

## BLOCKADE OF CLEARANCE OF IMMUNE COMPLEXES BY AN ANTI-Fc $\gamma$ RECEPTOR MONOCLONAL ANTIBODY

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Clearance of immune complexes by the mononuclear phagocyte system is important for maintaining normal host defenses against bacterial and viral assault (1), but also contributes to the pathogenesis of a variety of immune-mediated diseases. For example, removal from the circulation of IgG-coated erythrocytes and platelets by the MPS is the *sine qua non* of immune-mediated cytopenias (2, 3). On the other hand, abnormally decreased removal by the MPS of smaller, soluble immune complexes may play a role in the pathogenesis of immune complex-mediated tissue damage found in such autoimmune diseases as SLE (4).

Although the physicochemical nature and the size of immune complexes can influence rates of clearance and sites of deposition (reviewed in 5), interactions between immune complexes and the MPS *in vivo* are poorly understood. The inability to directly measure binding or internalization of immune complexes by cells in the liver and spleen has made the analysis of the molecular basis of immune complex clearance very difficult. Receptors for the Fc portion of IgG (Fc $\gamma$ R) and for complement (CR) undoubtedly play a role in the removal of immune complexes, but the relative importance of these receptors is not known.

There are three types of Fc $\gamma$ Rs on human leukocytes. A 72 kD receptor with high affinity for monomeric IgG is found on monocytes (6) and some resident macrophages (7). Two receptors exist with low affinity for monomeric IgG, one with broad electrophoretic mobility (51–73 kD) on neutrophils (8), natural killer cells (9), and macrophages (8); the other recently described (40 kD) on platelets (10), monocytes (10), and several tumor cell lines (11). All three bind immunoglobulin that is aggregated or complexed to antigen. The 51–73 kD receptor is recognized by mAb 3G8, which blocks ligand binding and has been very useful in the partial biochemical characterization of this receptor (8).

*In vitro* analysis of the role played by Fc $\gamma$ Rs in individuals with abnormally prolonged clearance of opsonized red cells (model particulate immune complexes) generally has been limited to studies of high-affinity Fc $\gamma$ Rs on monocytes.

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These studies, however, have shown no decrease in number or ligand-binding properties of high-affinity Fc $\gamma$ R<sub>s</sub> (12).

The distribution of the Fc $\gamma$ R recognized by mAb 3G8 on resident macrophages suggests that this receptor may be more relevant than are high-affinity Fc $\gamma$ R<sub>s</sub> with respect to the clearance of immune complexes. Support for this hypothesis has come from studies performed by Kurlander et al. (13) with mAb 2.4G2, which recognizes and blocks ligand binding to a murine low-affinity Fc $\gamma$ R (14). Kurlander showed that administration of 2.4G2 (both as intact IgG and as Fab fragments) dramatically prolonged clearance of several types of soluble immune complexes, but had no effect on clearance of monomeric IgG. These observations, however, may not be applicable to humans. Whereas both rodents and primates express low-affinity Fc $\gamma$ R<sub>s</sub> and CRs on their resident macrophages, only primates possess CRs for C3b and C4b (CR1) on their erythrocytes (15, 16). These erythrocyte CRs may also be important in clearance of immune complexes.

In this report, we describe results of experiments designed to investigate the role played by 51–73 kD Fc $\gamma$ R<sub>s</sub> in clearance of immune complexes by primates. We first examined localization in primate liver of cells reactive with mAb 3G8. We then established a model in primates in which we could accurately measure clearance of both particulate and soluble model immune complexes. Finally, we have used mAb 3G8 to examine the role in vivo of this receptor in immune clearance.

### Materials and Methods

*Animals.* Blood was obtained from healthy adult chimpanzees, rhesus monkeys, baboons, and gibbons maintained at the New York University Laboratory of Experimental Medicine and Surgery in Primates (LEMSIP), Tuxedo Park, NY; and from owl monkeys, African green monkeys, cebus monkeys, and stump-tailed macaques from the Atherosclerosis Research Center, Winston-Salem, NC. Chimpanzees used to study clearance of IgG-opsonized erythrocytes (RBC) were positive for blood group A<sup>c</sup>B<sup>c</sup>D<sup>c</sup>. All experimental procedures were performed at LEMSIP with animals that were lightly anesthetized with ketamine.

