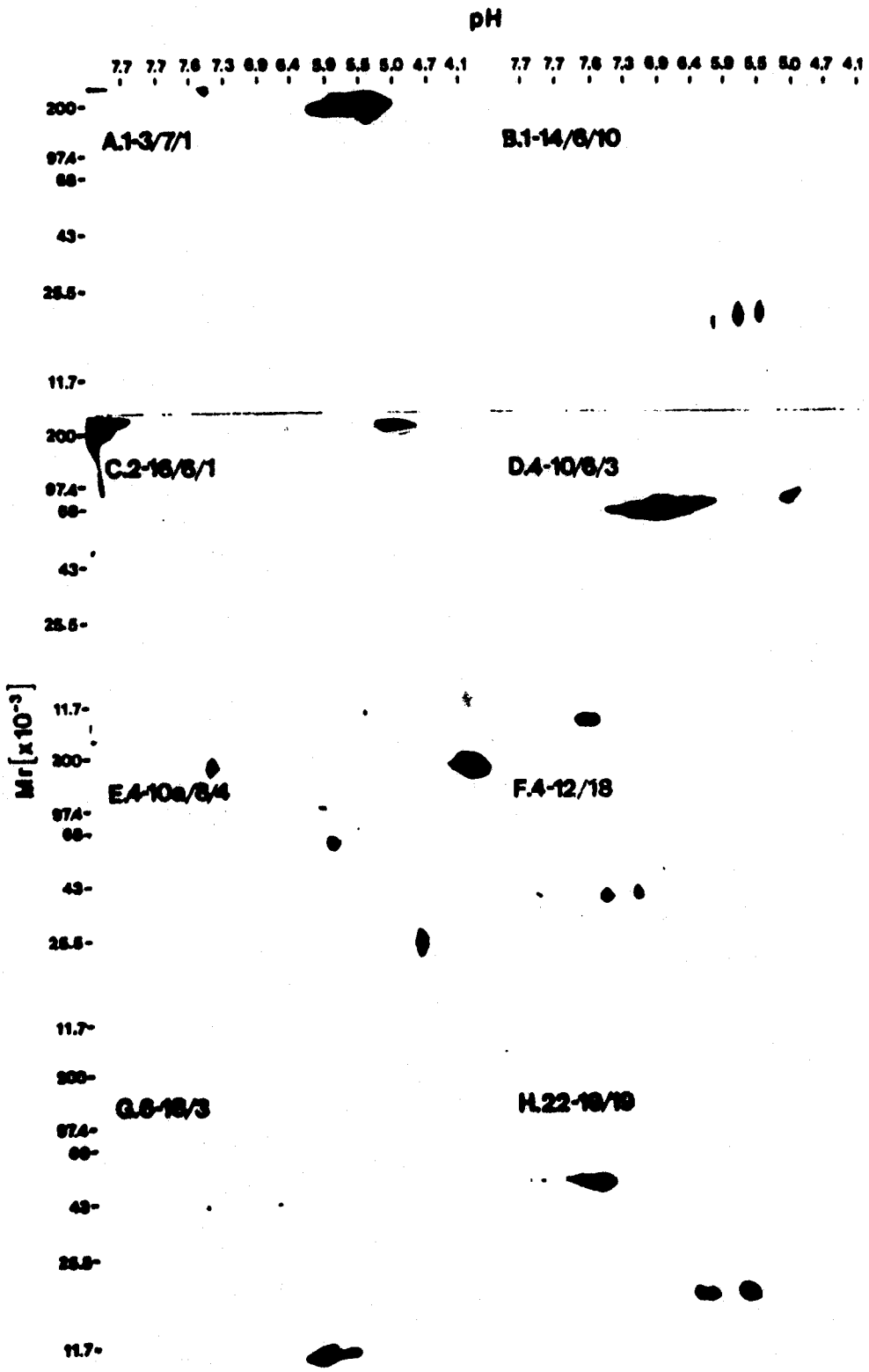
The 47 and 11 kDa polypeptides were not accessible to lactoperoxidase-catalyzed vectorial iodination. The 65 kDa polypeptide was weakly labeled with ^{35}S-methionine, but ^{35}S-methionine labeling of the 47 and 11 kDa polypeptides was not detected.

