

Isolation and Partial Characterization of Fc_γ-Binding Proteins of Human Leukocytes

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ABSTRACT We isolated and partially characterized three Fc-binding macromolecules from human leukocytes. Mononuclear cells from normal individuals and from five patients with chronic lymphocytic leukemia and neutrophils from normal donors were surface radiolabeled by using ¹²⁵I and lactoperoxidase. After detergent solubilization of the cells, Fc_γ-binding macromolecules were purified by repetitive affinity chromatography under a variety of conditions and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

Three radiolabeled macromolecules were isolated that retained specific ability to bind to Fc fragments. A 52,000–64,000-mol wt macromolecule was isolated from normal mononuclear and polymorphonuclear cells. A 43,000-mol wt band was characteristic of mononuclear cells, particularly from patients with chronic lymphocytic leukemia. A 33,000-mol wt molecule could be obtained from normal leukocytes under conditions that suggest it might be a proteolytic fragment.

INTRODUCTION

Cell surface receptors that bind the Fc portion of IgG (Fc_γ receptors)¹ are present on a variety of cells including lymphocytes (1–3), monocytes (4, 5), and neutrophils (1, 5). Fc_γ receptors are importantly involved in a variety of cell functions such as antibody-dependent cellular cytotoxicity (6) and the binding and phagocytosis of opsonized particles (7). Fc_γ receptors

may also be involved in the regulation of immune responses (8) and in intracellular penetration of autoantibodies (9).

Studies on the isolation and characterization of Fc_γ-binding proteins² have mainly used Fc_γ receptor-bearing cells from experimental animals (10–15) or from cell lines (15–23) and numerous, apparently different, molecules have been characterized. Rarely have Fc_γ-binding macromolecules been isolated from human peripheral blood leukocytes, either from normal individuals or from patients, and characterized by polyacrylamide gel electrophoresis (PAGE) in sodium dodecyl sulfate. One group has isolated both 120,000- and 60,000-mol wt Fc_γ-binding proteins from normal human mononuclear cells (24) and human T lymphocytes (25). But in other studies 28,000- or 30,000-mol wt Fc_γ-binding proteins were purified from patients with chronic lymphocytic leukemia (26, 27).

In this study, we have used an efficient radioiodination method (28) and a repetitive affinity chromatography procedure (12, 23) to isolate radiolabeled human Fc_γ-binding macromolecules. We have compared properties of Fc_γ receptors from normal mononuclear cells with Fc_γ receptors from the mononuclear cells of five patients with chronic lymphocytic leukemia and with Fc_γ receptors from normal neutrophils. We isolated a broad 52,000–64,000-mol wt band from the normal individuals' mononuclear and polymorphonuclear cells. We purified a more distinct 43,000-mol wt band from mononuclear cells of both normal individuals and patients with chronic lymphocytic leukemia. A 33,000-mol wt band, which retained Fc_γ-binding ability, could be obtained from most cells under conditions that suggest that it might be a proteolytic fragment.

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¹ *Abbreviations used in this paper:* BBS, 0.2 M borate, 0.15 M NaCl, pH 8 buffer; BSA, bovine serum albumin; DNase, deoxyribonuclease I; Fc_γ, receptors, receptors for the Fc portion of IgG; NP-40, Nonidet P40; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethyl sulfonyl fluoride; p33, 33,000 mol wt protein; p43, 43,000 mol wt protein; and p52–64, protein(s) in the 52,000- to 64,000-mol wt range.

² In this paper we interchangeably refer to Fc_γ-binding macromolecules and Fc_γ receptors. Definitive evidence that any isolated Fc_γ-binding macromolecule is a functional Fc_γ receptor has not yet been demonstrated.

METHODS

Isolation of leukocytes. Peripheral venous blood of normal donors, 400 ml, was collected into 100 ml of 0.15 M NaCl containing 6% (wt/vol) dextran and 5,000 U of heparin. Following dextran sedimentation of erythrocytes (at 37°C for 40 min) the leukocyte-rich plasma was layered onto Ficoll-Hypaque density gradients (29). After centrifugation at 1,600 g for 12 min, the interface (typically containing 70–85% lymphocytes and 10–25% monocytes) was recovered and was washed twice in 0.01 M phosphate-buffered saline, pH 7.4. Leukocytes that pelleted through the gradient (typically 90–95% neutrophils) were similarly washed and contaminating erythrocytes were removed by hypotonic (0.03 M NaCl) lysis for 20 s. An average of 4×10^8 mononuclear cells and 8×10^8 polymorphonuclear cells were obtained from each donor and >95% of cells were viable.

