IDENTIFICATION OF A SECOND CLASS OF IgG Fc RECEPTORS ON HUMAN NEUTROPHILS
A 40 Kilodalton Molecule also Found on Eosinophils

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Neutrophilic granulocytes, upon interacting with IgG immune complexes, respond in a variety of ways, including phagocytosis of the bound particles (1, 2), generation of a respiratory burst producing superoxide (O2-) and other reactive oxygen species (3), degranulation (4, 5) and ADCC (6, 7). Because the initiation of these events follows immune complex binding to plasma membrane FcR, analysis of the molecular nature of the FcR is fundamental to understanding these processes. An FcR for IgG on human neutrophils has been identified using an mAb (3G8) that inhibits IgG binding to the cells and precipitates a 51–73 kD molecule (8). Eosinophils, by contrast, bear a 43 kD FcR, which has been purified by affinity adsorption to IgG (9).

We have recently developed an mAb (IV3) that identifies on human monocytes, platelets, U937, and K562 cells a 40 kD FcR with low affinity for IgG (10–12), distinct from the 72 kD high-affinity FcR described on monocytes (13, 14), and distinct from the FcR on neutrophils recognized by mAb 3G8 (8). This antibody inhibits ligand binding to the 40 kD receptor, and on platelets, when crosslinked with a second antibody, causes platelet aggregation (12). Herein, we show that this antibody identifies a second class of FcR on human neutrophils, distinct from the molecule recognized by 3G8. The antibody also binds to eosinophils.

Materials and Methods

Chemicals and Reagents. Cytochrome C Type VI, superoxide dismutase, cytochalasin B, pepstatin, chymostatin, leupeptin, antipain, rabbit muscle actin, PMSF, and diisopropylfluorophosphate (DFP) were purchased from Sigma Chemical Co., St. Louis, MO; Dextran T500, Ficoll-Paque, Sepharose 4 B, CNBr-activated Sepharose, protein A–Sepharose CL-4 B from Pharmacia Fine Chemicals, Piscataway, NJ; ox erythrocytes from Colorado Serum Co., Denver, CO; tetanus toxin and papain from Calbiochem-Behring, La Jolla, CA; human anti–tetanus toxin antibody (Hyper-Tet) from Cutter Laboratories, Berkeley, CA; chloroglycouril from Pierce Chemical Co., Rockford, IL; carrier-free Na125I

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(IMS.300) from Amersham Corp., Arlington Heights, IL; affinity-purified goat F(ab')2 anti-murine Ig (anti-mIg), both fluoresceinated (FITC) and not, from Cappel Laboratories, Cockeysville, PA; RPMI 1640 from Gibco Laboratories, Grand Island, NY; FCS from Sterile Systems, Logan, UT; and a mixture of low molecular weight markers from Biorad Laboratories, Richmond, CA. Other chemicals were of analytical grade and were obtained commercially.

NP-40 lysis buffer contained 1% NP-40, 20 mM Tris, 110 mM NaCl, 10 mM EDTA, 2 mM PMSF, 10 µg/ml pepstatin, 10 µg/ml chymostatin, 10 µg/ml leupeptin, 10 µg/ml antipain, pH 7.1. Krebs Ringer phosphate (KRP) buffer consisted of 130 mM NaCl, 5 mM KCl, 1.2 mM MgSO4, 1 mM CaCl2, and 10 mM sodium phosphate buffer, pH 7.4. PBS was 145 mM NaCl in 20 mM phosphate buffer, pH 7.0. PBS-K contained 130 mM NaCl and 5 mM KCl in 10 mM phosphate buffer, pH 7.4.