The antigens recognized by both antibodies fractionated predominately in the aqueous phase after chloroform:methanol extraction; nevertheless, the antigenic activity associated with 4-10/6/3 was protease-resistant whereas the antigen detected with 4-13/3/7 was protease-sensitive. Possibly, one antigen is in a carbohydrate portion of the molecule and the other on the protein portion.

Although the antigens were heavily labeled with ^{125}I, antibody binding to either intact U937 cells or cell extract was rather low (Table 2). Thus, the fact that it was a major iodinated glycoprotein probably indicates a relatively large number of tyrosyl residues rather than a large number of molecules.

150 kDa Glycoprotein

Antibody 4-10a/8/4, IgG_{2a}, recognized a 150 kDa acidic protein (Fig. 3), one of the major iodinated components among the glycoproteins isolated from U937 cells. This glycoprotein was accessible to lactoperoxidase-catalyzed iodination of the cell surface and was metabolically labeled by ^{35}S-methionine. The two-dimensional electrophoretic pattern revealed a very heterogeneous charge distribution with the majority of the protein located at the acidic extreme in isoelectric focusing (4.1),


pH
7.7 7.7 7.6 7.3 6.9 6.4 5.9 5.5 5.0 4.7 4.1
A.1-3/7/1
B.1-14/6/10
C.2-16/6/1
D.4-10/6/3
E.4-10a/6/4
F.4-12/18
G.6-16/3
H.22-19/19
Mr [x10⁻³]
200-
97.4-
68-
43-
25.5-
11.7-

a smaller fraction at pH 7.2, and other material distributed between these two forms. Other proteins, seen as very minor bands on one-dimensional electrophoresis, were repeatedly present in the two-dimensional gels. These other molecules may be associated with the 150 kDa glycoprotein.

Indirect antibody binding assays indicated that p150 is present in large quantities in U937 cells. In addition to the cell surface component, there appeared to be an intracellular pool of antigen as indicated by the increase in binding of antibody to cell extract as compared to an equivalent number of cells (Figs. 1B and 2B).

The antigen recognized by antibody 4-10a/8/4 may be a surface differentiation marker for macrophages. The antigen marked a subpopulation of U937 cells, as detected by flow cytofluorimetry (Table 3). It also bound to a lesser extent on B cells, but not on the surface of T cells or peripheral blood granulocytes, monocytes, or lymphocytes. In contrast, high concentrations of the antigen were found in extracts of a wide variety of cells and tissues examined by a solid phase, antibody binding assay. This suggests that p150 is synthesized in virtually all cell types but is expressed on the surface of cells in a differentiation-specific manner.

40 kDa Cell Surface Polypeptide

Antibody 4-12/18, IgM, precipitated a surface-labeled polypeptide of 40 kDa from U937 cells (Fig. 3). This polypeptide was composed of three isomorphic components with isoelectric points of 7.7, 7.4, and 7.0 (Fig. 4). The antigen bound large amounts of antibody (Fig. 1B) and was a predominant component of the cell surface labeled by vectorial iodination. p40 was not immunoprecipitable from an iodinated concanavalin A-glycoprotein fraction of U937 cells, nor did it incorporate ^{35}S-methionine during metabolic labeling.

The antigen recognized by 4-12/18 was widespread. It was detected on the surface of U937 cells, T cell clone, B cell clone, and peripheral blood granulocytes and monocytes, but not lymphocytes (Table 3). p40 was also detected in most of the cell lines, cells, and tissues assayed by indirect, solid phase antibody binding.