Leukocytes were purified in a similar manner from the blood (10–50 ml) of five patients with chronic lymphocytic leukemia. In three experiments with normal donors, monocytes were depleted by incubating iron carbonyl particles with the heparinized blood-dextran mixture at 37°C as described (30) before erythrocyte sedimentation. In two experiments, fresh normal thymus tissue, obtained from a 6-mo-old patient and 18-mo-old patient undergoing open heart surgery, were minced in RPMI 1640 media, and the cell suspensions were layered onto Ficoll-Hypaque gradients. Thymocytes isolated from the interface were >95% small lymphocytes and were ~60% viable.

Lymphocytes and neutrophils were identified by using Jenner-Giemsa staining of cytocentrifuged cells. Monocytes were identified by using the 2,7-fluorenyldiamine procedure (31). In one experiment, cells that bound a mouse monoclonal anti-(human) monocyte antibody obtained from Bethesda Research Laboratories (Bethesda, Md.) were identified by using fluoresceinated antimouse antibody. In addition, esterase-positive cells were detected by staining with α -naphthyl acetate. Cell viability was assessed by exclusion of trypan blue dye.

Cell surface radioiodination. Cells were radiolabeled with ^{125}I using the modifications of the described lactoperoxidase method (28) except that each milliliter of iodination mixture contained 6×10^7 cells and 0.25 mCi of ^{125}I . The washed radiolabeled cells were solubilized at 0°C in 0.01 M phosphate, 0.15 M NaCl, pH 7.4 containing 1% (vol/vol) Nonidet P40 (NP-40) and 2 mM phenylmethyl sulfonyl fluoride (PMSF) as described (12, 23) with or without such proteinase inhibitors as: 3 mM EDTA, 10 mM diisofluorophosphate, one-third trypsin inhibitory U/ml aprotinin, 1 μM pepstatin, 1 mM 1,10-phenanthroline, and 100 mM iodoacetamide. After centrifugation at 30,000 g \times 20 min at 4°C, the supernatant fluid was used for affinity purification.

Immunoabsorbents. The preparation of immunoabsorbents with cyanogen bromide-activated Sepharose 2B was carried out as described (12) using the following proteins: human IgG, purified from fresh normal donor serum by $(\text{NH}_4)_2\text{SO}_4$ precipitation and gel filtration or purchased from N. L. Cappel Laboratories Inc. (Cochranville, Pa.); human (Fab) $_2$, human Fc, and human IgM, all from N. L. Cappel Laboratories Inc.; bovine serum albumin, egg albumin, and deoxyribonuclease I (DNase), all from Sigma Chemical Co. (St. Louis, Mo.). Human IgG-Sepharose contained 7–20 mg of protein per milliliter of packed beads; F(ab) $_2$ -Sepharose had 5.9 mg/ml beads; Fc-Sepharose had 5.6 mg/ml beads; and IgM-Sepharose had 2.7 mg/ml beads. Albumin-Sepharose immunoabsorbents contained 4–10 mg of protein per milliliter of beads. DNase-Sepharose contained 3.3 mg/ml of beads and had the capacity to bind at least 0.3 mg of rabbit actin (Sigma

Chemical Co.) per milliliter of immunoabsorbent. Before use the immunoabsorbents were incubated in 0.2 M borate 0.15 M NaCl buffer, pH 8 buffer (BBS) containing albumin (10 mg/ml) in order to minimize nonspecific binding (12) and were washed in BBS containing 1% NP-40.

Purification of Fc $_r$ receptor. All steps in receptor purification were carried out in a 4°C cold room unless specifically noted. Solubilized extracts of 3×10^8 – 2×10^9 radiolabeled cells were preincubated with 0.6 ml of an albumin-Sepharose immunoabsorbent for 30 min. The supernatant and a 1-ml wash with BBS-1% NP-40 containing the material unbound to albumin-Sepharose were then incubated with 0.6 ml of human IgG-Sepharose on a rotator overnight (unless otherwise noted). This immunoabsorbent was transferred to a 0.7 \times 4-cm glass column and washed with 24 ml of BBS containing 1% NP-40 (12, 23). The Fc $_r$ receptors were obtained in their active state by using elution with 0.5 N acetic acid containing 1% NP-40 followed by very rapid neutralization with 2 M Tris, pH 8.6, containing 1% NP-40 as described (12, 23).