The preparation and properties of mAb IV3 have been described (10-12). IV3 was used either as supernatant fluid from the culture of cloned cells or as IgG purified from ascites fluid by (NH4)2SO4 precipitation and ion-exchange chromatography. Fab fragments were made by digesting a 5 mg/ml solution in PBS containing 10 mM cysteine and 5 mM EDTA with papain (1:50 papain/protein) for 30 min at 37°C, and acetylating with 20 mM iodoacetamide. The digest was passed over a protein A-Sepharose column and was analyzed by SDS-PAGE. No intact IgG remained. IgG fractions of murine or myeloma mAb SGB (IgG1) (8), MOPC 141 (IgG2b), and anti-VK3b (IgG2b) (15) were purified from ascites fluids by ion-exchange chromatography. Purified IgG from mAb AML-2-23 (IgG2b) was generously provided by Dr. Michael Fanger, Dartmouth Medical School, Hanover, NH (16).

Immune complexes containing human IgG were prepared by incubating tetanus toxin (200 Lf/ml) with Hyper-Tet (200 Lf/ml) for 1 h at 37°C. Insoluble complexes were pelleted by centrifugation at 13,000 g for 1 min, washed once with PBS-K, and resuspended in the original volume of PBS-K. Protein concentration was determined by adding 0.1 M NaOH and measuring absorbance at 280 nm, assuming an extinction coefficient (1%) of 14. Pooled human IgG purified by ion-exchange chromatography was covalently linked to Sepharose 4B by a modification of the CNBr technique (17) at 7.6 mg IgG/ml Sepharose. Anti-mIg was linked to CNBr-activated Sepharose according to the manufacturer’s instructions at 1 mg protein/ml Sepharose. Before use, the Sepharose adsorbants were washed four times with 1% NP-40 in PBS containing 5 mM KI.

Cells. Human granulocytes were obtained from the peripheral blood of normal donors by separation from mononuclear cells on Ficol-Hypaque, sedimentation of erythrocytes using 3% dextran in PBS, and finally hypotonic lysis of residual erythrocytes. Preparations were >98% granulocytes. Most donors had normal numbers of eosinophils; the two used for the experiments of Fig. 1 and Table I had mild eosinophilia secondary to allergic rhinitis. Eosinophils for the experiments of Table II were obtained from the peripheral blood of a patient with idiopathic hypereosinophilic syndrome by dextran sedimentation and hypotonic lysis of erythrocytes. K562 cells, a myeloblastic line (18), were grown in stationary cultures as described (10). The cells were washed three times in PBS or PBS-K before use.

Radiolabeling and Affinity Adsorption. Granulocytes and K562 cells were surface-radioiodinated by the chloroglycouril method (19). 0.7 ml cells in PBS at 1.43 × 10^7 cells/ml were incubated with 1 mCi ¹²⁵I for 30 min at 0°C in a scintillation vial coated with 5 µg chloroglycouril. The reaction was quenched and the cells were washed three times with 5 mM KI in PBS. The cells were then lysed in NP-40 lysis buffer for 30 min at 0°C. For some experiments 2 mM DFP was added to the lysis buffer. Cell nuclei and other insoluble material were pelleted by centrifugation at 7,800 g for 20 min. Portions of labeled cell lysate (50 µl) were incubated for 1 h at 0°C with 50 µl mAb (IV3 supernatant, 3G8 IgG at 10 µg/ml, or a murine IgG2b myeloma, MOPC141, at 10 µg/ml), and then with 25 µl Sepharose–anti-mIg. Separate 50-µl portions of lysate were incubated with 25 µl Sepharose–human IgG. The Sepharose conjugates were washed seven times with 0.75 ml 1% NP-40/PBS/KI, and were incubated for 2 min in a boiling water bath with 80 µl Laemmli sample buffer (20) containing 20 mM DTT instead of 2-ME. The supernatant
was acetylated by adding 5 μl 1 M iodoacetamide, and was analyzed by SDS-PAGE and autoradiography as described (14). The molecular weight markers included in all gels were 125I-BSA, 125I-rabbit muscle actin, phosphorylase B, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme.

