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Research Article

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These results indicate that receptors for the Fc portion of human γG antibodies exist on both neutrophils and monocytes, and that γG antibodies differ in their ability to attach to these two cell types. Differences in the behavior of the γG antibodies studied may be related to differences in the density of antibodies on the erythrocyte surface and receptors on the phagocytic cells.

Receptors for Human γG Globulin on Human Neutrophils

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ABSTRACT Cell surface receptors for human γG antibodies directed against bacterial antigens were demonstrated on human neutrophils using an in vitro bacteriocidal-phagocytic assay. These results were confirmed by adherence of sensitized erythrocytes to monolayers of neutrophils or monocytes. Erythrocytes sensitized indirectly with antibacterial γG antibodies after passive sensitization with bacterial antigens adhered to both neutrophils and monocytes. Erythrocytes sensitized directly with conventional anti-D γG antibodies adhered only to monocytes, while those sensitized with the hyperimmune anti-CD γG antibody Ripley adhered to both monocytes and neutrophils. Adherence of anti-Rh or antibacterial γG antibodies to monocytes and neutrophils could be inhibited by whole γG, myeloma globulins of the γ_1 or γ_3 subclasses, or Fc fragments, but not by Fab fragment.

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INTRODUCTION

Studies from several laboratories have demonstrated cell surface receptors on human and animal monocytes or macrophages specific for homologous and heterologous γG antibodies. Receptors for γG antibodies have also been described on animal granulocytes (1–3), but the existence of these receptors on human granulocytes is controversial. Henson (3) demonstrated rosette formation around human neutrophils by erythrocytes sensitized with rabbit and guinea pig antibodies to sheep erythrocytes and also human anti-*Salmonella typhimurium* γG antibodies. In contrast to these results Loe-Buglio, Cotran, and Jandl (4), Cline and Lehrer (5), and Huber, Douglas, and Fudenberg (6), using erythrocytes coated with human anti-Rh antibodies, have reported receptors for human γG only on cells of the monocyte-macrophage line. The present work was undertaken to gain additional information on the surface receptors for γG on human phagocytic cells.

Two separate systems were used to detect attachment of γG antibodies to the cell surface: an in vitro bacteriocidal-phagocytic assay and a slide monolayer adherence technique. In the latter, γG antibodies with activity against bacterial and erythrocyte antigens were used to allow direct comparison of their properties. Our results indicate that receptors for human γG globulin exist on both human neutrophils and monocytes.

METHODS

Preparation of cells. Mixed human leukocyte preparations were obtained by sedimentation of heparinized blood from normal donors in 6% dextran. Highly purified human granulocytes and mononuclear cells were prepared from the blood of normal donors by the method of Boyum (7). After separation, the leukocytes were washed three times in Hanks' balanced salt solution and resuspended to the desired concentration in the same medium.

Bacteriocidal phagocytic assay. A modification (8) of the in vitro phagocytosis system of Maaløe (9) was used. Washed leukocytes, bacteria, and opsonin, either fresh normal human serum or isolated immune γG, were tumbled in plastic test tubes at 37°C. Phagocytosis was measured by the decline in the number of surviving bacteria in aliquots taken at intervals during a 2 hr period. Killing of bacteria in this system is dependent upon the intracellular bacteriocidal capacity of the leukocytes (10).

Bacteria. Cultures of *Staphylococcus aureus* and *Serratia marcescens* isolated from the blood of patients with subacute bacterial endocarditis (SBE) were maintained in Penassay broth. Bacteria and immune γG globulin used in phagocytic assays were isolated from the same patient. Staphylococcal culture filtrate used for the passive sensitization of erythrocytes was obtained from an 18 hr Penassay broth culture of *S. aureus*. The culture was centrifuged at 2000 rpm for 10 min and the supernate passed through a Millipore filter (GS 0.22 μ/25 mm). The filtrate was heated at 56°C for 45 min. For passive sensitization, 0.1 ml of washed, packed human erythrocytes (dce/dce) were incubated with the heat-inactivated filtrate for 1.5 hr at 37°C, washed three

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times with normal saline, and resuspended to a 0.5% suspension (11).