Assay of receptor activity. The Fc $_r$ receptor preparations eluted from human IgG-Sepharose were tested for binding activity at 4°C by measuring the ability of radiolabeled receptor to rebound to fresh human IgG-Sepharose. Nonspecific binding was estimated both by using albumin-Sepharose as a control and by determining the rebounding to human IgG-Sepharose in the presence of an excess of soluble human IgG. The assay was performed in plastic tubes as described (12, 23) except that human IgG-Sepharose was used. This assay system is analogous to the assay of Fc $_r$ receptor activity (32), which gave results comparable to two other independent methods of measuring receptor activity. Tubes containing highly purified Fc $_r$ receptor (receptor that had rebound to human IgG-Sepharose) were saved for elution with 1 ml of 0.5 N acetic acid or 6 M guanidine, both containing 1% NP-40 (12) and eluted material was analyzed by SDS-PAGE. To investigate specificity other immunoabsorbents were used, or potential inhibitors such as chicken IgG (N. L. Cappel Laboratories Inc.) were mixed with human IgG-Sepharose before the addition of labeled receptor.

Gel electrophoresis. SDS-PAGE was performed according to the method of Laemmli (33) in gradient slab gels. Preparation of the 6–18% linear gradient of polyacrylamide, of the 3% stacking gel, and of the samples for electrophoresis have been described (34). Gels were fixed, stained, destained, and autoradiographed as described (34) except that 50% (vol/vol) methanol, 10% (vol/vol) acetic acid, 3% (vol/vol) glycerol was used before gel drying. Calibration of molecular weights was performed using described protein standards (34) as well as ovalbumin (mol wt, 43,000), aldolase (mol wt, 40,000), and rabbit glyceraldehyde-3-phosphate dehydrogenase (mol wt, 36,000).

RESULTS

Mononuclear cell Fc $_r$ -binding macromolecules. Three discrete radiolabeled Fc $_r$ -binding macromolecules were isolated from human mononuclear cells of normal donors by using human IgG-Sepharose immunoabsorbents (Fig. 1a). The apparent molecular weights of the Fc $_r$ -binding macromolecules were ~33,000 (p33), 43,000 (p43), and 52,000–64,000 (p52–64) upon analysis in SDS-polyacrylamide gels. The p52–64 band was extremely broad and heterodisperse compared with the other radiolabeled bands.

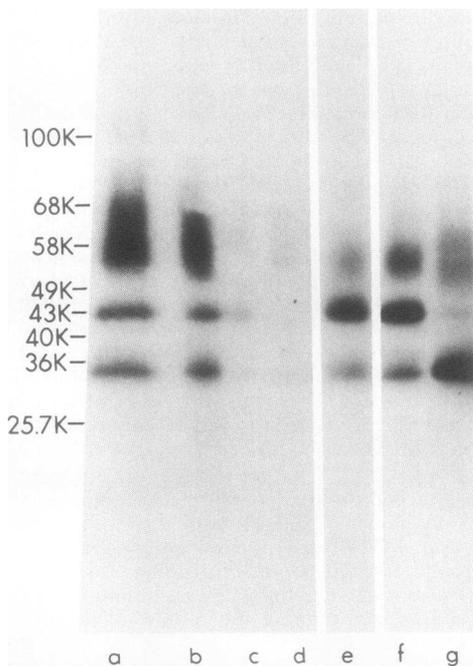


FIGURE 1 A composite of radioautographs illustrating general properties of mononuclear cell Fc_{γ} -binding macromolecules. (a) ^{125}I -labeled Fc_{γ} -binding molecules obtained from a single IgG-Sepharose affinity column. Neutralized aliquots from (a) were then incubated with: IgG-Sepharose (0.7 mg IgG bound to 0.1 ml beads), (b) without, or (c) with addition of 3 mg (4.3-fold excess) of soluble IgG. (d) A neutralized aliquot from (a) was incubated with BSA-Sepharose. Immunoabsorbents were washed and their eluates were analyzed by SDS-PAGE.

