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Granulocyte Fc_γ Receptor Recognition of Cell Bound and Aggregated IgG: Effect of γ-Interferon

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Granulocyte Fc_γ receptors are important components in the recognition of IgG-coated cells and immune complexes. Two proteins have been identified on resting human granulocytes which function as Fc_γ receptors, Fc_γRII (CD32) and Fc_γRIII (CD16). A third protein, Fc_γRI (CD64), is not constitutively expressed on resting granulocytes, but can be induced by activation with γ-interferon. We examined the role of these three Fc_γ receptors on human granulocytes in the binding of both IgG-sensitized erythrocytes and soluble oligomeric IgG. In these studies we employed anti-Fc_γ receptor antibodies which complete for the Fc_γRII and Fc_γRIII ligand binding sites. Preincubation of granulocytes with saturating concentrations of high-affinity anti-Fc_γRII monoclonal antibody did not alter the recognition of IgG sensitized human cells by granulocytes. Furthermore, ligand binding studies demonstrated that anti-Fc_γRII antibody altered neither the number nor the affinity of granulocyte binding sites for human trimeric IgG. In contrast, Fab anti-Fc_γRIII inhibited the binding of both IgG (anti-D) sensitized human RBCs and IgG sensitized sheep RBCs. Similarly, a reduction in the expression of Fc_γRIII by treatment with phosphatidyl-inositol specific phospholipase C reduced PMN recognition of IgG-sensitized cells. Also, anti-Fc_γRIII decreased the number of granulocyte binding sites for human IgG trimer without a change in receptor affinity. Fc_γRI, which was induced by γ-IFN, increased granulocyte recognition of both IgG sensitized RBCs and IgG trimer. These data suggest that Fc_γRIII is the primary Fc_γ receptor on granulocytes which recognizes IgG sensitized RBCs and low molecular weight complexes of IgG. With γ-interferon activated granulocytes, Fc_γRI appears to enhance this recognition process.

Key words: immune complexes, human granulocytes, receptors

INTRODUCTION

Fc_γ receptors on phagocytic cells are important in host defense [1–5]. Human peripheral blood polymorphonuclear cells (PMN) constituitively express two Fc_γ receptor proteins (Fc_γRII and Fc_γRIII), and a third (Fc_γRI) is expressed following PMN activation by γ-IFN. Fc_γRI (CD64) binds monomeric as well as aggregated IgG and also is expressed on cells of the monocyte-macrophage lineage. It is not detected on resting normal PMN [2–4]. Fc_γRII (CD32), a 40 kDA protein, binds oligomers but not monomers of IgG [2–4]. Similarily, Fc_γRIII (CD16), a 50–70 kDa protein, binds IgG only in multivalent form [2–4].

Human Fc_γ receptors have also been distinguished by their interaction with monoclonal antibodies. The function and relative importance of these Fc_γ receptors in the recognition and phagocytosis of particulate and soluble immune complexes by human granulocytes is uncertain. We examined the role of Fc_γRII and Fc_γRIII on human granulocytes using anti-Fc_γRII and anti-Fc_γRIII monoclonal antibodies which compete for the Fc_γRII and Fc_γRIII ligand binding sites, respectively. We employed IgG trimer as a model small molecular weight immune complex and IgG-sensitized erythrocytes as a model of IgG-coated particulate antigen. Human granulocytes

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were also treated with γ-IFN to induce the expression of $Fc_\gamma RI$, in order to assess the role of this Fc_γ receptor in the recognition of cell bound and aggregated IgG in activated granulocytes. The data suggest that $Fc_\gamma RIII$ is the primary Fc_γ receptor on granulocytes participating in the recognition of these model IgG-containing immune complexes and that γ-IFN induced expression of $Fc_\gamma RI$ enhances this recognition.

MATERIALS AND METHODS

Isolation of Granulocytes

Human granulocytes were isolated from heparinized blood by dextran sedimentation (Macrodex, Pharmacia, Piscataway, NJ), density gradient centrifugation (LSM, Bionetics Laboratory Products, Kinsington, MD), and adherence to tissue culture petri dishes as previously described [6]. Briefly, leukocyte and platelet rich plasma obtained after sedimentation with dextran was layered onto LSM and centrifuged for 30 min at 600*g*. Polymorphonuclear leukocytes were recovered from the pellet and had the appearance of granulocytes on Wright-Giemsa stain (Diff-Quick, Dade Diagnostic, Aquada, PR). More than 95% of the cells stained positively with anti-$Fc_\gamma RII$ and anti-$Fc_\gamma RIII$ as assessed by FACS analysis, and less than 1% were positive with anti-$Fc_\gamma RI$, CD3 and OKM1 monoclonal antibodies. Greater than 99% of the cells were viable as assessed by trypan blue dye exclusion.