**Fluorescence and Cell Sorting.** 10⁶ cells were incubated for 30 min at 4°C in 50 μl of antibody (either IV3 supernatant or 10 μg/ml solutions of purified IgG fractions of ascites fluid diluted in RPMI 1640 growth medium containing 10% FCS). The cells were washed three times in PBS containing 0.1% NaN₃, were resuspended and incubated for 30 min at 4°C in 50 μl FITC anti-mIg, and were washed a final three times. Freshly stained cells were analyzed on an EPICS C flow cytometer (Coulter EPICS Division, Hialeah, FL) using an argon laser at 500 mW power. Green fluorescence was collected through a 525-nm bandpass filter on 10,000–50,000 cells gated for low angle light scatter (to exclude erythrocytes, platelets, dead cells, and debris) and 90° light scatter (to exclude residual lymphocytes). Three-droplet sorting without coincidence correction at a flow rate of 300–500 cells/s was performed at a 32 kHz droplet-formation rate. The sorting logic was based on low-angle light scatter and green fluorescence alone, as some interference of the stream undulation with the 90° light scatter signal was observed. Cells were sorted into a 15-ml conical centrifuge tube containing 14 ml Ficoll/Hypaque and 0.5 ml RPMI 1640 culture medium with 10% FCS. After sorting, the supernatant layer of culture medium plus cells was aspirated, and the cells were deposited on a glass slide using a Shandon II cytocentrifuge. The cells were stained with Wright-Giemsa and were analyzed morphologically by light microscopy.

**Rosette Assays.** A 1% suspension of PBS-washed ox erythrocytes was incubated with a subagglutinating dose of the IgG fraction of a rabbit anti-ox erythrocyte antiserum. In a solution of PBS containing 5% heat-inactivated FCS, 25 μl of each antibody (40 μg/ml), 25 μl of cells (4 × 10⁶ cells/ml), and 50 μl of a 2% suspension of antibody-coated erythrocytes were incubated for 15 min at room temperature in V-bottom microtiter wells. The cells were pelleted at 50 g for 1 min and were incubated for 45 min at room temperature. Cells were resuspended and were evaluated microscopically at × 400 magnification in a hemocytometer for the number of erythrocytes bound to granulocytes. In some samples, granulocytes were so covered by bound erythrocytes that they could not be visualized; in these cases the total number of cells was assumed to be the mean for those samples where all granulocytes were visualized.

**Superoxide Generation Assay.** Continuous measurement of superoxide-dependent cytochrome C reduction (21, 22) was performed in a Varian DMS 100 double-beam spectrophotometer at 37°C. In the standard assay, both sample and reference cuvettes contained 2 × 10⁶ granulocytes and 100 nmol ferricytochrome C in 0.95 ml KRP buffer. Superoxide dismutase (20 μg) was present in the reference cuvette. All assay mixtures were first incubated with cytochalasin B (5 μl of 1 mg/ml DMSO solution) for 2 min in the presence or absence of inhibitors. 50 μl of immune complexes (175 μg) were added to both the sample and reference cuvette, and the absorbance change accompanying cytochrome C reduction was monitored at 550 nm. The rate of superoxide production was calculated from linear rate of absorbance change at 550 nm and the molar extinction coefficient for this reduction (21,000) (23).

**Results**

The capacity of mAb IV3 to bind to neutrophils and eosinophils was evaluated by flow cytometry. Peripheral blood granulocytes, incubated first with IV3 or 3G8 and then with FITC-anti-mIg, were analyzed for fluorescence with a flow cytometer. As seen in Fig. 1 b, all cells stained with IV3 were of uniform fluorescence and were considerably brighter (mean fluorescence was 42-fold brighter) than cells stained with a murine IgG2b (anti-Vδ3b), which has no specific affinity for granulocytes (Fig. 1 a). In contrast, granulocytes stained with 3G8 (Fig. 1 e) appeared as two populations, one with bright fluorescence and the
other with dim fluorescence, both considerably brighter than staining seen with anti-Vκ3b (mean fluorescence was, respectively, 358-fold and 9-fold brighter than with anti-Vκ3b).