In a separate experiment the solubilized material from ^{125}I -labeled mononuclear cells was incubated with IgG-Sepharose (e, f) at 4°C, or (g) at 22°C. Track (e) was obtained from a slab gel run without mercaptoethanol, all other tracks in this and subsequent figures were from reduced SDS-gels.

When material eluted from IgG-Sepharose was neutralized and reincubated with fresh IgG-Sepharose, identical bands were again isolated indicating that some of their ability to bind ligand (IgG) had been retained (Fig. 1b). Specificity for IgG was demonstrated because only soluble IgG inhibited rebinding of these macromolecules (Fig. 1c, Table I below) and because such macromolecules did not rebind to albumin-Sepharose (Fig. 1d) or to F(ab) $'_2$ -Sepharose (Table I below). Similar gel patterns were obtained whether or not samples had been reduced with mercaptoethanol before SDS-polyacrylamide gel analysis (Fig. 1e, f).

The specificity of the affinity reagents was also assessed by comparing mononuclear cells with cell types that lack Fc receptors. Human erythrocytes (in two experiments) and human thymocytes (in two experiments) were radiolabeled and solubilized in parallel with mononuclear cells. Three discrete radiolabeled macromolecules (as described above) were ob-

tained only from the mononuclear cells by using repetitive affinity chromatography. Little radioactivity and no distinct bands were isolated from an equal number or fivefold greater number of labeled erythrocytes or thymocytes.

Quantitation of ligand-binding ability of mononuclear cell Fc_{γ} receptor. The ability of eluted Fc_{γ} receptor to specifically rebind to IgG was assayed by using various protein-Sepharose conjugates. In 12 experiments $52.2 \pm 4.0\%$ (mean \pm SEM) of the radiolabeled Fc_{γ} receptor preparation bound to human IgG-Sepharose. By comparison, only $16.4 \pm 2.2\%$ of labeled receptor bound to albumin-Sepharose ($P < 0.005$, paired t test). In 5 of these experiments an excess of soluble human IgG (3 mg) was mixed with IgG-Sepharose (0.1-ml beads coupled to 0.7–0.8 mg IgG), and only $18.4 \pm 2.5\%$ of radiolabeled Fc_{γ} receptor was bound.

Soluble human IgG inhibited receptor binding in a dose-dependent manner (Table I). Specificity was demonstrated because neither IgM, nor albumin, nor chicken IgG inhibited receptor binding, nor did receptor preparations bind to F(ab) $'_2$ -Sepharose or IgM-Sepharose (Table I). Furthermore, nonquantitative studies with mononuclear cell Fc_{γ} -binding macromolecules in two experiments demonstrated that each band bound to IgG-Sepharose and Fc_{γ} -Sepharose but not to F(ab) $'_2$ -Sepharose (not shown).

Evidence that p33 may be a fragment of a larger molecule. The relative yields of the three Fc_{γ} -binding molecules were related to the temperature at which solubilized mononuclear cell extracts and IgG-Sepharose were incubated. When the incubation was carried out overnight at 4°C the p43 radiolabeled band was more prominent than the p33 band (Fig. 1f). When the overnight incubation with immunoabsorbent was at 22°C the p43 band was much less evident than the p33 band (Fig. 1g). These findings suggested that the p33 band might be a fragment of either or both of the larger macromolecules, presumably resulting from endogenous proteinase activity.

One alternative possibility, viz. that p33 and p43 are unrelated molecules, each with a maximal binding affinity at different temperatures, is excluded by experiments such as the one shown in Fig. 2. Fc_{γ} -binding macromolecules were isolated by using a single IgG-Sepharose column at 4°C and then mixed with fresh IgG-Sepharose at different temperatures. Both the p43 and p33 macromolecules were rebound to immunoabsorbent at 4°C (Fig. 2d) approximately as well as at 22°C (Fig. 2e). However, when the mixture of Fc_{γ} -binding macromolecules and IgG-Sepharose also included a solubilized extract of unlabeled mononuclear cells (to approximate the protease content of the original solubilized cell suspension), less of the p43 band and correspondingly more of the p33 band were isolated at 4°C (Fig. 2b). This effect was extreme at

TABLE I
Percentage of Eluted Mononuclear Cell Fc_γ Receptor Rebinding to Immunoabsorbents*