Serum and serum fractions. Six sera with known high titers of opsonic activity were selected from a panel of sera obtained from patients with subacute bacterial endocarditis. One serum (No. 7) was obtained from a patient with repeated bouts of septicemia. This serum had high titers of opsonic activity against both *S. aureus* and *S. marcescens*. The agglutinating titers of these sera with the bacteria isolated from the same patient ranged from 1:64 to 1:256. Two human sera with strong anti-D activity (indirect Coombs titers 1:256 and 1:1024) were obtained from the blood bank (Blood Services of New Mexico). The hyperimmune anti-CD serum Ripley (12) was kindly supplied by Dr. Marion Waller. This serum had a Coombs titer of 1:2048. Purified γG opsonins from the various sera were prepared by *O*-diethylaminoethyl cellulose (DEAE-cellulose) column chromatography using a 0.02 M phosphate buffer pH 8.2 (13). Isolated γG myeloma globulins were obtained by starch-block electrophoresis of sera from patients with multiple myeloma. The γG H-chain subclass was kindly determined by Dr. William Yount, The Rockefeller University, New York. Standard methods were used for Gm(a) and Gm(b) typing (14–16) of isolated myeloma proteins.

Slide adherence test. A modification of the method of Berken and Benacerraf (17) was employed. Plastic rings 4 mm in thickness with an I.D. of 9 mm were fixed to clean glass slides with vacuum grease (Spinco, Palo Alto, Calif.). Leukocytes (2.5×10^5) were added to the well and allowed to settle on the glass slide during an incubation period of 60 min at 37°C. The well was washed by immersion and gentle agitation in a beaker of Hanks' balanced salt solution to remove nonadherent cells. Human erythrocytes were sensitized directly with conventional anti-D γG antibodies, hyperimmune Ripley anti-CD γG antibodies, or indirectly with γG isolated from patients with SBE due to *S. aureus* after passive sensitization with staphylococcal culture filtrate. The erythrocytes were then washed three times in Hanks' balanced salt solution and added to the well as a 0.5% suspension. After incubation at 22°C for 1 hr, the slides were washed free of nonadherent erythrocytes. The rings were removed, the monolayers stained with Wright's blood stain, and the per cent of cells with adherent or ingested erythrocytes determined by light microscopy at a magnification of × 1000. The conditions employed were not optimal for ingestion of adherent cells; therefore, adherence and ingestion were scored equally. A total of 400 cells were counted for each slide. One observer counted 100 cells as a known and 100 as an unknown, a second counted 100 cells on two occasions as unknowns. Since the differences in the four counts showed no significant variation, an average of the four counts was used. Each experiment included controls of unsensitized erythrocytes and, if pertinent, erythrocytes sensitized with *S. aureus* antigens alone. The results were expressed as per cent positive cells on the test slide minus the per cent positive on the control slide. Control slides contained a mean of 4.3% positive monocytes (SEM 1.0, n = 28) and 3.1% positive neutrophils (SEM 0.5, n = 28).

Digestion of γG globulins. Pepsin digestion of γG globulin isolated by DEAE column chromatography was carried out at pH 4.1 for 24 hr with an enzyme-to-protein ratio of 1:100 and terminated by dialysis against several changes of 0.1 M phosphate buffer pH 7.4. The pepsin digested γG was free of Fc antigens as determined by immune diffusion in agar against goat anti-human Fc antisera (Hyland Laboratories, Los Angeles, Calif.).

Papain digestion was performed according to the method of Porter (18). Digestion was carried out for 24 hr using a 1:100 enzyme-to-protein ratio. The Fab fragments were separated by DEAE chromatography with a 0.01 M phosphate buffer pH 8.0, followed by gel filtration on Sephadex G-200 to remove undigested γG. Fc fragments were removed from the DEAE column with 0.4 M sodium chloride in phosphate buffer pH 8.0 after exhaustive elution with the 0.01 M phosphate buffer pH 8. The Fab preparation gave no precipitin lines against anti-Fc antisera when tested by immune diffusion in agar. The Fc preparation was free of light chain antigens as determined by immune diffusion against anti-K and λ antisera.