In three experiments on two individuals with mild hypereosinophilia, these two populations of 3G8-stained cells were sorted, fixed onto glass slides, stained with Wright-Giemsa, and analyzed microscopically for cell type (Table I). Of the brightly fluorescent 3G8-stained cells, nearly all (mean, 96%) were neutrophils, whereas the dimly fluorescent 3G8-stained cells were virtually all eosinophils (mean, 98%). The percentage of eosinophils in the IV3-stained population of cells after staining and sorting was comparable to the percentage of eosinophils in the original unstained cell suspension (Table I, Exp. 2), indicating that the eosinophils were not lost during the experimental procedure. We conclude that IV3 binds to both neutrophils and eosinophils.

The nature of the molecule bound by IV3 on granulocytes was analyzed by affinity adsorption and PAGE. Purified granulocytes, predominantly neutrophils with <5% eosinophils, were radioiodinated and lysed in nonionic detergent with or without an additional proteinase inhibitor, diisopropylfluorophosphate (DFP). The lysates were incubated with affinity adsorbants consisting of Sepharose sensitized with human IgG, IV3, 3G8, or a murine IgG2b (MOPC141) (see
Table 1

Flow Cytometric Analysis of Granulocytes Stained with IV3 and 3G8

<table>
<thead>
<tr>
<th>Exp.</th>
<th>mAb*</th>
<th>Intensity of staining</th>
<th>Cell type</th>
<th>Cell population</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>—</td>
<td>unsorted</td>
<td>80</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV3</td>
<td>100% bright</td>
<td>bright</td>
<td>97</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3G8</td>
<td>77% bright</td>
<td>sorted</td>
<td>2</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>23% dim</td>
<td>dim</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>none</td>
<td>—</td>
<td>unsorted</td>
<td>93</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV3</td>
<td>100% bright</td>
<td>bright</td>
<td>92</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3G8</td>
<td>89% bright</td>
<td>sorted</td>
<td>92</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11% dim</td>
<td>dim</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>none</td>
<td>—</td>
<td>unsorted</td>
<td>71</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV3</td>
<td>100% bright</td>
<td>bright</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3G8</td>
<td>83% bright</td>
<td>sorted</td>
<td>1</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>17% dim</td>
<td>dim</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Purified granulocytes were incubated first with mAb IV3 or 3G8 and then with FITC-anti-mlg.
* Antibody-stained cells were analyzed for fluorescence intensity by flow cytometry.
§ Cells were sorted according to the brightness of their fluorescence. The cell types, mostly neutrophils or eosinophils, in the sorted population were determined microscopically after staining with Wright-Giemsa.

Materials and Methods). Adsorbant-bound proteins were eluted and analyzed by SDS-PAGE and autoradiography. Fig. 2 shows that 3G8 bound a heterodisperse molecule ranging in molecular mass from 51–73 kD, as described in an earlier publication (8); its size was not altered by the presence of DFP. IV3, in contrast, bound a protein of 40 kD in the presence of DFP, and a protein of 33 kD in the
absence of DFP. Immobilized human IgG appeared to bind the proteins purified by both 3G8 and IV3; namely, a broad-based band between 51 and 73 kD unaltered by the presence of DFP, along with a 33 kD band which in the presence of DFP appeared as a 40 kD band. Only background precipitation was seen with MOPC141.

