ISOLATION OF THE RECEPTOR FOR IgG FROM A HUMAN MONOCYTE CELL LINE (U937) AND FROM HUMAN PERIPHERAL BLOOD MONOCYTES*

BY CLARK L. ANDERSON†

From the Immunology Unit, Department of Medicine, University of Rochester Medical Center, Rochester, New York 14642

Fc receptor-mediated endocytosis, the process whereby IgG immune complexes bind to the surface of the monocyte or macrophage and are quickly engulfed by the cell and transported to lysosomes, is an incompletely understood sequence of events (reviewed in 1, 2). It seems clear that analysis of the precise molecular events involved in this process should begin with characterization of the molecular entity on the plasma membrane responsible for binding the Fc portion of IgG, the Fc receptor (3). Because detailed molecular studies of the Fc receptor are likely to require large amounts of material and because normal human monocytes or macrophages are difficult to obtain in large number and in pure form, we have sought a model for Fc receptor structure among human monocyte cell lines. Studies of the human monocyte line U937 indicate that it should be useful in this regard. Binding and kinetic studies of IgG to the Fc receptor on U937 show that this cell line has an Fc receptor for IgG quite similar, if not identical, to the Fc receptor on normal human peripheral blood monocytes (4, 5).

In an effort to characterize more completely the Fc receptor on U937 cells, we have undertaken to isolate the receptor from detergent lysates of surface-radioiodinated cells by means of affinity chromatography on immobilized IgG. In critical experiments, purified human monocytes have been evaluated.

Materials and Methods

Cells. Cultures of U937 were obtained from Dr. Peter Ralph, Sloan-Kettering Institute for Cancer Research, Rye, NY; MOLT-4 from the Naval Biosciences Laboratory, Oakland, CA; HL60 from Dr. Robert Gallagher, National Cancer Institute, Bethesda, MD; and K562 from Dr. Peter Rowley, University of Rochester. These cells were maintained in spinner culture in RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 10% fetal calf serum (FCS)¹ (Sterile Systems, Logan, UT), penicillin (100 U/ml), and streptomycin (100 µg/ml), both from Gibco Laboratories. Human monocytes, obtained as a by-product of plateletpheresis of normal blood bank donors, were kindly purified (>90% homogeneous) by centrifugation-elutriation²

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Abbreviations used in this paper: BSA, bovine serum albumin; FcR, Fc receptors; FCS, fetal calf serum; IEF, isoelectric focusing; NaAC, sodium acetate; NAc, acetic acid; NP-40, Nonidet P-40; OA, ovalbumin; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; 2-ME, 2-mercaptoethanol.

² Scully, S. P., G. B. Segel, and M. A. Lichtman. Plasma membrane vehicles prepared from unadhered monocytes: characterization of calcium transport and the calcium ATPase. Cell Calcium. In press.
by Mr. Sean Scully, University of Rochester, and were then incubated at 37°C for 1 h in RPMI 1640 containing bovine serum albumin (BSA) (1 mg/ml) to dissociate in vivo bound IgG. All cells were then washed twice in phosphate-buffered saline (PBS) (20 mM phosphate, 145 mM NaCl, pH 7.0) in preparation for radiolabeling. More than 95% of these cells excluded trypan blue.

**Iodination.** The cells were radioiodinated by the chloroglycouril method (6). 1 ml of cells (10^7/ml in PBS) and 1 mCi ^125^I (IMS.300, Amersham, Arlington Heights, IL) in 10 μl were placed in a vial coated with 5 μg chloroglycouril and incubated for 30 min at 0°C. The reaction was quenched and the cells were washed three times in 5 mM KI in PBS. Cells were then lysed at 5.0 × 10^7/ml in 1% Nonidet P-40 (NP-40), 2 mM phenylmethylsulfonylfluoride (PMSF), and aprotinin (1 trypsin inhibitor unit [TIU]/ml) in PBS for 30 min at 0°C. The cell nuclei and unlysed material were sedimented at 7,800 g for 30 min. 5–10% of the starting radioiodine was contained in the lysate and 10% of lysate radioiodine was trichloroacetic acid (TCA) precipitable.