*Preparation of Antibodies.* 3G8, a murine IgG1 mAb (8), and MOPC 21, a murine IgG1 myeloma protein were isolated from ascites by precipitation with 45% ammonium sulfate followed by precipitation with caprylic acid (Sigma Chemical Co., St. Louis, MO) (17). Fab fragments were prepared by digestion with papain-Sepharose (Sigma Chemical Co.), and were purified by passage over protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) at pH 8.3 and by molecular sieve chromatography over a column of TSK 3000 (LKB Instruments, Inc., Rockville, MD). Proteins were >95% pure, and Fab fragments contained no detectable heavy chains, as judged by silver staining after SDS-PAGE (BioRad Laboratories, Richmond, CA). Preparations used for infusions were sterile and contained <0.25 ng of endotoxin per milligram of total protein, as determined by limulus amoebocyte assay (Associates of Cape Cod, Woods Hole, MA). Before use, antibody preparations were dialyzed against 0.15 M NaCl (USP), and aggregates were removed by ultrafiltration. mAb YZ-1 Fab (18) was the generous gift of Dr. Richard Jack, Harvard Medical School, Boston, MA.

*Preparation of Opsonized RBCs.* Chimpanzee RBCs were sensitized with chimpanzee anti-A<sup>c</sup>B<sup>c</sup>D<sup>c</sup> antiserum (generously supplied by Dr. W. W. Socha, LEMSIP). The number of molecules of IgG-RBC was determined using a solid-phase RIA. Briefly, 10<sup>8</sup> sensitized RBCs were lysed in PBS containing 1.0% NP-40 (vol/vol), 1.0% aprotinin (wt/vol), and 0.02% (wt/vol) NaN<sub>3</sub>. After removal of stroma by centrifugation (10,000 rpm, 15 min), serial dilutions of lysate were plated in 96-well microtiter plates (Dynatech Laboratories, Alexandria, VA) previously coated with affinity-purified goat Fab anti-human Ig (a

generous gift of Dr. N. Chiorazzi, The Rockefeller University). After incubation and washing,  $^{125}\text{I}$ -labeled anti-human Ig ( $10^6$  cpm/ml) was added. The approximate number of molecules of IgG-RBC was calculated from a standard curve constructed with purified human IgG.

A qualitative assessment of fixation of complement to the RBCs with the anti-A $^c$ B $^c$ D $^c$  antiserum was determined by incubating sensitized RBCs with whole fresh chimpanzee serum at 37°C for 60 min. RBC-bound C3 was detected by hemagglutination with an anti-human C3 antiserum (Cappel Laboratories, Malvern, PA).

Immediately preceding clearance studies, autologous RBCs were washed with 0.150 M NaCl and then adjusted to  $10^8$  cells/ml. Antiserum titered to yield either 2,000 or 20,000 IgG molecules per RBC (see Results), and  $^{51}\text{Cr}$  (New England Nuclear, Boston, MA) was added and incubated for 30 min at 37°C. The cells were washed and resuspended in 10 ml of 0.150 M NaCl. The cells were held on ice and used within 15 min.

*Protocol for Measuring Clearance of Opsonized RBCs.* Clearance studies of autologous, opsonized RBC were performed using minor modifications of methodology described by Frank, et al. (19).  $10^8$   $^{51}\text{Cr}$ -labeled, opsonized RBCs in 10 ml 0.15 M NaCl were injected into an antecubital vein as rapidly as possible (<5 s). Blood samples (5.0 ml) were drawn from the opposite antecubital vein 3, 5, 15, 30, 60, 90, and 120 min after injection to determine the baseline rate of clearance of sensitized RBCs. Antibody or saline was then infused over 30 min, and clearance of freshly sensitized RBCs was measured using an identical protocol.

*Preparation of Soluble Immune Complexes.*  $^{125}\text{I}$ -labeled double-stranded (ds)<sup>1</sup> DNA-anti-DNA soluble immune complexes (generously provided by Dr. Ronald Taylor, Department of Biochemistry, University of Virginia School of Medicine) were prepared as previously described (20, 21). Briefly,  $^{125}\text{I}$ -dsDNA of ~1,200–1,400 bp was incubated with anti-DNA antibodies purified from SLE plasma (Ma, previously described by Taylor and Morgan [22]) in ~40-fold antibody excess for 45 min at 37°C. The sedimentation coefficient, determined by sucrose density velocity sedimentation of the immune complexes, was <100 S.