The capacity of IV3 to interfere with an FcR-mediated granulocyte function was evaluated by rosette inhibition. As seen in Exp. 1 of Table II, IV3, when added alone, resulted in little or no inhibition of rosettes formed between granulocytes and rabbit IgG-sensitized ox erythrocytes, although K562 rosettes were completely inhibited. On the other hand, 3G8 resulted in nearly complete inhibition of rosette formation when a rosette was defined as $\geq 4$ RBC/cell, but less than full inhibition was noted if rosettes were defined as $\geq 1$ RBC/cell. Upon the addition of IV3 and 3G8 together, complete inhibition of rosette formation was noted. Exps. 2 and 3 (Table II) corroborate the results of Exp. 1, and further indicate that the combination of 3G8 plus other murine Ig of the same IgG2b subclass as IV3, either a myeloma protein (MOPC141) or an anti-granulocyte mAb (AML-2-23), do not duplicate the additive inhibitory effects of IV3 and 3G8; i.e., the combinations are no more inhibitory than 3G8 alone. Exp. 3, furthermore, indicates that Fab fragments of IV3 are as effective as the intact molecule, suggesting that the inhibitory effect is a direct result of Fab binding to the 40 kD molecule precipitated by IV3. Exp. 4 (Table II), performed with purified eosinophils, shows that rosettes formed with IgG-coated ox cells are completely inhibited by IV3, but not at all by 3G8. As shown, Fab fragments of IV3 are as effective as the intact IgG, eliminating the possibility that the Fc portion of IV3 is interacting with the eosinophil FcR.

As a further test of the ability of IV3 to inhibit FcR-mediated granulocyte function, we evaluated the effect of IV3 on immune complex–mediated superoxide production. As seen in Fig. 3, the addition of complexes made from tetanus
Time course of granulocyte superoxide (O$_2^-$) production stimulated by immune complexes. Granulocytes (2 × 10$^6$) incubated with cytochalasin B with and without IV3 mAb for 2 min were stimulated with 175 µg/ml immune complexes at zero time (arrow). Continuous measurement of superoxide dismutase-inhibitable cytochrome C reduction was performed as described in Materials and Methods. (a) No antibody, (b) IV3 IgG mAb (10 µg/ml), (c) IV3 Fab fragments (10 µg/ml).

**TABLE III**

<table>
<thead>
<tr>
<th>mAb</th>
<th>O$_2^-$ production (nmol/min/10$^6$)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.75</td>
<td>0</td>
</tr>
<tr>
<td>IV3</td>
<td>0.46</td>
<td>74</td>
</tr>
<tr>
<td>MOPC141</td>
<td>1.60</td>
<td>8.6</td>
</tr>
<tr>
<td>Anti-Vk3b</td>
<td>1.80</td>
<td>-2.9</td>
</tr>
<tr>
<td>AML-2-23</td>
<td>2.12</td>
<td>-21</td>
</tr>
</tbody>
</table>

Granulocytes incubated with cytochalasin B (5 µg/ml) in the presence or absence of each mAb (10 µg/ml) were stimulated with 175 µg/ml of immune complexes. The initial linear rates of superoxide production were measured by computing the slopes of curves, such as those seen in Fig. 3, relating cytochrome C reduction to time. The results are the average of duplicate determinations.

toxin and human IgG anti-tetanus toxin to purified granulocytes resulted in prompt release of superoxide into the medium, as measured by cytochrome C reduction. The addition of IV3 or Fab fragments of IV3 to the cells before the immune complex stimulus completely abrogated superoxide release. Two other murine IgG2b proteins (myeloma MOPC141 and anti-Vk3b mAb), and an IgG2b mAb (AML-2-23) that binds to granulocytes were incapable of duplicating the abrogation of immune complex-mediated superoxide release seen with IV3 (Table III). The slight enhancement of superoxide production seen with AML-2-23 in Table III is unexplained. When incubated alone with granulocytes, this antibody was unable to induce superoxide production. In other experiments, IV3 had no effect on superoxide release mediated by PMA, Con A, and FMLP (data not shown), indicating that the effect of IV3 is specific for the immune complex trigger of superoxide production.