**Affinity Chromatography.** Various proteins were cross-linked with Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) by a modification of the cyanogen bromide technique (7). The conjugation ratio varied from 2 to 4 mg protein/ml packed Sepharose beads. 0.5 ml of immunoadsorbant in an 8- × 100-mm column (Bio-Rad Laboratories, Richmond, CA) was washed at 4°C with 10 ml PBS, 5 ml BSA/PBS (5 mg/ml), 5 ml 0.5 M acetic acid (HAc) in 1% NP-40, and 75 ml 1% NP-40 in PBS. Radiolabeled lysate (0.3 ml or 6.0 × 10^7 cell equivalents) was applied to the immunoadsorbant and was mixed at 4°C by rotation of the column on its longitudinal axis overnight (sometimes for 6 h: results were no different). The immunoadsorbant was washed at 4°C with 75 ml 1% NP-40 in PBS and then eluted with 0.5 M HAc in 1% NP-40. Fractions (0.5 ml) of the acid eluate were collected in 100 μl of 2 M Tris in 1% NP-40. The radioiodine in the peak fractions was 90–100% TCA precipitable. The radiolabeled material eluted from Sepharose-IgG columns amounted to ~0.4% of the TCA-precipitable material applied. No additional radioactivity could be eluted with either 0.5 M NaSCN or 1% SDS. Urea (4.5 M in 50 mM Na acetate [NaAc], pH 6.0) eluted only p40-43. However, approximately sixfold more radioactivity remained attached to the column.

**SDS-PAGE, Isoelectric Focusing (IEF), and Autoradiography.** SDS-PAGE was performed on slab gels according to Laemmli (8). The acid eluate samples containing 2% SDS, 10% glycerol, 5% 2-mercaptoethanol (2-ME), and 0.001% bromphenol blue were immersed in boiling water for 2 min and applied to the stacking gels. Molecular weight markers were myosin (200,000), β-galactosidase (130,000), phosphorylase B (94,000), BSA (68,000), ovalbumin (43,000), soybean trypsin inhibitor (21,000), and lysozyme (14,300), from Bio-Rad Laboratories and chloramine T-radioiodinated human IgG1 (4). After being stained, de-stained, and dried, the gels were apposed to pre-fogged Xomat AR X-ray film (Eastman Kodak Co., Rochester, NY) and sandwiched between two intensifying screens (Lightning Plus Chronex, Dupont, Wilmington, DE) at -70°C. Two-dimensional gel electrophoresis was performed in 3- × 100-mm cylindrical gels in the first dimension and on a 10% SDS-PAGE slab gel in the second dimension, exactly as described by O'Farrell (9). The samples contained 9.5 M urea, 5% 2-ME, 2% NP-40, 1.6% pH 5–7 ampholine, 0.4% pH 3.5–10 ampholine (LKB, Rockville, MD). For some experiments, the protocol of Ames and Nikaido (10) was followed to maximize dissociation of proteins before application of the sample to the IEF gel. Thus, samples containing 2% SDS, 50 mM Tris (pH 6.8), and 0.5 mM CaCl2 were immersed in boiling water for 2 min. Urea (18.8 mg/25 μl lysate) and 2 vol of a solution containing 9.5 M urea, 1.6% pH 5–7 ampholine, 0.4% pH 3.5–10 ampholine, 5% 2-ME, and 8% NP-40 were then added to this lysate. Results using this system were comparable to the O'Farrell gels except that the basic end of the gel was extended, as noted by the authors. All gels displayed have their basic ends to the left.