RESULTS

Bacteriocidal phagocytic assay. In order to clarify the γG receptors on human granulocytes highly purified preparations of these cells containing 90–98% neutrophils, 2–6% eosinophils, and 0–5% mononuclear cells were used as the phagocytic cells in an in vitro bacteriocidal phagocytic assay system. In each instance controls indicated that the bacteria used were not killed in the presence of the γG opsonins or granulocyte preparations alone, or if tubes containing bacteria, opsonin and cells were held stationary during the incubation period. Gamma G globulin isolated from serum of four patients with SBE due to *S. aureus* and one with septicemia due to *S. marcescens* were used with bacteria isolated from the same patient. Granulocytes from four normal donors, which were incapable of killing the bacteria in the absence of opsonin, were capable of killing 90% of the inoculated organisms in the presence of isolated immune γG. Using phagocytic cells from a single donor D.C., no significant difference was noted in the opsonic action of six different γG preparations. These results are entirely analogous to those found previously using mixed leukocytes in place of purified granulocytes (8). Wrights stained slides were prepared from aliquots of the phagocytic mixture at 10 and 30 min. Ingestion of bacteria by granulocytes was demonstrated in tubes in which bacteria were killed, but not in those in which bacterial colony counts were unchanged indicating that killing of bacteria was accomplished intracellularly after ingestion by granulocytes rather than extracellularly. Bacteria were not killed by mononuclear cell preparations containing 80–90% lymphocytes, 5–10% monocytes, and 0–5% granulocytes either alone or in combination with γG opsonins.

In a second set of experiments, the phagocytic-bacteriocidal activity of mixed leukocyte preparations containing granulocytes, monocytes, and lymphocytes in approximately the same proportions as found in peripheral blood was compared directly to that of granulocyte preparations (> 96% neutrophils) obtained from the same blood sample (Fig. 1). Immune γG preparations or a

1:5 dilution of fresh normal serum were used as opsonins. The two cell preparations were adjusted to give equal numbers of neutrophils in each tube. No difference was noted between the ability of mixed leukocytes or purified granulocytes to phagocytize and kill bacteria with any of the four γG preparations tested. With both cell preparations, killing of *S. aureus* and *S. marcescens* was more rapid in the presence of fresh normal serum than when immune γG was used as opsonin. Heating the fresh normal serum at 56°C for 30 min reduced its opsonic activity to a level below that of immune γG. As in previous experiments, bacteria were not killed in the presence of either cells or opsonin alone.

Phagocytosis and killing of bacteria by granulocytes in the presence of immune γG could be inhibited by nonimmune γG (Cohn fraction II) or its Fc subunit but not by the Fab fragment (Table I). The addition of Cohn fraction II in a concentration similar to that found in human serum (12 mg/ml) completely inhibited the killing of *S. aureus* in the presence of immune γG. The Fc subunit in approximately equal molar concentration reduced γG mediated phagocytosis and killing from 90%

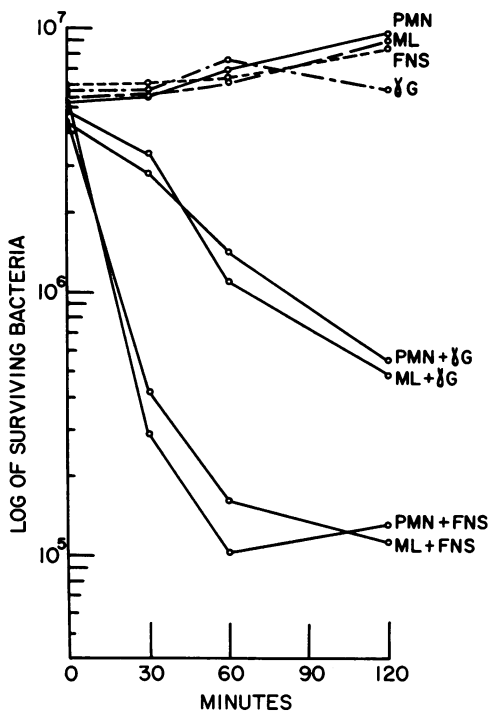

FIGURE 1 Comparison of phagocytosis and killing of *Staphylococcus aureus* by purified granulocytes (PMN) and mixed leukocytes (ML) in the presence of fresh normal serum (FNS) and immune γG (γG). The purified granulocyte preparation contained 95% neutrophils, 4.5% eosinophils, and 0.5% mononuclear cells. The mixed leukocyte preparation contained 60% neutrophils, 30% lymphocytes, 5% eosinophils, and 5% monocytes. The two cell preparations were adjusted to give 5×10^6 neutrophils in each tube.