68 kDa Antigen

Antibody 4-19/4, IgM, reacted with a 68 kDa component present in the iodinated total U937 cell extract (Fig. 3). This antigen was not immunoprecipitated from the

FIG. 4. Autoradiograms of two-dimensional gels of U937 proteins immunoprecipitated by hybridoma antibodies. Proteins were immunoprecipitated as in Fig. 2. They were dissolved in lysis buffer and subjected to equilibrium isoelectric focusing and gel electrophoresis as described (26). **A:** ^{125}I-labeled glycoprotein precipitated by antibody 1-3/7 1; **B:** ^{125}I-labeled surface protein precipitated by antibody 1-14/6/10; **C:** ^{125}I-labeled glycoprotein precipitated by antibody 2-16/6/1. **D:** ^{125}I-labeled glycoprotein precipitated by antibody 4-10.6/3. Identical results were obtained with antibody 4-13/3/7. **E:** ^{125}I-labeled glycoprotein precipitated by antibody 4-10a/8 4; **F:** ^{125}I-labeled surface protein precipitated by antibody 4-12 18; **G:** ^{125}I-labeled glycoprotein precipitated by antibody 6-18/3; **H:** ^{125}I-labeled glycoprotein precipitated by antibody 22-19/19. The indicated molecular weights correspond to the positions of the marker proteins, as listed in Fig. 3, which were applied in a parallel lane of the second-dimension gel.

purified glycoprotein fraction, surface labeled proteins, or ^{35}S-methionine labeled proteins.

The chemical composition of the antigen may be unusual. Antigenic activity fractionated predominately into the protein fraction after chloroform:methanol extraction, but was resistant to digestion by trypsin or pronase in a Triton X-100 extract of U937 cells (Table 2). It was also resistant when proteins were first denatured by chloroform:methanol extraction and then subjected to pronase digestion.

45, 30, and 25 kDa Polypeptides

The antigenic target of antibody 5-8/9, IgM, was not identified by immunoprecipitation. However, the molecular weight of the antigen could be assigned by measuring antigenic activity of the U937 cell extract fractionated into components in 2 mm slices of a polyacrylamide gel. Antigen was detected at 45, 30, and 25 kDa. The antigenic activity fractionated predominately in the aqueous phase after chloroform:methanol extraction. It was sensitive to trypsin and pronase in a Triton X-100 extract of U937 cells; therefore, the antigen has properties of a protein.

This antigen had a unique distribution when analyzed by flow cytofluorimetry: it was detected on the surface of approximately 30% of U937 cells and a T cell clone, and 12% of peripheral blood lymphocytes, but was not reactive with a B cell clone, granulocytes, or monocytes (Table 3).

40 and 11 kDa Protein Complex

Polypeptides of 40 and 11 kDa were precipitated by antibody 6-18/3, IgG$_1$ (Fig. 3). Under nonreducing conditions, these polypeptides migrated as a single polypeptide of 50 kDa. The 40 kDa polypeptide was comprised of a series of isomorphic subunits between pI 7.7 and 5.5. The 11 kDa polypeptide migrated at pI 6.4 with a minor component at pI 5.9 (Fig. 14).

Both polypeptides were metabolically labeled using ^{35}S-methionine and could be immunoprecipitated from the medium of cells which were incubated with ^{35}S-methiunine. Thus, these polypeptides were secreted by U937 cells. Neither polypeptide was labeled at the cell surface using lactoperoxidase.

The antigen was present in low concentration and was detected only in U937 and a myeloma cell line by the indirect antibody binding assay (data not shown).

19 kDa Component

The antigenic target of antibody 16-8/9/4, IgG$_{2a}$, was identified as a molecule of 19 kDa, based on antibody binding to material eluted from 2 mm slices of a polyacrylamide gel containing 200 μg U937 cell extract (Fig. 5). Immunoprecipitation of the molecule was not detected.