**Reagents.** The following reagents were obtained from Sigma Chemical Co., St. Louis, MO: BSA, fraction V powder; sperm whale skeletal muscle myoglobin; chicken egg white lysozyme; ovalbumin (OA); bovine pancreas deoxyribonuclease I; aprotinin; phenanthroline; pepstatin A; and PMSF. Chloroglycouril (Iodogen) was obtained from Pierce Chemical Co., Rockford, IL. NP-40 was obtained from Particle Laboratory, Elmhurst, IL. Vibrio cholerae neur-
aminidase was obtained from Cal-Biochem-Behring Corp., La Jolla, CA. Pooled human IgG was purchased as Pentex human gamma globulins, fraction II, from Miles Laboratories, Inc., Elkhart, IN, and was further purified by DEAE cellulose ion-exchange chromatography. A human IgG1 myeloma protein (Woo) and Fab fragments from the same protein were purified as described (4). A human IgG2 myeloma (Web) purified in the same manner was a generous gift of Dr. George Abraham, University of Rochester. IgM was purified from a patient (Hoe) with macroglobulinemia by means of ion-exchange chromatography and gel filtration such that the preparation contained <0.2% IgG by nephelometric analysis using anti-IgG-precipitating antibody. The medium in which U937 cells were incubated with IgG1 to saturate Fc receptors (Fig. 1) was a balanced salt solution (11) containing BSA (1 mg/ml) and NaN3 (0.2%) (BBA). Unless otherwise indicated, Sepharose-IgG columns were prepared with purified pooled human IgG.

Results

Analysis of Sepharose-IgG Binding Material from Detergent Lysates of a Human Monocyte Line, U937. Detergent lysates of surface-radioiodinated U937 cells were applied to Sepharose-IgG columns. The columns were washed free of unbound radioactivity and bound 125I material was eluted with acid. Analysis of the acid eluates by SDS-PAGE and autoradiography revealed that most of the radioactivity moved as a single broad band with an apparent molecular weight at its densest portion of 72,000 (p72). A second heterodisperse band, variable in quantity but always <10% of the total amount of radioactivity seen on the gels, had an apparent molecular weight of 40,000–43,000 (p40–43). Lane 1 in Fig. 1B shows a typical pattern. Samples prepared in the absence of 2-ME gave identical results. Similarly, samples carried through the entire procedure in the presence of inhibitors of the major classes of cellular proteinases (12) (1 mM

![Fig. 1](image-url)

(A) Acid eluates from Sepharose-IgG columns. Two batches of U937 cells were radioiodinated. One batch had been incubated before iodination with IgG1 (4 × 10^7 cells/ml BBA, 10 μg IgG1/ml BBA, 30 min, ice bath) to saturate the FcR (circles); the other had been incubated with an IgG-free protein solution (IgM 10 μg/ml BBA) and was labeled while FcR were unoccupied by IgG (squares). After iodination, the batch of cells with unoccupied FcR was incubated with IgG to saturate the receptors. Both batches were incubated at 10^7 cells/ml RPMI 1640 containing FCS, penicillin, and streptomycin at 37°C for 60 min to allow IgG to dissociate and were then lysed in NP-40. The lysates were applied to individual Sepharose-IgG immunoadsorbant columns. The columns were washed, bound radioactivity was eluted with acid, and fractions were counted. (B) SDS-PAGE of acid eluate peaks. Peak fractions of acid-eluted radioactivity from the two columns described in A were evaluated by autoradiography of SDS-PAGE slab gels. Equal volumes of eluate from the two Sepharose-IgG columns were applied to lane 1 (366 cpm) and lane 2 (164 cpm). Lanes 2 and 3 received equal (164 cpm) amounts of radioactivity. Lane 2 = cells labeled with FcR occupied (○) by IgG1. Lanes 1 and 3 = unoccupied (○). Numbers to right of autoradiograph indicate apparent molecular weight × 10^-3. T = top of 5–15% gradient gel. D = bromphenyl blue dye front.
iodoacetamide, 1 mM phenanthroline, 1 mM PMSF, 1 μM pepstatin A, and 1 TIU/ml aprotinin) yielded gel patterns unchanged from those normally seen when the routine inhibitors, PMSF and aprotinin, were used.