TABLE I
Inhibition of the Opsonic Activity of γG by Cohn Fraction II and γG Subunits

Inhibitor	Concentration of inhibitor	Concentration of γG opsonin	% Bacteria killed at 120 min
	mg/ml	*mg/ml*	
—	—	—	0
—	—	2	90
F_{II}	12	2	0
Fab	8	2	90
Fc	4	2	10

to 10% of the inoculated organisms. Digestion of immune γG with pepsin abolished its ability to mediate phagocytosis and killing of bacteria by granulocytes.

Slide adherence test. Experiments with the bacteriocidal phagocytic assay described above suggested that a receptor for γG opsonins existed on neutrophils. To clarify these results a third set of experiments was performed using the slide adherence test. This technique allowed direct visualization of the cells involved and also comparison of the effect of human γG antibodies directed against erythrocyte antigens with those directed against bacterial antigens.

Monolayers were prepared from mixed leukocyte, and highly purified granulocyte or mononuclear cell preparations. The slides were washed to remove contaminating erythrocytes and lymphocytes which did not adhere to glass. Cells in the monolayers of mixed leukocytes or highly purified monocytes or granulocytes were 96% viable as determined by trypan blue dye exclusion.

Erythrocytes (DCe/DCe) sensitized directly with γG isolated from two subjects with anti-D activity adhered only to mononuclear cells (Table II). In 15 experiments a mean of 36.2% of the mononuclear cells were positive while only 3.8% of the neutrophils had adherent erythrocytes. Problems in the precise differentiation of monocytes from cells of the lymphocyte line by light microscopy alone made it impossible to state from these experiments that lymphocytes were not involved; however, the vast majority of positive cells had the morphologic characteristics of monocytes. Erythrocytes (dce/dce) first sensitized passively with staphylococcal antigens and then with γG isolated from patients with SBE due to *S. aureus* adhered to both monocytes and neutrophils. No significant difference in positive reactions was found between the monocytes (46.9%) and neutrophils (43.9%). Erythrocytes (DCe/DCe) sensitized directly with hyperimmune Ripley anti-CD γG also adhered to both monocytes and neutrophils (Fig. 2). 48% of the monocytes were positive compared to 35.1% of the neutrophils ($0.05 > P > 0.02$). No significant difference was found between the number of positive neutrophils

TABLE II

Adherence of Erythrocytes Sensitized with Three γG Antibodies to Monocytes and Neutrophils

Sensitizing γG antibody	Number of tests	% Positive				P value
		Monocytes		Neutrophils		
		$\bar{m}$	SEM	$\bar{m}$	SEM	
Anti-D	15	36.2	4.4	3.8	1.1	$P < 0.0001$
Ripley	22	48.7	3.2	35.1	4.9	$0.05 > P > 0.02$
Anti-staph*	19	46.9	4.9	43.9	4.7	$P > 0.5$

* Erythrocytes first passively sensitized with staphylococcal antigen.

using erythrocytes coated with anti-staphylococcal or Ripley γG ($P > 0.2$). Adherence to or ingestion of erythrocytes coated with anti-D γG or anti-staphylococcal γG antibodies was rarely observed on basophils or eosinophils. These findings indicated that receptors active in phagocytosis mediated by human γG exist on both human neutrophils and monocytes.