Protein-Sephadex conjugate	Soluble proteins	Experiment no.			
		1	2	3	4
	mg protein				
BSA-Sephadex	—	25.3±0.7	13.7±1.1	20.6±2.4	25.9±1.0
IgG-Sephadex	—	56.4±0.9	50.8±9.9	67.6±1.3	66.3±2.2
IgG-Sephadex	IgG (3.0)		13.9±2.5		23.5±2.1
IgG-Sephadex	IgG (1.0)	25.9±2.0			33.0±1.5
IgG-Sephadex	IgG (0.3)				48.9±1.4
IgG-Sephadex	IgM (1.0)				65.4±4.2
IgG-Sephadex	BSA (3.0)				71.0±4.1
IgG-Sephadex	Chicken IgG (3.0)				67.7±3.9
F(ab) ₂ -Sephadex	—			27.1±3.4	
IgM-Sephadex	—				24.6±4.1

* Mean values of triplicate determinations ±SD are given. The total radioactivity added to each immunoabsorbent in experiments 1–4 was 6,719, 1,646, 2,106, and 1,927 cpm, respectively.

22°C (Fig. 2c), such that the p43 band was barely evident. In the latter case the isolated macromolecules resembled the Fc_γ-binding bands obtained from a single immunoabsorbent that had been incubated with labeled solubilized cell extracts only at 22°C (Fig. 2f and Fig. 1g).

Another variable that affected the relative yield of the three Fc_γ-binding macromolecules was the length of time that solubilized cell extracts were incubated with IgG-Sephadex (Fig. 3). In the briefest incubation periods (<4 h at 4°C) the p43 band appeared to be selectively isolated.

The inclusion of certain proteinase inhibitors in the solubilization buffer allowed for the recovery of more radioactivity in the p43 band than in the p33 band. In six experiments, the solubilization step was evaluated using PBS 1% NP-40, 2mM PMSF with or without a combination of aprotinin, iodoacetamide, and EDTA. As with lowering solubilization temperature, the proteinase inhibitors (especially the iodoacetamide) increased the amount of radiolabel associated with the p43 band while decreasing the label associated with p33 (Fig. 4). Additional proteinase inhibitors (phenanthroline, diisofluorophosphate, and pepstatin) did not further alter the observed pattern nor could any combination of inhibitors entirely avoid some copurification of the p33 band.

Fc_γ-binding macromolecules from human lymphocytes. Because both lymphocytes and monocytes were present in the "mononuclear cell preparations," the cell type that produced the p43 and p52–64 bands was not established. In three experiments, the peripheral blood was divided into two equal samples, one of which was incubated with iron carbonyl particles, which resulted in removal of >80% of the monocytes from mononuclear cell preparations. Then from both

samples, ¹²⁵I-labeled Fc_γ-binding macromolecules were isolated. The amount of Fc_γ receptor and its SDS-PAGE pattern were similar from each sample (not

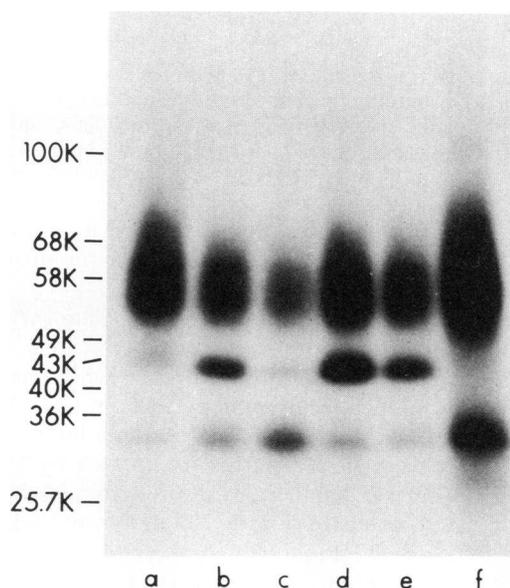


FIGURE 2 Effect of temperature on isolation of mononuclear cell Fc_γ-binding macromolecules. (a) ¹²⁵I-labeled Fc_γ-binding proteins obtained from a single affinity column (after incubation overnight at 4°C). Neutralized aliquots from (a) containing partially purified Fc_γ receptor were premixed (5 min at 4°C) with: (b, c) 1 ml containing solubilized mononuclear cells (10⁸ cell equivalents), or (d, e) 1 ml of solubilization buffer. These mixtures were then incubated with IgG-Sephadex overnight at 4°C (b, d), or at 22°C (c, e). Immunoabsorbents were washed and their eluates were analyzed by SDS-PAGE. (f) For comparison, Fc_γ binding macromolecules obtained from a single affinity column as in (a) except that incubation was at 22°C instead of 4°C.