*Experimental Protocol for Clearance of Soluble Immune Complexes.*  $^{125}\text{I}$ -dsDNA-anti-DNA or  $^{125}\text{I}$ -dsDNA alone was injected into an antecubital vein as rapidly as possible (<5 s). Blood samples (5.0 ml) were then drawn from the opposite antecubital vein every 30 s for the first 3 min, then at 5, 7.5, 10, 20, and 30 min. As above, each animal received intravenous antibody or saline over 30 min, then a second clearance study was performed using an identical protocol.

Each 5.0 ml sample of blood was immediately heparinized, placed on ice, and then centrifuged (2,000 rpm, 5 min). Cell pellets were resuspended in cold PBS, and centrifuged again (2,000 rpm, 5 min) within 10 min. Cell pellets, which contained RBC- and leukocyte-bound immune complexes, were saved and counted. Plasma and supernatants from washed cells were processed within 15 min to determine the amount of labeled dsDNA that was antibody-bound, free, or degraded. Aliquots (1.0 ml) were mixed with one-half vol of 15% TCA and allowed to precipitate at room temperature. Another 1.0 ml aliquot and saturated ammonium sulfate (for a final concentration of 40%, vol/vol) were allowed to precipitate on ice. These samples were centrifuged at 5,000 rpm for 20 min to recover precipitates and supernatants, which were counted separately. The third aliquot was saved unaltered.

*Determination of Cell Numbers.* Absolute numbers of circulating leukocytes were calculated using complete blood counts and differential counts determined by microscopic examination of stained blood smears.

*Murine Immunoglobulin in Serum and Bound to Cells.* Levels of murine immunoglobulin in serum were determined using a solid-phase sandwich RIA as previously described (23). Neutrophils and mononuclear cells were isolated from chimpanzee and human blood using a two-step Ficoll-Hypaque density centrifugation technique (24). Neutrophils and mononuclear cells were incubated with appropriate mAbs (see below), followed by fluorescein-conjugated goat anti-mouse Ig, or fluorescein-conjugated anti-rabbit Ig (Cappel

<sup>1</sup> Abbreviations used in this paper: ds, double-stranded; IC, immune complex.

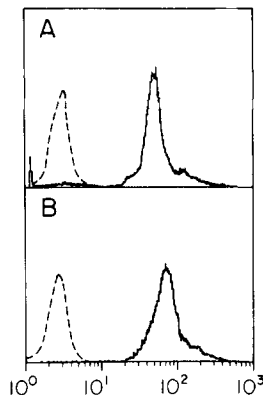


FIGURE 1. Comparison of indirect immunofluorescence of human (A) and chimpanzee (B) neutrophils. In this flow cytometric analysis, cell number is represented on the ordinate and fluorescent intensity on a log scale on the abscissa. The dotted lines represent background staining with anti-mouse Ig-FITC alone, and the solid lines show staining with mAb 3G8 IgG.

Laboratories). Cells were analyzed on a FACS IV flow cytometer (Becton-Dickinson Immunocytometry Systems, Mountain View, CA), which was calibrated before each run with 7.5  $\mu$ m fluorescent beads (Flow Cytometry Standards Corp., Research Triangle Park, NC). The mAbs used included 3G8, Leu-7 (a generous gift of Dr. Robert Evans, Sloan-Kettering Memorial Cancer Center, New York) (25), and phycoerythrin-conjugated Leu-M3 (Becton-Dickinson Immunocytometry Systems) (26).

**Immunohistological Studies.** Sections of chimpanzee and human tissue were fixed either in acetone or in a mixture of 4% (vol/vol) paraformaldehyde, 0.075 M lysine, and 0.01 M periodate, as described previously (27). Thin sections were applied to multiwell slides and incubated with mAbs. Bound antibody was detected using diaminobenzidine- $H_2O_2$  after incubation with peroxidase-conjugated goat F(ab')<sub>2</sub> anti-mouse Ig or biotin-conjugated horse anti-mouse Ig and avidin-peroxidase complexes (Vector Laboratories, Inc., Burlingame, CA).

**Cytotoxicity.** Chimpanzee and human neutrophils ( $2 \times 10^5$  cells/ml) were incubated with mAb 3G8 IgG (10  $\mu$ g/ml) in 96-well microtiter dishes, and washed. Baby rabbit complement (Pel-Freeze, Rogers, AR) was then added for 30 min. The cells were washed again, and cell death was determined by counting cell number, and using trypan blue exclusion.