**Labeling of p72 Is Inhibited by In Situ FcR Binding of IgG.** Whether the material eluted from the Sepharose-IgG columns had specific binding affinity for IgG and might therefore represent soluble FcR was approached in several ways. First, an attempt was made to block iodination of the FcR by labeling the cells while the FcR were occupied by IgG. One portion of cells was radiolabeled after saturation of the FcR by IgG, and the bound IgG was then allowed to dissociate from the cells by incubation at 37°C. Another portion of cells was labeled before binding and subsequent dissociation of IgG. Both batches of cells were then lysed in detergent and the lysates were applied to IgG affinity columns. Fig. 1A shows that only ~40% as much radioactivity was acid-eluted from Sepharose-IgG when the cells were labeled while their FcR were occupied with ligand, as compared with cells labeled with receptors unoccupied. Furthermore, when equal amounts of radioactivity (Fig. 1B, lanes 2 and 3) or equal volumes (Fig. 1B, lanes 1 and 2) from the two columns were evaluated by SDS-PAGE and autoradiography, an even more striking inhibition of radiolabeling of p72 was apparent. No inhibition of p72 labeling was seen when cells were incubated with IgM or BSA before labeling. These results suggested that occupation of the FcR in situ by IgG obscured access to one or more tyrosine groups critical for labeling of p72. p72 is thus a likely candidate for the FcR. The density of p40-43 was not perceptibly altered, however, which indicates that labeling of this protein was probably unimpeded by FcR occupation. In the eluate from cells labeled while the FcR were occupied, a small amount of an additional band (p50) was seen which co-migrated with the IgG heavy chain. This extra band, presumed but not proven to be 125I-γ, is most likely a result of binding to the affinity column of 125I-IgG present in the lysate of cells labeled while the FcR were occupied, since dissociation conditions were such that 20% of the bound IgG may still have been cell-bound at the time of lysis (4).

**Analysis of Sepharose-IgG Binding Material from Other Cell Types.** To evaluate further the specificity of p72 for IgG, a human T cell line (MOLT 4) that bears no FcR for IgG was analyzed in identical fashion to U937 above. The peak of radioactivity eluted from Sepharose-IgG after application of a MOLT 4 lysate was 10% of what was eluted when U937 lysate was applied, and no bands were discerned on autoradiographs of SDS-polyacrylamide gels, as seen in Fig. 2.

Other cells were evaluated as well (Fig. 2). The IgG-binding material from HL60

![Fig. 2. Composite of autoradiographs of SDS-PAGE analysis of Sepharose-IgG-binding material from several types of cells. Detergent lysates of radioiodinated preparations of MOLT-4 cells (M), U937 cells (U), HL60 cells (H), K562 cells (K), and normal human peripheral blood monocytes (Mo) were applied to Sepharose-IgG. Equal counts per minute of acid eluates from the columns were analyzed by SDS-PAGE and autoradiography. Numbers indicate molecular weight × 10⁻³.](image-url)
a human promyelocytic line, was similar in molecular weight to that seen with U937, except that p72 seemed more heterodisperse. K562 (14), an erythroblastic cell line that binds monomeric IgG but with kinetic characteristics different from U937 (C. L. Anderson, unpublished observation), yielded very little radioactivity upon elution of the affinity columns with acid, and no p72 appeared in the gels. Whether K562 cells bear an FcR with different molecular characteristics or whether p72 is present but unlabeled has not been evaluated. A faint p40–43, however, was apparent in K562 lysates. Analysis of normal human peripheral blood monocytes highly purified by centrifugation-elutriation showed a pattern very similar to U937. The principal band from monocyte lysates seemed to move in SDS slightly more slowly than the U937 molecule. As with U937 cells, p40–43 was variably recovered from monocytes (present in two of three lysates).