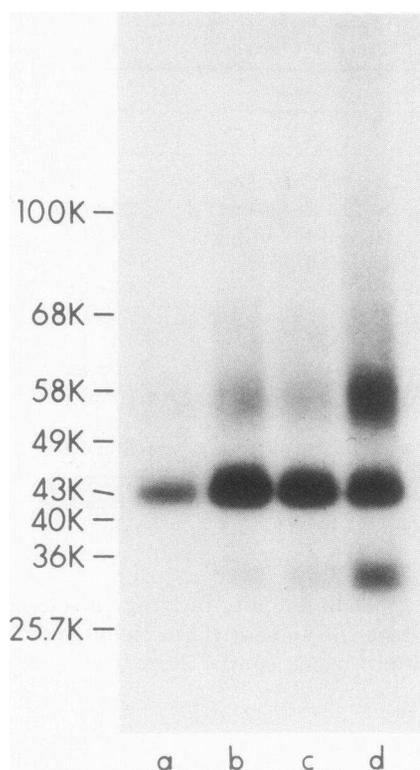


FIGURE 3 Effect of incubation time on isolation of mononuclear cell Fc_{γ} -binding macromolecules. Solubilized material from ^{125}I -labeled mononuclear cells was incubated (at $4^{\circ}C$) with IgG-Sepharose for: (a) 0.5 h, (b) 1.5 h, (c) 4 h, or (d) 18 h. Immunoabsorbents were washed and eluates were analyzed.

shown). Thus, it appeared that at least a large proportion of the labeled Fc_{γ} receptor in each of the discrete bands was derived from lymphocytes.

To identify which bands might be associated with B lymphocytes, similar studies were performed with mononuclear cells from five patients with chronic lymphocytic leukemia. Lymphocytes from two of the patients had been examined in immunofluorescent studies and were both positive for surface immunoglobulin. The p43 band was the predominant radiolabeled macromolecule (Fig. 5) isolated from each patient with chronic lymphocytic leukemia.

To determine whether the p43 band was similar to actin, Fc_{γ} -binding macromolecules were obtained from mononuclear cells of normal individuals by using a single IgG-Sepharose column and were incubated with Sepharose coupled to DNase. Although insolubilized DNase is known to bind actin specifically (35), the DNase-Sepharose did not bind Fc_{γ} receptor preparations, particularly not the p43 band. Furthermore, in four experiments mononuclear cell Fc_{γ} receptor was isolated by using replicate affinity columns in the usual manner and some columns received additional washes with 2 mM Hepes buffer (pH 7.4, containing 1% NP-40), which is known to remove bound actin (22,

36). However, no labeled receptor was removed (not shown). Based on these criteria the p43 band does not appear to be actin.

Fc $_{\gamma}$ -binding macromolecules from human neutrophils. Two discrete radiolabeled Fc_{γ} -binding macromolecules, p52-64 and p33, were isolated from normal human peripheral blood neutrophils (Fig. 6). A similar SDS-PAGE pattern was observed in 10 other experiments in which neutrophil purity was $>95\%$. Unlike mononuclear cells, purified neutrophils did not exhibit prominent p43 bands nor did proteinase inhibitors effectively inhibit the appearance of the p33 band (Fig. 6b). However, the isolated neutrophil Fc_{γ} -binding macromolecules appeared to have ligand-binding ability similar in specificity to mononuclear Fc_{γ} receptor (Table II).

DISCUSSION

The results of this study conclusively demonstrate that three radiolabeled Fc_{γ} -binding macromolecules can be isolated from peripheral human leukocytes. We have also shown that different macromolecules or combinations of molecules may be isolated depending on such variables as the cell type, the temperature and time of the incubation of solubilized cell extracts with

