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

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A B S T R A C T 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, Fcγ-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 (Fcγ receptors)1 are present on a variety of cells including lymphocytes (1-3), monocytes (4, 5), and neutrophils (1, 5). Fcγ 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). Fcγ 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 Fcγ-binding proteins2 have mainly used Fcγ receptor-bearing cells from experimental animals (10-15) or from cell lines (15-23) and numerous, apparently different, molecules have been characterized. Rarely have Fcγ-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 Fcγ-binding proteins from normal human mononuclear cells (24) and human T lymphocytes (25). But in other studies 28,000- or 30,000-mol wt Fcγ-binding proteins were purified from patients with chronic lymphocytic leukemia (26, 27).

In this study, we have used an efficient radiiodination method (28) and a repetitive affinity chromatography procedure (12, 23) to isolate radiolabeled human Fcγ-binding macromolecules. We have compared properties of Fcγ receptors from normal mononuclear cells with Fcγ receptors from the mononuclear cells of five patients with chronic lymphocytic leukemia and with Fcγ 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 Fcγ-binding ability, could be obtained from most cells under conditions that suggest it might be a proteolytic fragment.

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1Abbreviations used in this paper: BBS, 0.2 M borate, 0.15 M NaCl, pH 8 buffer; BSA, bovine serum albumin; DNase, deoxyribonuclease I; Fcγ receptors, receptors for the Fc portion of IgG; NP-40, Nonidet P40; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethyl sulfonil 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.

2In this paper we interchangeably refer to Fcγ-binding macromolecules and Fcγ receptors. Definitive evidence that any isolated Fcγ-binding macromolecule is a functional Fcγ 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 × 10^7 mononuclear cells and 8 × 10^9 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 cytospun unfixed cells. Monocytes were identified by using the 2,7-fluorenediamine procedure (31). In one experiment, cells that bound a mouse monoclonal anti-human monococyte 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 125I 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 125I. 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 diisulfophosphorafate, one-third trypsin inhibitory U/ml aprotinin, 1 μM pepstatin, 1 mM 1,10-phenanthroline, and 100 mM iodosacamide. After centrifugation at 30,000 g × 20 min at 4°C, the supernatant fluid was used for affinity purification.

Immunoadsorbents. The preparation of immunoadsorbents 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 (NH₄)₂SO₄ precipitation and gel filtration or purchased from N. L. Cappel Laboratories Inc. (Cochraneville, 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; Fab(α)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 immunoadsorbents 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 immunoadsorbent. Before use the immunoadsorbents 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 Fc₆ receptor. All steps in receptor purification were carried out in a 4°C cold room unless specifically noted. Solubilized extracts of 3 × 10^7–2 × 10^8 radiolabeled cells were preincubated with 0.6 ml of an albumin-Sepharose immunoadsorbent for 30 min. The supernatant and a 1-ml wash with BBS containing albumin 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 immunoadsorbent was transferred to a 0.7 × 4-cm glass column and washed with 10 ml of BBS containing 1% NP-40 (12, 23). The Fc₆ 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 Fc₆ receptor preparations eluted from human IgG-Sepharose were tested for binding activity at 4°C by measuring the ability of radiolabeled receptor to rebind to fresh human IgG-Sepharose. Nonspecific binding was estimated both by using albumin-Sepharose as a control and by determining the rebinding 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 Fc₆ receptor activity (32), which gave results comparable to the other independent methods of measuring receptor activity. Tubes containing highly purified Fc₆ 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 immunoadsorbents 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 Fc₆-binding macromolecules. Three discrete radiolabeled Fc₆-binding macromolecules were isolated from human mononuclear cells of normal donors by using human IgG-Sepharose immunoadsorbents (Fig. 1a). The apparent molecular weights of the Fc₆-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.
IgG bound single FIGURE 1

tralized by obtained bands this and from macromolecules (Fig. Sepharose (e, some of these below)).

experiments) were assayed by comparing the specificity of 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, receptor. The ability of eluted Fc, receptor to specifically rebind to IgG was assayed by using various protein-Sepharose conjugates. In 12 experiments 52.2±4.0% (mean±SEM) of the radiolabeled Fc, receptor preparation bound to human IgG-Sepharose. By comparison, only 16.4±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±2.5% of radiolabeled Fc, 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, binding macromolecules in two experiments demonstrated that each band bound to IgG-Sepharose and Fc, 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, 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 immunoadsorbant 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, 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 immunoadsorbent at 4°C (Fig. 2d) approximately as well as at 22°C (Fig. 2e). However, when the mixture of Fc, 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

FIGURE 1 A composite of radioautographs illustrating general properties of mononuclear cell Fc, binding macromolecules. (a) 125I-labeled Fc, 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. Immunoadsorbents were washed and their eluates were analyzed by SDS-PAGE.