**Discussion**

The principal conclusion to be drawn from these data is that mAb IV3, raised against the K562 FcR, defines a previously undescribed FcR on human neutro-
The evidence is of three sorts. First, IV3, which binds to all neutrophils, precipitates from neutrophil lysates, a 40 kD molecule virtually identical in size to the FcR recently defined on other cell types using this antibody. That is, IV3 has identified a 40 kD low-affinity FcR for IgG on monocytes and U937 cells (10) and on platelets (12). Normal B cells and cells of a B cell line (Raji) bear an FcR of nearly identical molecular mass to p40 that is not recognized by IV3 (10, 24). The 40 kD molecule precipitated by IV3 from neutrophil lysates is obviously distinct from the molecule recognized by mAb 3G8 (Fig. 2), which, as previously described (8), recognizes a neutrophil FcR with a molecular mass of 51–73 kD.

Both membrane proteins, the one precipitated by IV3 and the one precipitated by 3G8, can be purified using Sepharose-human IgG (Fig. 2), although judging from observations with the U937 40 kD receptor (10), Sepharose must be highly substituted with IgG to purify the 40 kD molecule. The use of Sepharose-IgG having a relatively low IgG-Sepharose ratio likely accounts for prior failures to purify the 40 kD molecule from neutrophil lysates (8, 9). Whether the requirement for high ligand-Sepharose ratio is due to the low affinity of p40 for IgG, receptor multivalency, or receptor self-association, needs further evaluation.

The data of Fig. 2 also indicate that p40 and p51–73 differ in their susceptibility to proteolytic degradation. In the absence of DFP during detergent lysis of the cells, p40 appears as a 33 kD molecule, whereas the molecular mass of p51–73 is unaffected by the absence of DFP. In studies of a 43 kD FcR purified from eosinophil-enriched populations (most likely the receptor recognized by IV3), Kulczycki et al. (9) also found a 33 kD degradation product appearing after FcR purification in the absence of DFP.

The second line of evidence supporting our contention that IV3 defines a newly recognized FcR on neutrophils is that the antibody is capable of specifically inhibiting the binding of rabbit IgG-coated erythrocytes to neutrophils. Although high concentrations (10 μg/ml) of IV3 alone are incapable of inhibiting the formation of FcR-dependent rosettes (Table II), it is quite clear that the partial inhibition seen with 3G8 is made complete by the addition of IV3. That is, the two antibodies acting in concert completely inhibit the binding of ligand to the neutrophil. We suggest that antibody-coated erythrocytes are binding to both molecules, but that the p40 FcR, because of low affinity for its ligand or low numbers of receptors per cell, makes a lesser contribution than p51–73 to the formation of rosettes. Two observations indicate that the Fc portion of IV3 is not inhibiting ligand binding. First, Fab fragments of IV3 inhibit rosettes as effectively as the intact molecule. Second, other antibodies of the same subclass as IV3, including one that binds to a determinant on neutrophils, do not show the same synergistic effect in concert with 3G8. The inhibitory effect of IV3 most likely results from direct binding of IV3 Fab fragments to the ligand-binding site on the p40 molecule.

The third observation indicting p40 is a neutrophil FcR is the capacity of IV3 to inhibit neutrophil immune complex-mediated superoxide production (Fig. 3). This effect, as with rosette formation, was independent of the Fc portion of IV3. Fab fragments of IV3 being sufficient for inhibition. Moreover, IV3 had no effect on superoxide generation by agents other than immune complexes, such as Con A, PMA, and FMLP, indicating that the antibody was interfering...
selectively with the FcR-mediated response, most likely by blocking immune complex binding to the cells. Although IV3 alone was unable to stimulate superoxide generation, preliminary experiments indicate that crosslinking IV3 with F(ab')2 fragments of anti-mIg results in superoxide release from neutrophils. How these two molecules, p40 and p51–73, are structurally related to subserve FcR-mediated activities is so far unclear. Both seem to be involved in the same FcR functions. Both IV3 and 3G8 were required for complete inhibition of rosette formation, although the predominant inhibitory effect was seen with 3G8. Although nearly complete inhibition of immune complex–induced superoxide release was caused by IV3, preliminary studies indicate that 3G8, too, is capable of inhibiting immune complex–mediated superoxide generation. The most likely explanation for this cooperation is that p40 and p51–73 are two distinct FcR that cooperate to bind opsonized erythrocytes and to generate superoxide.