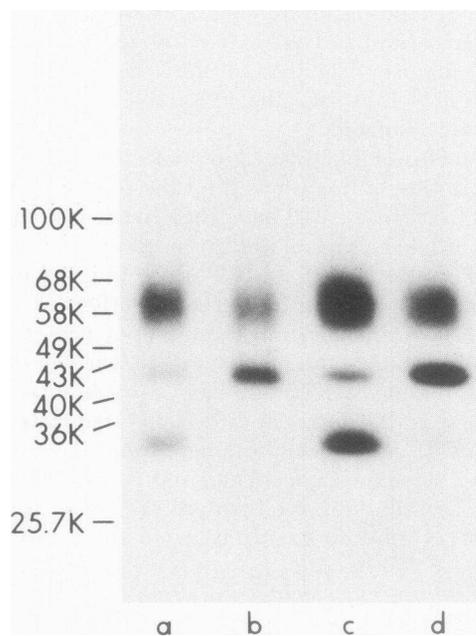


FIGURE 4 Effect of proteinase inhibitors on isolation of mononuclear cell Fc_{γ} -binding macromolecules. ^{125}I -labeled mononuclear cells were solubilized in PBS containing 1% NP-40 and 2 mM PMSF (a) without additional proteinase inhibitors, or (b) with EDTA, aprotinin, and iodoacetamide. Eluted material from a single affinity column is shown. (c) Repetitive affinity chromatography of material from (a). (d) Repetitive affinity chromatography of material from (b). All samples were run on the same gel at the same time.

the initial IgG-Sepharose affinity column, and the choice of proteinase inhibitors. Attention to these variables would partially solve the central problem in the study of isolated Fc_γ receptors, namely, the diversity of macromolecules that have been proposed as Fc receptors (10–27).

The broad p52–64 band was obtained from neutrophils, from mononuclear cell preparations containing lymphocytes and monocytes, and from mononuclear cell preparations depleted of monocytes. Relatively little of the p52–64 band was obtained from B cells of patients with chronic lymphocytic leukemia. It appears that B cells, at least leukemic B cells, express relatively less p52–64 protein and more p43 macromolecules than other cell types. The p52–64 band is similar in size and apparent heterogeneity to some of the previously identified Fc_γ-binding macromolecules of mononuclear and macrophage-like cells (12, 14, 15, 18, 21, 24) and to Fc_ε receptors (34, 37–42). It is interesting to note that for the Fc_ε receptor the bulk of the apparent heterogeneity results from N-linked oligosaccharides (42). Some groups have resolved two bands within the broad bands characterized as Fc_γ receptors (21) and Fc_ε receptors (39).

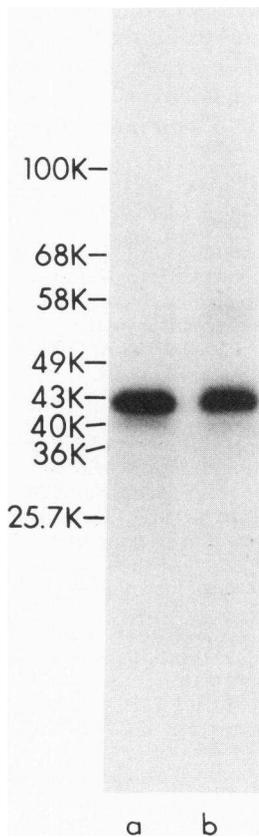


FIGURE 5 Fc_γ-binding macromolecules obtained from lymphocytes of two different patients with chronic lymphocytic leukemia, by using single affinity columns.

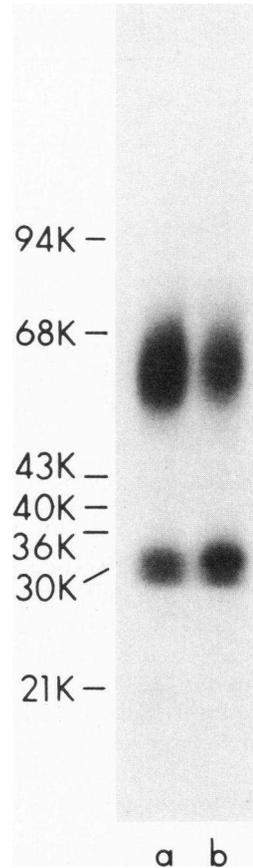


FIGURE 6 Polymorphonuclear cell Fc_γ-binding macromolecules obtained by repetitive affinity chromatography. ¹²⁵I-labeled neutrophils were solubilized in PBS containing 1% NP-40 and 2 mM PMSF (a) without additional proteinase inhibitors, or (b) with EDTA, aprotinin, pepstatin, and iodoacetamide.