Preparation of Granulocyte Monolayers

One million PMNs in 1 ml of RPMI-1640 (M.A. Bioproducts, Walkersville, MD) were plated in petri dishes (35 mm, Falcon, Oxnard, CA) and allowed to adhere for 1 hr at 37°C in a 5% CO_2 atmosphere. Plates were then washed with RPMI-1640 prior to study. Monolayers contained 0.8–1 × 10^6 cells of which more than 95% were granulocytes.

γ-IFN Treatment of Granulocytes

Granulocytes, 1 × 10^6 per ml in RPMI-1640 containing 10% heat-inactivated FCS (FCS, GIBCO Laboratories, Grand Island, NY) and 500 U of purified human γ-IFN (10^6 antiviral U/ml, Interferon Sciences, Inc., New Brunswick, NJ), were incubated for 24 hr at 37°C in a 5% CO_2 atmosphere. Cells were then washed with RPMI-1640 containing 10% heat-inactivated FCS and resuspended in the appropriate buffer to be employed either in the equilibrium binding studies with ^{125}I-IgG or in the studies of the recognition of human IgG-coated RBCs (Rh^+) and rabbit IgG-coated sheep RBCs.

Preparation of Antibodies and Immunoglobulins

Human anti-D (Rh+) antibody from a single donor was obtained from Ortho Pharmaceutical (Raritan, NJ) and rabbit IgG anti-sheep erythrocyte antibody from Diamedix (Miami, FL). Anti-$Fc_\gamma RII$ (IV.3) and anti-$Fc_\gamma RI$ (32.2) monoclonal antibodies were the generous gift of Drs. Clark Anderson (Ohio State University, Columbus, Ohio) and Dr. Michael Fanger (Dartmouth University, Hanover, NH). Anti-$Fc_\gamma RIII$ monoclonal antibody 3G8 was the generous gift of Dr. Jay C. Unkeless (Mount Sinai Hospital, NY). Fab preparations of anti-$Fc_\gamma RIII$ and anti-$Fc_\gamma RII$ were prepared by papain digestion as previously described [7]. IgG antibodies were purified by immunoaffinity chromatography and radiolabeled with ^{125}I [8,9]. Anti-$Fc_\gamma RI$, anti-$Fc_\gamma RII$ and anti-$Fc_\gamma RIII$ monoclonal antibodies were used in flow cytometry studies to assess the expression of each receptor.

Human IgG was isolated from fresh human serum by Sephacryl S-300 gel filtration and QAE ion exchange chromatography (Pharmacia, Piscataway, NJ), as previously described [9]. IgG fractionated as IgG monomer on Sephacryl S-300 and appeared as IgG by Ouchterlony analysis, immunoelectrophoresis or polyacrylamide disc gel electrophoretic analysis [8,10]. The IgG was radiolabeled with ^{125}I (New England Nuclear Corporation, Boston, MA) to a specific activity of 0.16 μCi/μg using chloramine-T. Prior to use, all monomeric IgG preparations were centrifuge in a Beckman airfuge (Beckman Instruments, Fullerton, CA), at 100,000*g* for 15 min to remove IgG aggregates.

IgG trimer was prepared as previously described [10]. Briefly, purified IgG (70 mg/ml in 0.2 M Tris-HCl buffer, pH 8.4) was incubated with a 16-fold excess of dimethyl suberimidate (Sigma Chemical Co., St. Louis, MO) for 1 hr at 30°C. The reaction was then quenched by the addition of a 5-fold molar excess glycine (with respect to dimethyl suberimidate), and the mixture immediately applied to a tandem column of Sephadex G-200 (Pharmacia, Piscataway, NJ) and Ultrogel AC22 (LKB Instruments, Inc., Gaithersburg, MD), and fractionated into oligomers of defined molecular weight. Pools of IgG trimer were made and concentrated to 5–10 mg/ml prior to their use. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis and refractionation by gel filtration chromatography revealed that the major protein was trimeric IgG (mol wt = 450,000).