Use of Immunoadsorbents Other than Sepharose-IgG. The binding specificity for IgG of p72 and p40–43 from U937 lysates was evaluated in still another manner. Proteins such as BSA that have no capacity either to bind to U937 or to inhibit the binding of IgG to U937 cells (using the assay in refs. 4 and 5) were conjugated to Sepharose and tested for their ability to purify p72 and p40–43 from U937 lysates. In a typical experiment, the amount of radiolabel eluted from a Sepharose-BSA column was 36% of that eluted from a molar equivalent Sepharose-IgG column (Fig. 3 A), and SDS-PAGE analysis of the material eluted from a Sepharose-BSA column showed no discernible bands (Fig. 3 B). Similarly, immunoadsorbents prepared with lysozyme, whale myoglobin, and chicken OA, proteins that, like BSA, do not inhibit 125I-IgG-U937 binding, yielded results identical to Sepharose-BSA, i.e., no discernible bands were seen on autoradiograms of the SDS gels used to analyze the acid eluates from the columns.

Binding Activity of the Isolated Receptor. After elution from the Sepharose-IgG column the radioactive peak, >90% of which was accounted for by p72, was evaluated for its ability to rebind to IgG. As much as 42% of the TCA-precipitable radioactivity in the eluate was capable of binding back onto Sepharose-IgG during a single incubation,
and on repeat analysis by SDS-PAGE this material had an apparent molecular weight of 72,000. Table I shows the results of a radioassay in which soluble IgG and OA were tested for their ability to inhibit the rebinding of 125I-eluate to Sepharose-IgG. As can be seen, 34% of the 125I-eluate was capable of binding to Sepharose IgG. Pooled human IgG inhibited this binding by ~70% in a dose-responsive fashion, whereas OA did not inhibit binding. The small amount of 125I-eluate bound to Sepharose-OA was not diminished by a large excess of soluble OA, but was partially inhibited by IgG. The significance of this small degree of binding to OA is not clear, but certainly no discernible bands were eluted from Sepharose-OA as described above in Fig. 3. Several explanations are possible, one of which is that the soluble receptor in association with soluble IgG has less nonspecific affinity for Sepharose-OA.

Other proteins were tested for their ability to inhibit the binding to Sepharose-IgG of 125I-eluate from Sepharose-IgG columns. Fab fragments from a human IgG1 myeloma (Woo), IgM purified from a patient (Hoe) with macroglobulinemia, and a human IgG2 myeloma protein (Web) showed considerably less (≤1%) ability to inhibit the binding of 125I-eluate to Sepharose-IgG compared with an IgG1 myeloma (Woo), which inhibited efficiently (Fig. 4). These three minimally inhibitory proteins were similarly inefficient at inhibiting the binding of 125I-IgG1 to U937 cells; i.e., the amount of protein required to inhibit by 50% was 100-fold greater than the amount of IgG1 required.

**Heterodispersity of the p72 Band.** The broadness of p72 as it appeared in autoradi-

| Beads              | Inhibitor | 125I-eluate bound to beads |
|--------------------|-----------|---------------------------|
|                    | mg/ml     | % mean ± SD               |
| Sepharose-IgG      | Buffer    | 34.2±1.6                  |
| Sepharose-IgG      | IgG       | 5.6±0.2                   |
| Sepharose-IgG      | IgG       | 9.7±1.9                   |
| Sepharose-IgG      | IgG       | 18.5±2.0                  |
| Sepharose-IgG      | OA        | 32.9±1.3                  |
| Sepharose-OA       | Buffer    | 8.2±2.3                   |
| Sepharose-OA       | IgG       | 1.3±0.6                   |
| Sepharose-OA       | OA        | 7.4±0.5                   |
| None               | Buffer    | 2.1±1.0                   |