**Blockade of Rosette Formation.** Chimpanzee and human neutrophils were plated on 60-well microtiter plates previously coated with 100  $\mu$ g/ml poly-L-lysine (Sigma Chemical Co.). Then the cells were incubated with either mAb 3G8 IgG or saline for 30 min at 4.5°C. After washing, 10  $\mu$ l of a 2% suspension of IgG-sensitized SRBC was added and incubated at room temperature for 30 min. Nonrosetted RBCs were removed, and rosetted cells (at least three RBCs attached to cell surface) were counted.

**Statistical Analysis.** Paired and nonpaired *t* tests were used as noted. For measurement of clearance rates, the logarithm of the percentage of radioactivity remaining in the blood was plotted as a function of time, and the clearance half-time was calculated.

## Results

**mAb 3G8 Binds to Chimpanzee Neutrophil Fc $\gamma$ R<sub>s</sub>.** We surveyed neutrophils from eight great ape and monkey species for the presence of neutrophil Fc $\gamma$ R<sub>s</sub> that resembled those on human neutrophils with respect to reactivity with mAb 3G8, as well as to function. Only neutrophils from the chimpanzee (*Pan troglodytes*) expressed the antigen recognized by mAb 3G8. Fig. 1 represents a flow-

cytometric analysis demonstrating that >95% neutrophils from both human and chimpanzees reacted with mAb 3G8 and were of similar fluorescence intensity.

Fc $\gamma$ R<sub>s</sub> on chimpanzee neutrophils also resembled human 51–73 kD Fc $\gamma$ R<sub>s</sub> with respect to function in vitro. In parallel experiments, 97  $\pm$  1.8% chimpanzee and 98  $\pm$  2.3% human neutrophils formed rosettes with IgG-coated RBCs. Furthermore, mAb 3G8 inhibited rosetting to a similar degree in both species: 82  $\pm$  6% in the chimpanzee and 87  $\pm$  4.5% in the human. In neither species was mAb 3G8 cytotoxic to neutrophils in the presence of complement.

*Tissue Distribution of Fc $\gamma$ R in Man and Chimpanzee.* We examined sections of human spleen, liver, and lymph nodes for cells recognized by mAb 3G8. Sections of spleen (not shown) showed striking segregation of reactivity to red pulp, the region that contains the majority of resident macrophages (28). White pulp, populated predominantly by lymphocytes, was negative. Sections of lymph node were also negative, suggesting that resident macrophages of different organs may differ in their expression of the 3G8 epitope.

Liver sections of both humans (not shown) and chimpanzees (Fig. 2) stained in a pattern consistent with the localization of Kupffer cells (29). Therefore, we concluded that the 3G8 epitope was present in tissue sites important in clearance of immune complexes. Furthermore, at least in liver, staining of chimpanzee and human tissue appeared comparable.

*Effects of mAb 3G8 on Clearance of Opsonized RBCs.* In our initial experiments, we observed that clearance of opsonized RBCs from the circulation of the chimpanzee was dependent on the number of molecules of IgG per RBC. Whereas RBCs sensitized with 2,000 molecules of IgG per RBC were cleared with a  $t_{1/2}$  of >70 h (Fig. 3), RBCs sensitized with 20,000 molecules of IgG per RBC were cleared with a baseline  $t_{1/2}$  of 50–100 min. Consequently, to examine effects of mAb 3G8 on clearance, all subsequent experiments were performed using RBCs coated with 20,000 molecules of IgG per RBC.

Clearance of IgG-opsonized RBCs before and after animals received infusions of saline, mAb 3G8, and MOPC 21 is shown in Fig. 4. Clearance curves before and after infusions of saline (Fig. 4A), and 1 mg/kg of MOPC 21 (Fig. 4B) were virtually superimposable. Thus, neither prolonged anesthesia nor the prior administration of opsonized RBCs per se had an appreciable effect on the subsequent clearance of opsonized RBCs. Results obtained after infusing MOPC 21, an IgG1 murine myeloma protein, indicated that a nonspecific murine immunoglobulin also had no effect on clearance.