Preparation of IgG-Coated RBCs

Human RBCs (Rh+) from a single donor were sensitized with anti-D antibody as previously described [11,12]. In brief, 1 × 10^8 RBCs in 1 ml of 0.01 M ethylenediaminetetracetic acid (EDTA) buffer were incubated with defined amounts of anti-D serum for 1 hr at 37°C. After washing twice, RBCs were standardized to 1 × 10^8/ml in HBSS, and 1 ml added to the granulocyte monolayers. Similarly, 1 × 10^9 sheep RBCs in 1 ml of 0.01 M EDTA buffer were sensitized by adding 32.4 μg of rabbit IgG anti-sheep RBC antibody in 1 ml at 37°C for 1 hr. The IgG-sensitized RBCs were washed twice and

resuspended in HBSS to a final concentration of 4×10^8/ml and overlayed on the granulocyte monolayers.

The number of human IgG/RBC (Rh+) was determined as previously reported [13,14]. RBCs (Rh+) sensitized as above (3,800 IgG/RBC) were incubated for 30 min at 37°C with known amounts of ^{125}I rabbit anti-human IgG, and the number of IgG/RBC determined by reference to previously established standard curves [13,14]. The number of rabbit IgG per RBC was determined by incubating rabbit IgG, radiolabeled with ^{125}I by the chloramine T method as above, with sheep RBCs and determining the bound radioactivity after washing the sensitized RBCs [13,14]. In our experiments sheep RBCs were sensitized with 1,900 IgG/RBC.

Granulocyte Recognition of Human IgG-Coated RBCs (Rh+) and Rabbit IgG-Coated Sheep RBCs

The in vitro recognition of IgG-coated RBCs was assessed as previously reported [11,12,15]. Briefly, either 1×10^8 IgG-coated human RBCs (Rh+) or 4×10^8 IgG-sensitized sheep RBCs were added to monolayers containing 1×10^6 granulocytes. To assess the role of granulocyte Fc_γRII and Fc_γRIII, monolayers were preincubated with either anti-Fc_γRII or anti-Fc_γRIII monoclonal antibodies which inhibit Fc_γRII or Fc_γRIII ligand binding, respectively, or isotype controls [16,17]. Granulocytes and IgG-coated RBCs were incubated at 37°C in a 5% CO_2 atmosphere for 12 hr, washed with the appropriate buffer, and stained with Wright-Giemsa. Granulocytes (200) were counted under light microscopy to assess the number of IgG-sensitized RBCs bound/granulocyte and the % granulocytes binding at least three RBCs were determined. Binding of IgG (anti-D)-coated RBCs and rabbit IgG-coated sheep RBCs by human granulocytes and its alteration by anti-Fc_γ receptor monoclonal antibodies or isotype controls were performed in parallel. Experiments were also performed with shorter incubation times and under these conditions similar results were observed.

Equilibrium Binding Studies With ^{125}I-IgG

All assays were performed in HBSS containing 0.1% gelatin, 10 mM EDTA, and 0.1% NaN_3, as previously described [8–10,14]. Before each assay, washed granulocytes were incubated for 30 min at 37°C in HBSS buffer to permit cell-associated IgG to dissociate from the surface. Cells were centrifuged and resuspended to a concentration of 1×10^6 granulocytes per ml in the assay buffer. Increasing concentrations of radioiodinated IgG were added in the presence or absence of 100-fold excess of unlabeled IgG. The amount of cell-associated and unbound ^{125}I-IgG were quantitated after rapidly centrifugating the granulocytes through a layer of silicone oil in microfuge tubes. Specific binding was defined as the cell-associated radioactivity inhibited by the presence of a 100-fold excess of unlabeled IgG and represented more than 70% of the total cell-associated counts. Receptor number and affinity constants were determined by Scatchard analysis. Similar methodology was employed using radiolabeled ^{125}I-IgG anti-Fc_γRII and ^{125}I-IgG anti-Fc_γRIII monoclonal antibodies in the presence or absence of a 50-fold excess of either unlabeled IgG anti-Fc_γRII or unlabeled IgG anti-Fc_γRIII in order to measure the number of granulocyte Fc_γRII and Fc_γRIII sites, respectively. To determine whether monomeric and/or trimeric IgG bound to a single class or multiple classes of Fc_γ receptors, specific binding data were analyzed by Scatchard plots as previously described [18].