Addition of Cohn fraction II to the well containing the cell monolayer before or at the same time of addition of the sensitized erythrocytes inhibited rosette formation. With concentrations as low as 50 μg/ml, inhibition was virtually complete. The minimum inhibitory concentration (lowest concentration which reduced the number of leukocytes showing rosette formation by 50% or more) was found to be between 12.5 and 25 μg/ml. Fraction II was equally effective in inhibiting rosette formation by Ripley-sensitized erythrocytes around monocytes and neutrophils. The Fc subunit of γG obtained by papain digestion of Cohn fraction II was found to inhibit 50% of the rosette-forming activity at a concentration of 4 mg/ml; however, no inhibition of rosette formation by Fc fragment was noted below this concentration. The Fab subunit of γG showed no inhibition at concentrations of 2, 4, and 8 mg/ml.

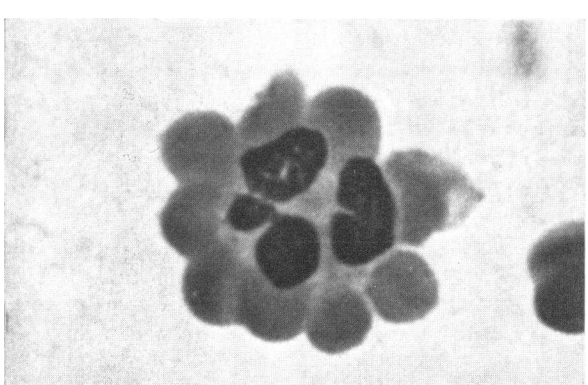

FIGURE 2 Adherence of erythrocytes sensitized with Ripley γG to a human neutrophil.

In an attempt to define the subclass of the γG antibodies active in promoting adherence of antigen to phagocytic cells, a panel of isolated myeloma globulins was examined for ability to inhibit rosette formation by Ripley-coated erythrocytes. Serial dilutions of myeloma proteins were added to cell monolayers and incubated for 30 min at 37°C before adding the sensitized erythrocytes. The minimum inhibitory concentration of all six γ_1 myelomas tested was 12.5 μg/ml. The minimum inhibitory concentration of all four of the γ_3 myelomas tested was also 12.5 μg/ml. Both γ_1 and γ_3 myelomas completely inhibited rosette formation at concentrations of 50–500 μg/ml. None of the four γ_2 or the three γ_4 myeloma proteins tested had any significant inhibiting effect at concentrations up to 500 μg/ml. No difference was apparent in the ability of myelomas of either the γ_1 or γ_3 subclass to inhibit rosette formation around monocytes or neutrophils. Contamination of γ_1 myelomas with γG globulin of the γ_3 subclass was checked by Gm typing. Gm(b) antigens were not present in the γ_1 preparations. Similarly no Gm(a) antigens could be detected in the γ_3 myeloma preparations. The lack of antigens unique to the γ_3 subclass in γ_1 myeloma preparations indicated that the similarity of the inhibiting activity of these two subclasses was not due to cross-contamination by γG globulin of the γ_1 or γ_3 subclass. If erythrocytes were sensitized with conventional anti-D γG instead of Ripley γG the pattern of inhibition by myeloma globulins was the same.

The possibility that an intermediate opsonic substance, produced and released by the leukocytes, combined with the γG-erythrocyte complex via the Fc portion of γG and then attached to a non-γG receptor on the cell was considered. To test this possibility leukocyte monolayers were incubated for 2 hr with the following metabolic inhibitors: sodium azide (0.01 mole/liter and 0.1 mole/liter), puromycin (10 μg/ml), and actinomycin D ($10–10^{-8}$ μg/ml). The monolayers were then washed and erythrocytes sensitized with Ripley antibody added. The sensitized erythrocytes were suspended in Hank's balanced salt solution which contained the inhibitor in