In contrast, infusion of 1 mg/kg of mAb 3G8 IgG (Fig. 4C) increased the  $t_{1/2}$  from an average of 90 min to 4,095 min, a 48-fold increase ( $p < 0.005$ ). There was a large difference in the magnitude of effect in the two animals studied. 3G8 Fab fragments (1.0 mg/kg) (Fig. 4D) also increased the  $t_{1/2}$  significantly, from an average of 130 min to 1,772 min ( $p < 0.001$ ). Although the magnitude of increase (13.6-fold) was not as great as that seen with intact mAb 3G8 IgG, we concluded that the effect of 3G8 was at least in part due to Fab-mediated blockade of the 51–73 kD Fc $\gamma$ R.

Fig. 5 summarizes the changes in  $t_{1/2}$  in all experiments using sensitized RBCs before and after the indicated infusions, including the additional control infusion of YZ-1 Fab, an anti-CR 1 mAb. The small change in  $t_{1/2}$  seen with this antibody

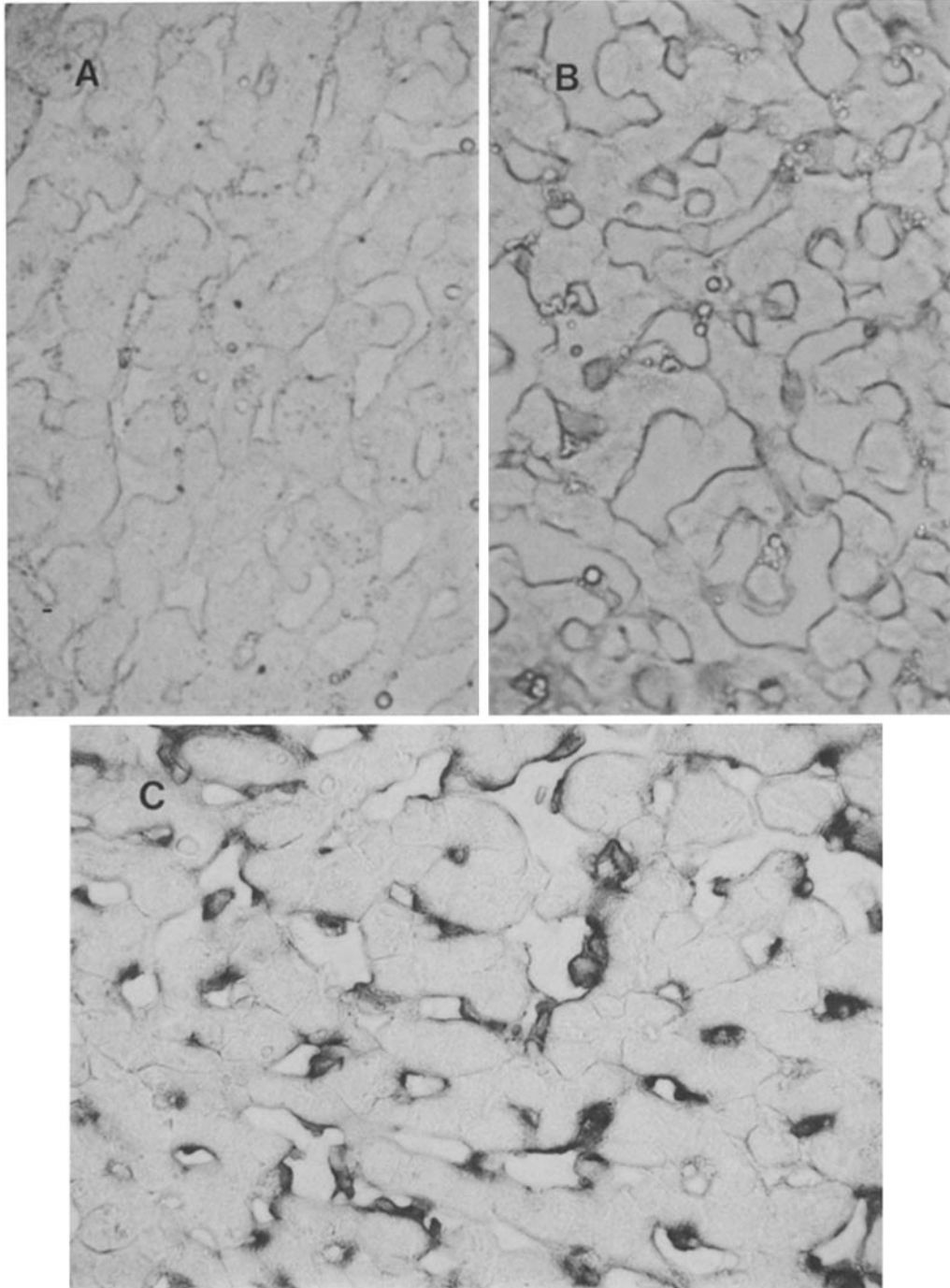


FIGURE 2. Immunoperoxidase staining of chimpanzee liver. Chimpanzee liver sections stained with control mAb (A), mAb 3G8 Fab (B), and 9.3 (anti-class II) (C). Staining in a sinusoidal pattern typical for the distribution of Kupffer cells is seen with both mAb 3G8 and 9.3.

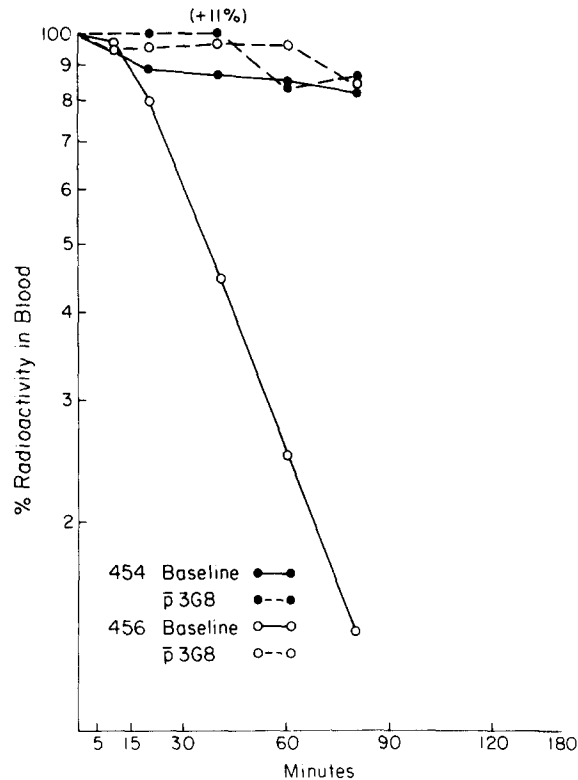


FIGURE 3. Effect on clearance of opsonized RBCs of two doses of opsonizing antibody, and the effect on clearance of mAb 3G8 IgG infusion.  $t_{1/2}$  of clearance in chimpanzee 456 was 50 min using the larger dose of opsonizing anti-RBC antibody (○—○). After infusion,  $t_{1/2}$  increased to 1,410 min (○---○). Using the smaller dose of opsonizing antibody,  $t_{1/2}$  in chimpanzee 454 was >70 h before mAb 3G8 infusion (●—●), and not significantly different from the postinfusion  $t_{1/2}$  (●---●).