Flow Cytometry

Monoclonal antibodies directed against Fc_γRIII (mAb 3G8), Fc_γRII [mAb IV.3], and Fc_γRI [mAb 32.2] were utilized in indirect immunofluorescence binding studies to assess protein expression of these receptors on granulocytes. The cells were incubated with the antibodies for 30 min at 4°C and washed twice with phosphate buffered saline (PBS) containing 0.1% bovine serum albumin and 0.02% sodium azide. Bound antibody was labeled by incubation with an FITC-labeled goat anti-mouse antibody (TAGO, Inc., Burlingame, CA) for 30 min at 4°C. The cells were again washed twice and then fixed with 4% paraformaldehyde until analyzed by flow cytometry. Flurorescence was measured by a FACStar cytometer with Consort-30 software (Becton-Dickinson, Mountain View, CA) as previously described [19].

Statistics

To determine whether the difference between mean data was significant a Wilcoxon rank sum test for paired data was applied [20].

RESULTS

Effect of Anti-Fc_γRII and Anti-Fc_γRIII on Granulocyte Recognition of IgG Sensitized RBCs

We evaluated the role of Fc_γRII and Fc_γRIII in the binding of human IgG anti-D sensitized RBCs and rabbit IgG-sensitized sheep RBCs, using anti-Fc_γRII and anti-Fc_γRIII monoclonal antibodies. We first determined the amount of monoclonal antibody required to occupy all granulocyte Fc_γRII and Fc_γRIII sites by measuring that amount necessary for saturation under conditions of equilibrium binding. Using radiolabeled anti-Fc_γRII and anti-Fc_γRIII, we observed that granulocytes express $40.3 \pm 7.1 \times 10^3$ Fc_γRII binding sites/cell (Kd = 0.73 ± 0.04 nM) and $255.1 \pm 19.6 \times 10^3$ Fc_γRIII binding sites/cell (Kd = 5.95 ± 1.45 nM). Based on these calculations, 2.1 μg/ml of anti-Fc_γRII and 17.0 μg/ml of anti-Fc_γRIII saturated 95% of the granulocyte binding sites. We then employed concentrations of anti-Fc_γRII and anti-Fc_γRIII

TABLE I. Recognition of IgG (Anti-D)-Sensitized Human RBCs and Rabbit IgG-Sensitized Sheep RBCs by Granulocytes*

	% granulocytes binding RBCs	
	Human RBCs	Sheep RBCs
Anti-FcγRII (0.06 μg/ml)	28 ± 2	68 ± 4
0.12	27 ± 3	66 ± 3
1	26 ± 2	65 ± 4
3	26 ± 2	64 ± 3
6	25 ± 4	62 ± 3
Anti-FcγRIII (0.5 μg/ml)	28 ± 3	64 ± 4
1	26 ± 4	39 ± 2
5	8 ± 3	17 ± 3
10	7 ± 3	10 ± 1
80	—	1 + 1
Anti-FcγRIII (Fab) (0.5 μg/ml)	30 ± 4	66 ± 5
5	17 ± 2	52 ± 3
25	7 ± 1	26 ± 2
50	0	9 ± 3
No anti-FcγR mAb	28 ± 3	71 ± 4

*The binding and/or phagocytosis of IgG sensitized RBCs by granulocytes was performed at 37°C for 12 hr. Percentages of granulocyte binding ≥3 RBCs or phagocytosing RBCs after preincubation with either IV.3 anti-FcγRII, 3G8 anti-FcγRIII, or 3G8 anti-FcγRIII Fab monoclonal antibodies are shown. The data represent the mean ± SEM of four experiments.

which would saturate between 30 and 98% of granulocyte Fc_γRII and Fc_γRIII.