the same concentration as that in the chamber. No decrease in adherence to monocytes or neutrophils was noted after treatment with either of these three widely different metabolic inhibitors. Adherence of sensitized erythrocytes to these metabolically blocked cells could be inhibited by γ_1 myeloma globulin. Furthermore, preincubation of Ripley-sensitized erythrocytes with the supernatant medium from normal monocytes or neutrophils did not enhance their adherence to leukocytes. To determine if inhibition of rosette formation by myeloma proteins was due to a binding of the myeloma protein to the cell surface, or interaction of the myeloma with an intermediate in the medium, leukocyte monolayers were incubated with γ_1 myelomas for 30 min and then washed to remove nonadherent myeloma protein before adding sensitized erythrocytes. The minimum inhibiting concentration of γ_1 globulin was the same, 12.5 μg/ml, whether the leukocytes were washed before adding sensitized erythrocytes or the inhibiting myeloma was left in the final test mixture. A parallel set of monolayers was prepared as control and treated identically except that Hanks' balanced salt solution without sensitized erythrocytes was added. After the final incubation, Hanks' balanced salt solution was removed from the wells and assayed for γ_1 globulin concentration by determining its ability to inhibit agglutination in a Gm(f+) test system. The same γ_1 myeloma Gm(f+) was used as a standard. Supernates from wells which had not been washed after incubation with γ_1 myeloma contained the expected 6–100 μg/ml of γ_1 Gm(f+) globulin. The wells which had been washed all contained less than 3 μg/ml of γ_1 Gm(f+) globulin. The initial concentration of γ_1 globulin to which the cells were exposed appeared to be the crucial factor in inhibition rather than the γ-globulin free in the medium, suggesting that inhibition was due to binding of the myeloma globulin to a receptor on the cell surface.

To further clarify the mechanisms of inhibition of adherence by γG globulin, erythrocytes were passively sensitized with staphylococcal antigens and then incubated with Cohn fraction II (1 mg/ml) for 1 hr at 37°C. The erythrocytes were then washed three times in Hanks' balanced salt solution and added to monolayers of mixed leukocytes. In four identical experiments erythrocytes adhered to 44% (SEM = 7.7) of the monocytes and 45% (SEM = 8.6) of the neutrophils. If the passively sensitized erythrocytes were incubated with isolated γ_1 myeloma instead of Cohn fraction II, no rosette formation occurred. Similar concentrations of Cohn fraction II or γ_1 myeloma added to wells with erythrocytes sensitized directly with Ripley antibody completely inhibited rosette formation. Cohn fraction II had a direct agglutination titer of 1 : 4 with staphylococcal-antigen-sensitized erythrocytes whereas γ_1 myelomas had no agglutinating effect. It thus appeared that the ability to inhibit rosette formation was dependent primarily on γG molecules which did not react directly with antigens on the erythrocytes. These experiments also indicated that nonspecific attachment of γG to the staphylococcal filtrate sensitized erythrocytes through combination of the Fc portion of γ_1 globulins with the small amount of staphylococcal protein A (19) which might adhere to the cell surface was not a factor in promoting adherence of the sensitized erythrocytes to leukocytes.

Treatment of phagocytic cells with trypsin. Treatment of phagocytic cells with trypsin has been shown to inactivate complement receptors on the cell surface but not receptors for γG (3, 20–22). In our experiments, treatment of either mixed leukocytes or granulocytes with concentrations of trypsin from 0.1 to 1.0% did not decrease phagocytosis and killing of bacteria in the presence of γG opsonins but did decrease killing in the presence of fresh normal serum. Rosette formation with Ripley-sensitized erythrocytes was not diminished by trypsin treatment of monocytes or neutrophils. These experiments indicate that in both assay systems, receptors for γG were not destroyed by trypsin digestion and that rosette formation by γG-sensitized erythrocytes around neutrophils was not dependent on ancillary complement action.

DISCUSSION

The results reported here indicate that human neutrophils as well as mononuclear cells have surface receptors capable of binding the Fc portion of human γG antibodies. There is however a difference in the ability of γG antibodies to attach to these two cell types. Gamma G antibodies directed against *S. aureus* or the hyperimmune anti-Rh antibody Ripley attach to both neutrophils and mononuclear cells while conventional anti-Rh antibodies will attach only to mononuclear cells. In light of these results it appears that the difference in cell types active in binding γG antibodies noted by Henson (3), who demonstrated receptors for human anti *S. typhimerium* γG antibodies on neutrophils, and Huber et al. (6) or LoBuglio et al. (4) who found adherence of erythrocytes sensitized with conventional anti-Rh antibodies only on monocytes and macrophages, may have been due to the different γG antibodies used by these investigators. The hyperimmune anti-CD antibody Ripley behaves differently than the other anti-Rh antibodies tested. It is capable of mediating attachment of erythrocytes to neutrophils. It also differs from other anti-Rh antibodies in that it is heterozygous for most Gm factors (23), reacts with most human rheumatoid factors (24), and is capable of fixing complement (25). These differences in the functional capacity of γG antibodies to attach to different types of phagocytic cells

may possibly be important in the integration of humoral and cellular defense mechanisms.