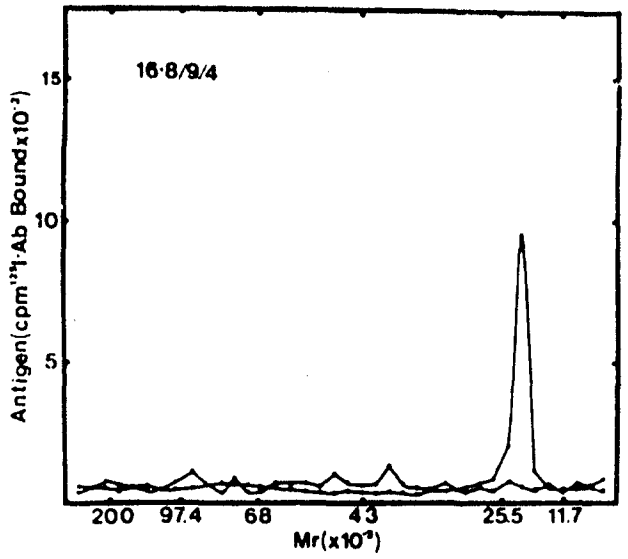


FIG. 5. Antibody 16-8/9/4 binds to a 19 kDa protein. Crude U937 homogenate 200 μg, was fractionated by NaDodSO$_4$-polyacrylamide gel electrophoresis and protein fractions were eluted from 2 mm slices and the eluate was adsorbed to wells of a microtiter plate as described (23). Antigen was measured using the solid phase indirect antibody binding assay (12) with 50 μl of 16-8/9/4 culture supernatant *(upper curve)* or 50 μl of P3-X63Ag8 culture supernatant *(lower curve)*, and 50 μl of ^{125}I-labeled goat anti-rat IgG secondary antibody (0.25 μg/ml, 3.8 × 10^4 cpm/ng).

The antigen appeared to be very hydrophobic and was entirely soluble in the organic phase of a chloroform:methanol extract. However, the antigen extracted by Triton X-100 was sensitive to digestion by pronase or trypsin.

55, 50, and 18 kDa Glycoprotein Complex

Antibody 22-19/19, IgG$_{2a}$, immunoprecipitated three polypeptides of 55, 50, and 18 kDa from the iodinated glycoprotein fraction of U937 cells (Fig. 3). Under nonreducing conditions, these polypeptides migrated as an 80 kDa polypeptide; a minor component of 90 kDa was also observed. The 50/55 kDa doublet focused at pI 7.6. The 18 kDa peptide separated into three components: pI 6.4, 6.3, and 5.7 (Fig. 4). There is evidence that these glycoproteins actually exist as a 80 and 90 kDa complex. Following ^{35}S-methionine metabolic labeling, the only immunoprecipitated polypeptide was 80 kDa, even under reducing conditions. This suggests that the 55, 50, and 18 kDa components are proteolytic fragments generated during the purification of glycoproteins, and are associated under nonreducing conditions by sulfhydryl linkages. When cells were labeled by lactoperoxidase-catalyzed iodination, the major ^{125}I-polypeptide was the 90 kDa component, suggesting that this subunit was preferentially exposed at the cell surface. The relationships between these polypeptides may be clarified by pulse-chase analysis and protein purification.

Flow cytofluorimetric analysis revealed that the expression of this antigen is restricted to cells of the myelomonocytic lineage (Table 3). The antibody reacted

with 33% of U937 cells, 52% of monocytes, and 67% of neutrophils, but not with T and B cell clones or peripheral lymphocytes.

SUMMARY

Monoclonal antibodies that react with many of the major cell surface proteins of a human macrophage cell line have been prepared. These include antibodies that immunoprecipitate nine proteins comprising the majority of the major cell glycoproteins or the cell surface proteins detected by vectorial labeling with ^{125}I. These immunoprecipitation antibodies are of special value as reagents that can be used for the purification and characterization of the proteins.

We have also begun to define the specificity of expression of the antigens. Several of the antibodies, (1-3/7/1, 4-10a/8/4, 5-8/9, 22-19/19), react with U937 cells to a limited degree which may indicate that a subpopulation of macrophages has been recognized. Other antibodies, (1-14/6/10, 4-12/18, 5-8/9), are notable in their high level of reactivity with a T cell clone. At least one antibody, 22-19/19, reacts with only myelomonocytic cells, and may be useful in defining the development of these cells.

We also have evidence that the proteins precipitated by 1-3/7/1 and 6-18/3 are secreted by U937 cells. The effects of these proteins on other cells is of obvious importance.

A variety of other macrophage functions can be modeled by the U937 cell line including activation, endocytosis, chemotaxis, and antibody-dependent cytotoxicity. These monoclonal antibodies may be useful in the study of these processes as well as for the characterization of the plasma membrane of the human macrophage.

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