The relatively narrow p43 band was derived from normal mononuclear cell preparations and from B cells of chronic lymphocytic leukemia patients. Removal of monocytes from preparations by using iron-carbonyl particles did not alter the relative intensity of the p43 band. This is consistent with the proposal that the p43 band is predominantly derived from B lymphocytes. Previous studies indicate that actin (43,000 mol wt) can be present on lymphocyte surfaces (43) and that actin may interact with IgG (44). In our study the p43 band does not resemble actin—at least as it has been previously characterized (22, 35, 36).

The p33 Fc_γ-binding macromolecule obtained from mononuclear cells appears to be a proteolytic fragment because its yield is increased in the absence of aprotinin, iodoacetamide, and EDTA and by incubation for longer periods or higher temperatures. Further studies are required to determine whether the p33 band is derived from the p43 band, the p52–64 band, or from both. Studies with incubation temperature and pro-

TABLE II
Percentage of Eluted Neutrophil Fc_γ Receptor Rebinding to Immunoabsorbents*

Protein-Sephadex conjugate	Soluble proteins	Experiment no.			
		1	2	3	4
	<i>mg protein</i>				
BSA-Sephadex	—	12.5±0.6	12.2±2.0	16.3±2.4	24.4±3.2
IgG-Sephadex	—	64.3±1.3	36.1±9.0	54.9±1.6	41.9±0.1
IgG-Sephadex	IgG (3.0)		12.6±0.9		19.9±0.2
IgG-Sephadex	IgG (1.0)	24.4±4.7			24.4±2.3
IgG-Sephadex	IgG (0.3)				33.3±1.7
IgG-Sephadex	IgM (1.0)				41.9±2.0
IgG-Sephadex	BSA (3.0)				41.1±2.4
IgG-Sephadex	Chicken IgG (3.0)				38.3±2.8
F(ab) ₂ '-Sephadex	—			16.6±1.2	
IgM-Sephadex	—				18.5±1.7

* Mean values of triplicate determinations ±SD are given. The total radioactivity added to each immunoabsorbent in experiments 1–4 was 4,383, 21,146, 3,903, and 5,324 cpm, respectively.

teinase inhibitors (Figs. 2 and 4) suggest an inverse relationship between the p33 and p43 bands; however, little or no p33 band was obtained from chronic lymphocytic leukemia cells (Fig. 5). Fc_γ-binding macromolecules in the 33,000-mol wt region were always obtained from polymorphonuclear cells.³ Neutrophils contain a large amount and variety of proteolytic enzymes and presumably proteolysis occurs either before solubilization or in spite of proteinase inhibitors during solubilization.

We have not isolated macromolecules smaller than 33,000 daltons as reported in previous studies with mononuclear cells of normal donors (24) and chronic lymphocytic leukemia patients (26, 27). We suspect that the previous studies may have proteolytically degraded that Fc_γ receptor because cell lysates were incubated at 37°C (24, 26) or were subjected to freezing and thawing and overnight dialysis (27).

Several groups have used antigen-antibody precipitates or aggregated IgG coupled to Sephadex for isolation of Fc_γ receptors from human and animal cells, and they obtain macromolecules in the 100,000–130,000-mol wt range (11, 13, 20, 24, 25). In those studies, however, “control” immunoprecipitates and aggregates formed with F(ab)₂ fragments may have been significantly smaller than those formed with IgG, allowing some doubt as to specificity of the putative receptor. Such large macromolecules have not been obtained when Fc_γ-binding macromolecules were purified by using unaggregated IgG coupled to Sephadex (12, 18, 21, 23) or by using an antibody to Fc_γ receptor (15).

In this study specificity is documented because

³ The present experiments do not establish whether the 33,000-mol wt molecules from neutrophils are identical to the 33,000-mol wt macromolecules obtained from mononuclear cells.

radiolabeled Fc_γ-binding macromolecules bind to IgG-Sephadex and, in some studies, to Fc_γ-Sephadex but not to F(ab)₂'-Sephadex nor to other immunoabsorbents. Soluble IgG interacts with soluble receptor as assayed by inhibition of subsequent binding to immunoabsorbents, but F(ab)₂ fragments, a non-mammalian IgG, and other proteins do not. This study demonstrates that three macromolecules that retain ligand binding activity for Fc_γ fragments can be isolated from human leukocytes and further investigation is required to determine their functional significance.

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