Granulocytes bound IgG (anti-D)-coated RBCs in a dose-response manner which depended on the concentration of IgG per RBC. No significant alteration in the recognition in IgG (anti-D)-coated RBCs was observed following preincubation of granulocytes with anti-Fc_γRII monoclonal antibody over a range of monoclonal antibody concentrations (0.06-6.0 μg/ml) (Table I). These data suggest that Fc_γRII does not play a primary role in the recognition of human anti-D-coated RBCs by granulocytes. In contrast, preincubation of granulocytes with anti-Fc_γRIII monoclonal antibody inhibited the recognition of IgG (anti-D)-sensitized RBCs. Thus, Fc_γRIII appears to be the major Fc_γ receptor on freshly isolated, resting granulocytes involved in the recognition of IgG (anti-D)-coated RBCs.

Granulocytes also bound and phagocytozed rabbit IgG-sensitized sheep RBCs, and this interaction also depended upon the concentration of IgG per RBC. Anti-Fc_γRIII monoclonal antibody also inhibited the binding of rabbit IgG-coated sheep erythrocytes in a dose-dependent manner (Table I). In addition, similar studies were performed using Fab anti-Fc_γRIII. Fc_γRII preferentially binds murine IgG of the isotype IgG1 and IgG2b. Therefore, Fab anti-Fc_γRIII was utilized, because the anti-Fc_γRIII employed was isotype IgG1 and could bind to Fc_γRII. However, no significant differences were observed in the inhibition of PMN binding of IgG anti-D coated RBCs and rabbit IgG sensitized sheep RBCs by either anti-Fc_γRIII or Fab anti-Fc_γRIII.

Fc_γRIII is a protein that is associated with the PMN membrane through a phosphatidyl-inositol (P-I) linkage [21,22]. Fc_γRIII can be partially removed from the PMN surface by incubation with phospholipase C (PI-PLC) [23]. We preincubated granulocytes with concentrations known to decrease Fc_γRIII protein expression [23]. Granulocytes which were preincubated with PI-PLC bound less IgG coated RBCs (buffer = 68 ± 4%; 0.02U/ml PI-PLC = 47 ± 2%, 0.2 U/ml PI-PLC = 27 ± 4% and 2 U/ml PI-PLC = 18 ± 2% PMN binding 3 or more rabbit IgG sensitized RBCs). Similar data were observed with IgG anti-D sensitized human RBCs.

We previously observed that Fc_γRI on human monocytes is the major Fc_γ receptor which participates in the binding of IgG (anti-D) sensitized RBCs [14]. Fc_γRI is not present on resting granulocytes, but its expression can be induced upon exposure to the cytokine γ-interferon. Since γ-interferon may be elaborated during activation of immune complex disease or during infection, we studied granulocytes following exposure to γ-interferon. Analysis by flow cytometry indicated that γ-interferon induced the expression of Fc_γRI on the granulocyte surface in 60–70% of the cells. Mean fluorescence intensity with anti-Fc_γRI was increased from 6 to 8 MFI units before γ-interferon treatment and in the absence of γ-interferon to 18-26 MFI units following γ-interferon treatment.

Our data suggest this granulocyte Fc_γRI, induced by treatment with γ-interferon plays a role in the binding of IgG (anti-D) coated human RBCs (Fig. 1). When γ-interferon treated granulocytes were preincubated with anti-Fc_γRIII, the binding of anti-D coated RBCs was only

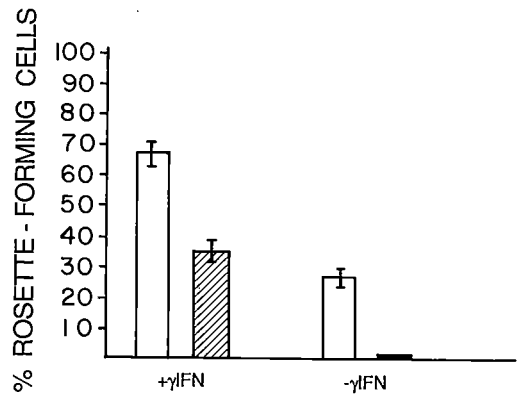

Fig. 1. Recognition of IgG (anti-D) sensitized RBCs by granulocytes (PMNs) at 37°C. PMNs were incubated for 24 hr in the presence (+γIFN) or absence (−γIFN) of 500 U/ml of γ-interferon. PMN were preincubated with either buffer (both empty rectangles) or anti-Fc_γRIII (10 μg/ml) (both shaded rectangles). The percentage (±SEM) of PMNs binding ≥3 RBCs/PMN is shown for four experiments.