A radioassay was devised whereby 0.05 ml of 125I-eluate from a Sepharose-IgG column and 0.05 ml of various potential inhibitor proteins were incubated a 4°C for 4 h with 0.05 ml of 50% Sepharose-protein conjugates in PBS containing 1% NP-40. The beads were washed free of unbound material by repeated centrifugation and resuspension in fresh medium. The amount of 125I-eluate bound to the beads was counted and expressed as percent (mean of triplicates ± SD) of total TCA-precipitable radioactivity added to the beads.
Inhibition of binding of $^{125}$I-eluate to Sepharose-IgG by several proteins. The experimental protocol was the same as for the experiment described in Table I. The amount of radioactivity bound in the presence of a large amount (6.1 mg/ml) of IgG was subtracted from all values and the percent inhibition of binding was computed by the formula percent Inh = \[(1 - (a + b)) \times 100\], where $a$ = mean counts per minute of triplicate tubes containing inhibitor proteins and $b$ = mean of triplicate samples with medium instead of inhibitor protein.

ographs of SDS gels suggested the presence of multiple proteins of similar apparent molecular weight. This broadness was evaluated in two ways. To determine whether $\gamma$-particle scatter in the autoradiograms might be obscuring multiple peaks in the p72 region, cylindrical gels were transversely sliced and the radioactivity of the individual slices was counted. At least three peaks and shoulders were invariably seen in the p72 area between 66,000 and 90,000, which suggests that microheterogeneity accounted for the breadth of p72 seen on autoradiographs. Furthermore, two-dimensional analysis by IEF and electrophoresis in SDS (9, 10) revealed up to 12 radioactive spots in the p72 area between the isoelectric point of actin and the basic end of the gel. The spots increased in apparent molecular weight as they progressed toward the acidic end of the gel, which suggests quantum additions of charged carbohydrate residues (15). Two-dimensional gel autoradiograms of eluates from U937 cells and monocytes are shown in Fig. 5.

Neuraminidase digestion of the $^{125}$I-eluate from Sepharose-IgG or of intact cells resolved the isoelectric point heterogeneity to one or two spots at the basic end of the gel, which indicates that terminal sialic acid residues were responsible for most of the charge heterogeneity (Fig. 6). However, the breadth of p72 as seen on SDS-PAGE remained unchanged after neuraminidase although the band moved slightly more rapidly (not shown), which suggests that removal of the sialic acid residues resulted in more SDS binding (hence faster mobility) (16), but that considerable heterogeneity still remained, perhaps but not necessarily because of core carbohydrate. Lysates prepared from U937 cells grown in the presence of tunicamycin (1 $\mu$g/ml, 48 h) showed results similar to neuraminidase digestion, i.e., p72 moved more rapidly in SDS and resolved to two spots on two-dimensional gels (not shown).

Nature of p40-43. Although the exact identity of p40-43 is uncertain, several observations deserve comment. First, p40-43 was a minor constituent representing 0–10% of the radioactivity seen on the gels and was often at the threshold of detection such that even with prolonged exposure the autoradiograms yielded poor detail.
Fig. 5. Autoradiograms of O'Farrell two-dimensional gels analyzing the acid eluates from Sepharose-IgG columns onto which detergent lysates of radioiodinated U937 cells (A) and purified human peripheral blood monocytes (B) were applied. Basic ends of gel are to the left, acid to the right. The second dimension is in 10% polyacrylamide. Spots at extreme left and right margins are $^{125}$I-$\gamma$ chains in the molecular weight marker lanes. Arrows mark spots moving coincident with Coomassie-staining actin.
Fig. 6. Neuraminidase effect on charge heterogeneity of $^{125}$I-eluate. $^{125}$I-eluate at pH 5.5 (corrected by addition of 2 M Tris) containing 1 mM calcium chloride was divided into two portions. One portion (A) received neuraminidase (0.17 IU/ml), PMSF (1 mM) and aprotinin (1 TIU/ml). The other (B) received buffer. Both were incubated at 37°C for 2 h. The radioactivity was precipitated with acetone and prepared for two-dimensional gels by the method of Ames and Nikaido (12). Arrows indicate actin.
Attempts to increase incorporation of radioiodine into p40-43 have to date been unsuccessful. Second (not shown), on occasion two separate bands in the p40-43 area could be discerned on SDS-PAGE, one very sharp band at 43,000 mol wt (p43), coincident with the only Coomassie-staining band seen on the gels identified as actin by DNAase affinity, and another broader band moving more rapidly at ~40,000 mol wt (p40). On two-dimensional gels, the Coomassie-stained actin spot (bi-lobed in some gels suggest β and γ actin) was coincident with the most acidic of the several p40-43 spots seen on the autoradiograms (Fig. 5). The remainder of the p40-43 spots (p40), often as many as five to seven distinct spots, was spread between actin and the basic end of the gel, and in most experiments seemed to move with a higher apparent molecular weight (seen faintly in Fig. 5B). Third (not shown), passage of the 125I-elu ate over Sepharose-DNAase, which is able to bind actin (17), resulted in the disappearance of the entire p40-43 band on SDS-PAGE and all of the p40-43 spots on two-dimensional gels, but p72 was unchanged.