In a separate experiment the solubilized material from 125I-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-

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22°C (Fig. 2c), such that the p43 band was barely evident. In the latter case the isolated macromolecules resembled the Fcγ-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 Fcγ-binding macromolecules was the length of time that solubilized cell extracts were incubated with IgG-Sepharose (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.

**Fcγ-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 Fcγ-binding macromolecules were isolated. The amount of Fcγ receptor and its SDS-PAGE pattern were similar from each sample (not

**TABLE I**
Percentage of Eluted Mononuclear Cell Fcγ, Receptor Rebinding to Immunoadsorbents*

<table>
<thead>
<tr>
<th>Protein-Sepharose conjugate</th>
<th>Soluble proteins</th>
<th>Experiment no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg protein</td>
<td>1</td>
</tr>
<tr>
<td>BSA-Sepharose</td>
<td>—</td>
<td>25.3±0.7</td>
</tr>
<tr>
<td>IgG-Sepharose</td>
<td>—</td>
<td>56.4±0.9</td>
</tr>
<tr>
<td>IgG-Sepharose</td>
<td>IgG (3.0)</td>
<td>13.9±2.5</td>
</tr>
<tr>
<td>IgG-Sepharose</td>
<td>IgG (1.0)</td>
<td>25.9±2.0</td>
</tr>
<tr>
<td>IgG-Sepharose</td>
<td>IgG (0.3)</td>
<td></td>
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<tr>
<td>IgG-Sepharose</td>
<td>IgM (1.0)</td>
<td></td>
</tr>
<tr>
<td>IgG-Sepharose</td>
<td>BSA (3.0)</td>
<td></td>
</tr>
<tr>
<td>IgG-Sepharose</td>
<td>Chicken IgG (3.0)</td>
<td>71.0±4.1</td>
</tr>
<tr>
<td>F(ab)′γ-Sepharose</td>
<td></td>
<td>27.1±3.4</td>
</tr>
<tr>
<td>IgM-Sepharose</td>
<td></td>
<td>24.6±4.1</td>
</tr>
</tbody>
</table>

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

**FIGURE 2** Effect of temperature on isolation of mononuclear cell Fcγ-binding macromolecules. (a) 125I-labeled Fcγ-binding proteins obtained from a single affinity column (after incubation overnight at 4°C). Neutralized aliquots from (a) containing partially purified Fcγ receptor were preincubated (5 min at 4°C) with: (b, c) 1 ml containing solubilized mononuclear cells (10⁶ cell equivalents), or (d, e) 1 ml of solubilization buffer. These mixtures were then incubated with IgG-Sepharose overnight at 4°C (b, d), or at 22°C (c, e). Immunoadsorbents were washed and their eluates were analyzed by SDS-PAGE. (f) For comparison, Fcγ binding macromolecules obtained from a single affinity column as in (a) except that incubation was at 22°C instead of 4°C.

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bands 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 Fcγ-binding macromolecules appeared to have ligand-binding ability similar in specificity to mononuclear Fcγ receptor (Table II).

DISCUSSION

The results of this study conclusively demonstrate that three radiolabeled Fcγ-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 3](image1.png)  
**Figure 3** Effect of incubation time on isolation of mononuclear cell Fcγ-binding macromolecules. Solubilized material from 125I-labeled mononuclear cells was incubated (at 4°C) with IgG-Sepharose for: (a) 0.5 h, (b) 1.5 h, (c) 4 h, or (d) 18 h. Immunoadsorbents were washed and eluates were analyzed. (not shown). Thus, it appeared that at least a large proportion of the labeled Fcγ 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, Fcγ-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 Fcγ receptor preparations, particularly not the p43 band. Furthermore, in four experiments mononuclear cell Fcγ 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.

Fcγ-binding macromolecules from human neutrophils. Two discrete radiolabeled Fcγ-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 Fcγ-binding macromolecules appeared to have ligand-binding ability similar in specificity to mononuclear Fcγ receptor (Table II).
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, 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_y-binding macromolecules of mononuclear and macrophage-like cells (12, 14, 15, 18, 21, 24) and to Fc, receptors (34, 37-42). It is interesting to note that for the Fc, 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, receptors (21) and Fc, receptors (39).

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, 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-
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, binding macromolecules in the 33,000-mol wt region were always obtained from polymorphonuclear cells. 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, 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 Sepharose for isolation of Fc, 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, binding macromolecules were purified by using unaggregated IgG coupled to Sepharose (12, 18, 21, 23) or by using an antibody to Fc, receptor (15).

In this study specificity is documented because radiolabeled Fc, binding macromolecules bind to IgG-Sepharose and, in some studies, to Fc, Sepharose but not to F(ab)’2-Sepharose nor to other immunoadsorbents. Soluble IgG interacts with soluble receptor as assayed by inhibition of subsequent binding to immunoadsorbents, 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, fragments can be isolated from human leukocytes and further investigation is required to determine their functional significance.

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