TABLE II. Granulocyte Binding of IgG Trimer: Effect of Anti-FcγRII Monoclonal Antibodies*

	Fcγ binding sites	Affinity constant (Ka)
Buffer	181,000	$1.4 \times 10^7\ M^{-1}$
FcγRII antibody	144,000	$1.0 \times 10^7\ M^{-1}$
FcγRIII antibody	10,000	$1.4 \times 10^7\ M^{-1}$

*Granulocytes were preincubated with anti-FcγRII (6 μg/ml) and anti-FcγRIII (50 μg/ml) or buffer control prior to the equilibrium binding of trimeric IgG.

partially inhibited, while no effect of anti-Fc_γRII was noted. These data suggest that Fc_γRI likely plays a role in the binding of these IgG-coated cells.

Effect of Anti-Fc_γRII and Anti-Fc_γRIII on Granulocyte Binding of Soluble IgG

We also studied the role of Fc_γRII and Fc_γRIII in the binding of soluble human IgG ligand. In these experiments we employed trimers of human IgG, as both Fc_γRII and Fc_γRIII recognize oligomeric, but not monomeric IgG [2]. Human granulocytes were preincubated with either anti-Fc_γRII or anti-Fc_γRIII antibody, and the number of Fc_γ receptor binding sites and affinity constants determined. As noted above, these concentrations of anti-Fc_γRII and anti-Fc_γRIII are sufficient to saturate granulocyte Fc_γRII and Fc_γRIII, respectively. Anti-Fc_γRII did not significantly alter the number of granulocyte binding sites or affinity for trimeric IgG (Table II). These results were observed using a range of monoclonal antibody concentrations (0.06–6.0 μg/ml). On the other hand, the number of granulocyte binding sites for IgG trimer was decreased >90% by preincubation with Fab anti-Fc_γRIII (Table II). This effect was observed in four of four normal granulocyte donors. Thus, granulocyte Fc_γRIII also appears to be largely responsible for the binding of trimeric IgG.

We next studied the binding of soluble IgG to granulocytes preincubated with γ-interferon, so as to express Fc_γRI. PMNs were cultured in the presence of γ-interferon (500 U/ml) for 24 hr and then preincubated with saturating concentrations of Fab anti-Fc_γRIII. The binding of trimeric IgG to these cells was then assessed. A curvilinear plot was observed with a two site fit for a high and low affinity binding site, consistent with IgG trimeric binding to Fc_γRIII (low affinity) and Fc_γRI (high affinity). The lowest affinity binding site was inhibited by preincubation with Fab anti-Fc_γRIII.

DISCUSSION

The precise functions of the Fc_γ receptors on human granulocytes are only now beginning to be elucidated. We utilized monoclonal antibodies, competing for either the Fc_γRII or the Fc_γRIII ligand binding sites, to assess the role of these receptors in the granulocyte binding of IgG ligand [11,24,25]. We first determined the amount of these monoclonal antibodies necessary for saturation of granulocyte Fc_γRII and Fc_γRIII as well as their affinity constants. Using this information, we assessed whether anti-Fc_γRII or anti-Fc_γRIII altered the recognition of IgG ligand. Our data suggest that granulocyte Fc_γRIII rather than Fc_γRII is primarily involved in the recognition of IgG sensitized RBCs and oligomeric IgG.

These results could be observed if IgG aggregates or IgG sensitized RBCs displaced granulocyte bound anti-Fc_γRII. However, we employed concentrations of high-affinity anti-Fc_γRII monoclonal antibody substantially greater than that necessary to saturate 95% of the Fc_γRII sites. In addition, we observed that the affinity of the monoclonal antibody is much greater than that of IgG trimer. Under these conditions, it is unlikely that anti-Fc_γRII antibody were displaced from the granulocyte surface. Although the affinity of human IgG coated RBCs for the granulocyte is unknown, it is also unlikely that these RBCs displaced this high-affinity monoclonal antibody from the granulocyte surface at the high anti-Fc_γRII antibody concentrations used.