Discussion
These experiments, designed to isolate solubilized FcR from U937 cells and human monocytes, describe the means for isolation and some of the characteristics of two molecules (or sets of molecules), p72 and p40-43. Four lines of evidence support the conclusion that one of these molecules, p72, constitutes at least a part of the FcR for IgG. (a) Occupation of the in situ FcR by IgG before the labeling procedure blocked labeling of p72. This phenomenon has also been demonstrated with the IgE receptor on rat mast cells (18). (b) Using the same protocol, the p72 molecule could not be isolated from MOLT 4, a human T cell cell line that does not express FcR. (c) p72 was not purified using control affinity chromatographic columns prepared with proteins such as BSA, myoglobin, OA, and lysozyme, proteins that show no capacity to bind to U937 cells. (d) Once eluted from Sepharose-IgG, p72 was able to rebind to Sepharose-IgG in a manner that indicated specificity identical to the IgG FcR on the membrane of viable U937 cells; i.e., binding was inhibited by IgG1 but not by IgG2, Fab fragments of IgG1, IgM, or OA.

Although p72 and p40-43 were not found in eluates from the non-Ig control affinity adsorbants mentioned above, both of these molecules were recovered (albeit less efficiently than from IgG columns) from immunoadsorbants prepared with IgM, Fab fragments of IgG1, and IgG2. The Ig preparations used for these immunoadsorbants showed minimal but definite (~1%) capacity to inhibit the binding of IgG1 to U937 cells. Thus, it is quite possible that a small amount of IgG contamination may have resulted in effective affinity purification of FcR from detergent lysates since the estimated molar ratio of immunoadsorbant to soluble receptor in these experiments was ~20,000:1. Experiments testing this possibility by altering the ratio of ligand to receptor were inconclusive. Alternatively, it is also possible that solubilization of the FcR had resulted in a broadening of its specificity (previously suggested in ref. 3) such that binding occurred to homologous regions of immunoglobulins such as IgM, IgG2, and Fab fragments. However, the most quantitative data available (Fig. 4) indicate that the soluble receptor maintains its in situ specificity. Final resolution of this question must await further experimentation.

Although the data strongly suggest that p72 is involved in soluble FcR activity, the participation of the p40-43 band in ligand binding and its relationship to p72 are
considerably less certain. Incorporation of radiolabel into p40-43 has been too variable and inefficient for the necessary definitive studies. Nevertheless, clearly at least some of the p40-43 material is actin as judged by the criteria that some of the radiolabeled p40-43 co-migrates with Coomassie-stained actin on two-dimensional gels and that it binds to DNAase. That some actin may be radiolabeled is not surprising even though the chloroglycouril iodination procedure is thought to label only surface molecules (19), since cytoplasmic labeling may have occurred in the few (≤5%) nonviable cells. The resulting radiolabeled actin would be expected to bind to IgG immunoadsorbants (20). Actin has also been described as a cell surface constituent in some cells (21).