The second major conclusion to be inferred from the data is that eosinophils as well as neutrophils bear a 40 kD FcR recognized by mAb IV3. It is clear from the information in Fig. 1 and Table I that eosinophils, which constitute the 3G8-dim population of granulocytes, are recognized by IV3. Moreover, this conclusion is corroborated by the results of the single experiment with eosinophils in Table III, which shows that IV3 but not 3G8 is capable of inhibiting the binding of IgG-coated RBC to eosinophils. Fab fragments of IV3 are adequate for inhibition, suggesting that IV3 inhibits ligand-receptor interaction by binding directly to the receptor. Although we have not determined the molecular mass of the molecule(s) precipitated by IV3 from purified eosinophils, Kulczycki (9) has described a 43 kD molecule purified by affinity to Sepharose-IgG, which we propose is most likely the FcR recognized by IV3. Further evaluation of this issue is in progress.

In agreement with Fleit et al. (8), our data in Fig. 1 indicate that 3G8 binds to eosinophils, the mean fluorescence being ninefold brighter than cells stained with anti-Vk3b. Other workers, however, have found that eosinophils are negative when stained with 3G8 (25). We have no explanation yet for this discrepancy, although the sensitivity of the assays and the methods of eosinophil purification are obviously different. The single rosette experiment with eosinophils in Table III indicates that, if the molecule recognized by 3G8 is present on eosinophils, it is too sparsely represented to contribute significantly to the binding of opsonized erythrocytes.

Several lines of evidence suggest that the 40 kD FcR recognized by IV3 is the human homologue of a class of murine receptors recognized by mAb 2.4G2, and which on macrophages is termed FcRII (26, 27). (a) p40 on human mononuclear phagocytes has affinity for mIgG2b and mIgG1, but not for mIgG2a (10, 11); FcRII on murine macrophages show this same pattern of specificity (27, 28). (b) Human B cells bear an FcR of ~40 kD, which, at least on the cells of one B cell line (Daudi), has serologic crossreactivity with p40 on U937 cells (10); 2.4G2 recognizes a subset of murine B cells (26). (c) 2.4G2 binds to and inhibits FcR function on murine eosinophils and neutrophils (29) just as does IV3 in humans, as shown by the data herein.
Summary

We describe a newly recognized 40 kD FcR for IgG on human neutrophilic granulocytes. An mAb (IV3) developed against the IgG FcR of K562 cells, and specific as well for a 40 kD FcR on human monocytes and platelets, was found to purify by affinity adsorption a 40 kD protein from detergent lysates of surfacelabeled neutrophils. This protein, proteolytically degraded to 33 kD when purified in the absence of diisopropylfluorophosphate, is distinct from the 51–73 kD protein precipitated by the anti-neutrophil FcR mAb, 3G8, previously described by others. Complete inhibition of binding of rabbit IgG-coated erythrocytes to neutrophils was achieved only when both antibodies, IV3 and 3G8, were used. Fab fragments of IV3 were as effective inhibitors as the intact molecule. IV3 IgG or Fab fragments completely and selectively inhibited immune complex–mediated generation of superoxide by human neutrophils; superoxide generation by other stimulants was not abrogated by IV3. This antibody (IV3) bound also to human eosinophils and completely inhibited the binding of IgG-coated erythrocytes to eosinophils. IV3 appears to define the human homolog of the murine macrophage FcRII identified initially by mAb 2.4G2 and present in the mouse on both neutrophils and eosinophils.

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