The mechanism behind the ability of antibacterial or Ripley antibodies to attach to neutrophils, and the failure of conventional anti-Rh γG antibodies to do so is at present obscure. Two explanations appear plausible: (*a*) differences in spatial arrangement of the bacterial and erythrocyte antigens or of the γG receptors on the monocytes and neutrophils determine which cells will be involved, or (*b*) there are structural differences in the Fc portion of the antibodies which match with two types of receptors, monocytes having both types and neutrophils only one. The work of Phillips-Quagliata, Levine, and Uhr (*2*) supports the first theory. They suggest that the binding of antigen-antibody complexes to cell surface receptors is due to strengthening of an unstable bond between individual antibody molecules and cell receptors by the additive effect that occurs when many antibody molecules attached to a polyvalent antigen form multiple antibody-cell receptor bonds. The density of the Rh antigens on the erythrocyte surface may be insufficient to obtain the packing of anti-D antibody necessary for contact of exposed Fc structures with the minimum number of receptors on the neutrophil surface. This would imply, however, that monocytes require fewer antibody-cell receptor bonds than neutrophils to achieve this stabilizing additive effect or that the distribution of receptors on the monocyte allows better contact with the sparsely distributed anti-Rh antibodies than those on neutrophils.

Experiments reported here which demonstrate that γ_1 and γ_3 myeloma globulins are capable of inhibiting rosette formation of conventional anti-D γG sensitized erythrocytes around monocytes, and also Ripley-sensitized erythrocytes around monocytes and neutrophils suggest that the same γG receptor is active in all three situations. The pattern of γG H-chain subgroup inhibition demonstrated here for both monocyte and neutrophil receptors confirms previous work by Huber and Fudenberg (*20*) who studied γG receptors on monocytes using conventional anti-Rh coats. Further studies quantitating the exact number of γG molecules on the erythrocyte surface and comparing these results with the ability of the γG to adhere to different cell types may offer a method for resolving this question.

The probability that this receptor is specific for the Fc portion of γG and not complement or a complement-like intermediate opsonin produced by the phagocytic cells is increased by the fact that it is not inactivated by trypsin or metabolic inhibitors (*26*), and it can be inhibited by myeloma proteins adherent to the leukocytes. In addition, studies of the supernatants from leukocyte preparations provided no evidence for an intermediate activating mechanism between cell leukocyte receptor and opsonic γG.

The concentrations of Fc piece required to inhibit adherence of sensitized erythrocytes to monocytes or neutrophils were much higher than those of whole γG. It is possible that the Fc structures active in combination with the cell surface receptors were partially destroyed during the 24 hour papain digestion, or that these structures require the configuration conferred by the tertiary structure of the whole molecule for their full activity. Previous studies in our laboratory have indicated that these structures can be inactivated by acetylation, carbamylation, amidination (*27*), or mild reduction (*28*).

The possibility that receptors for γG antibodies of the γ_2 and γ_4 subclasses also exist on leukocytes cannot be excluded on the basis of data presented here. Inhibition of rosette formation by myeloma globulins using erythrocytes sensitized with anti-Rh antibodies which are primarily of the γ_1 and γ_3 subclasses (*29*) might not reveal these sites. Similarly, the small amount of γ_4 anti-Rh antibody in Ripley γG might be masked in inhibition studies using γ_4 myeloma globulins by the presence of much larger amounts of anti-Rh antibody of the γ_1 and γ_3 subclasses. However, recently presented evidence suggests that myelomas of the γ_2 and γ_4 subclasses do not bind to human monocytes (*30*).

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