In a previous study, anti-Fc_γRIII only partially inhibited the binding of IgG coated ox RBCs to human granulocytes, while anti-Fc_γRII did not alter this interaction [4]. Our data on the affinity of binding of anti-Fc_γRIII to PMN may explain this partial inhibition of IgG coated ox RBCs to PMN by anti-Fc_γRIII. In the study above, at the highest concentrations of anti-Fc_γRIII that were employed, only 84% of Fc_γRIII would be expected to be occupied, approximately corresponding to the extent of inhibition of rosette formation observed. Our data with human IgG in an allogeneic system, using high concen-

trations of anti-$Fc_\gamma RIII$, which saturated >98% of granulocyte $Fc_\gamma RIII$ (Table I), demonstrated virtually complete inhibition of binding.

These results are in keeping with little $Fc_\gamma RII$ participation in the binding of both these IgG coated cells and trimers of IgG in the presence of granulocyte $Fc_\gamma RIII$. Similarly, our data and the data of others indicate that $Fc_\gamma RII$ both on human monocytes and granulocytes, in contrast to $Fc_\gamma RII$ on human macrophages, does not play a major role in the binding of IgG coated cells or human trimeric IgG under physiologic conditions [4,14,25,26,31]. These data do not, however, suggest that PMN $Fc_\gamma RII$ cannot mediate the binding of IgG containing immune complexes. Rather, it appears that PMN $Fc_\gamma RII$ plays a lesser role in the recognition of IgG complexes when PMN $Fc_\gamma RIII$ and/or $Fc_\gamma RI$ are expressed. It is important to note that these data do not preclude an effect of $Fc_\gamma RII$ in phagocytosis, only the first step of which involves IgG ligand recognition and binding. Granulocyte $Fc_\gamma RIII$, being a P-I linked protein rather than an integral membrane protein [21,22,23], may not be as important in mediating phagocytosis as it is in the initial granulocyte IgG recognition step. Our experiments indicate that PI-PLC treatment of granulocytes decreases both the expression of $Fc_\gamma RIII$ protein and the recognition by granulocytes of IgG coated cells.

The $Fc_\gamma RIII$ dependent binding of human IgG coated RBCs required a long incubation period (12 hours) and a relatively large amount of human IgG (anti-D)/RBC. This may be due to the low affinity of PMN $Fc_\gamma RIII$, or to the low density of the D antigen on the human RBC membrane resulting in less cross-linking of $Fc_\gamma RIII$. The surface area of sheep RBCs is approximately 25% of that of human RBCs. Therefore, in our experiments, sheep RBCs coated with 1,900 IgG per RBC have approximately twice the density of IgG on their surface than the IgG coated human RBCs employed (3,800 IgG per RBC). This increased density of IgG may facilitate the interaction with $Fc_\gamma RIII$, particularly since $Fc_\gamma RIII$ preferentially interacts with aggregates of IgG of increasing size [5,26]. The absence of $Fc_\gamma RII$ function is not due to decreased availability of $Fc_\gamma RII$ protein. Using either ^{125}I-IgG anti-$Fc_\gamma RII$ or analysis by flow cytometry we and others have observed approximately 40,000 $Fc_\gamma RII$ sites on the granulocyte surface. This low availability of the $Fc_\gamma RII$ ligand binding site on granulocytes has been previously observed with $Fc_\gamma RII$ on U-937 cells [25] and monocytes [14], where $Fc_\gamma RII$ is more functionally active in the cold and under low ionic strength conditions. $Fc_\gamma RII$ on PMN, however, has been shown to be involved in cell triggering [2,4,27,28], signal transduction [21], lymphocyte proliferative responses [29] and NK cell mediated cytotoxicity [30].

The high affinity 72 kDa $Fc_\gamma RI$ has been shown to efficiently mediate IgG ligand-binding in vitro [2,14,16]. $Fc_\gamma RI$ expression can be enhanced by γ-IFN on monocytes [19,32,33], while granulocytes can be induced by γ-IFN to express $Fc_\gamma RI$ [33,34]. γ-IFN treated neutrophils can mediate increased ADCC by such induced human $Fc_\gamma RI$ [27]. Thus, $Fc_\gamma RI$ may play a role augmenting $Fc_\gamma RIII$ mediated immune complex clearance when granulocytes or monocytes/macrophages are activated during inflammatory reactions. This may be more evident in tissues where the concentration of circulating IgG, for which $Fc_\gamma RI$ has substantial affinity, is decreased.

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