The identity of the p40-43 material that did not co-electrophorese with actin is currently a mystery. Most of the experiments indicated that this material (p40) moved in SDS with a slightly greater apparent molecular weight than actin. Moreover, according to the two-dimensional gels, a significant fraction, often a majority, of the p40 material had isoelectric points far more basic than actin. Multiple forms of actin have been described but none >0.1 pH unit more basic than β plus γ actin (22). Artifacts known to produce charge heterogeneity have not been evaluated in the present study, although several such as deamidation, carbamylation by cyanate in urea, and oxidation of cysteine to cysteic acid all produce artifactual spots in the acidic direction (22). Despite the observations that the p40 material is too basic and too fast-moving in SDS to be actin, it is clear that the entire p40-43 band (and spots) was removed by passage over Sepharose-DNAase. Useful speculation includes the possibilities that these p40 spots represent either FcR subunits or perhaps transducers of receptor function similar to the 42,000 Mr guanosylnucleotide-binding protein (23).

The first step in analyzing the structure of p72 is to determine whether it is a single protein. Although electrophoresis in SDS of p72 showed multiple bands when the radioactivity in sliced gels was counted, two-dimensional gels revealed a long row of spots, seen with both U937 and monocytes, that resolved to one or two spots at the basic end of the gel upon treatment of the lysate or the intact cells with neuraminidase or upon growth of the cells in tunicamycin. Although the mobility of p72 in SDS remained broad after either tunicamycin or neuraminidase, p72 likely consists of a single protein bearing multiple and various glycosyl residues. A high content of carbohydrate in FcR molecules is not an unusual finding. Other reports of FcR isolation describe broad bands in SDS-PAGE which when evaluated critically show either a multiplicity of spots by IEF, spots that are more basic after digestion with neuraminidase, or a high content of carbohydrate by direct assay (cited in ref. 3).

An important aspect of these experiments, anticipated from the ligand binding studies (4, 5), is that the molecular details of the FcR isolated from both U937 cells and purified human monocytes are quite similar. Although p72 from monocytes seems to move in SDS with a slightly greater apparent molecular weight, the patterns of charge microheterogeneity of p72 from the two cells were nearly identical. Moreover, purified FcR from the two cells could not be distinguished by the amount or pattern of p40-43 material. Thus, U937 remains a useful model of human monocyte FcR structure.

Summary

The Fc receptors for IgG from a human monocyte line (U937) and from highly purified human peripheral blood monocytes were solubilized, purified, and partially
characterized. Both sources of cells gave indistinguishable results. Two molecules (or sets of molecules), one of about 72,000 mol wt and the other of 40,000–43,000 mol wt were discerned on autoradiograms of sodium dodecyl sulfate (SDS)-polyacrylamide gels analyzing acid eluates from Sepharose-IgG columns over which detergent lysates of radioiodinated cells had been passed. The larger of the two molecules, p72, accounted for \( \geq 90\% \) of the radioactivity. This component was noted to be heterodisperse both by size on SDS gels and by charge on isoelectric focusing gels. The charge heterogeneity, being virtually eliminated by neuraminidase and tunicamycin, was probably due to variable glycosylation. Several lines of evidence indicated that p72 is probably all or part of the Fc receptor: (a) radiolabeling of this molecule using chloroglycouril was blocked by occupation by IgG of the Fc receptor; (b) in soluble form this molecule expressed ligand specificity identical to the in situ receptor; (c) the molecule was not recovered from affinity adsorbants bearing proteins that do not bind to the Fc receptors, nor (d) from a human T cell line that does not bear Fc receptors. The smaller of the two molecules isolated, p40–43, is at least in part actin. Its relationship to p72 is not understood.

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