(also of the IgG1 subclass) suggests that the change seen with mAb 3G8 infusions was not due simply to the nonspecific effect of interactions between phagocytes and antiphagocyte antibodies, or even to interactions between receptors involved in some types of phagocytosis and antireceptor antibodies in general. Fig. 5 also demonstrates that infusion of as little as 0.125 mg/kg of mAb 3G8 significantly increased the  $t_{1/2}$ . Effects of infusing 0.25 and 0.5 mg/kg were indistinguishable from those observed after infusion of 1.0 mg/kg.

*Murine Immunoglobulin in Serum and on Cells.* 36 min after infusion, the level of circulating mAb 3G8 IgG and 3G8 Fab in serum was >2.0  $\mu$ g/ml (Fig. 6), a concentration presumably sufficient to react with all of the 51–73 kD Fc $\gamma$ R<sub>s</sub> on circulating leukocytes and tissue macrophages (8). Both mAb 3G8 Fab fragments and 3G8 IgG disappeared rapidly from the circulation, such that no mouse Ig was detectable after 8 d. The myeloma protein MOPC 21 disappeared at a slower rate, suggesting that the rapid rate of disappearance of mAb 3G8 was not due to nonspecific catabolism of foreign immunoglobulin, but rather to removal by circulating and tissue cells that bear Fc $\gamma$ R<sub>s</sub> recognized by mAb 3G8.

Because 51–73 kD Fc $\gamma$ R<sub>s</sub> are expressed on all mature neutrophils, mAb 3G8

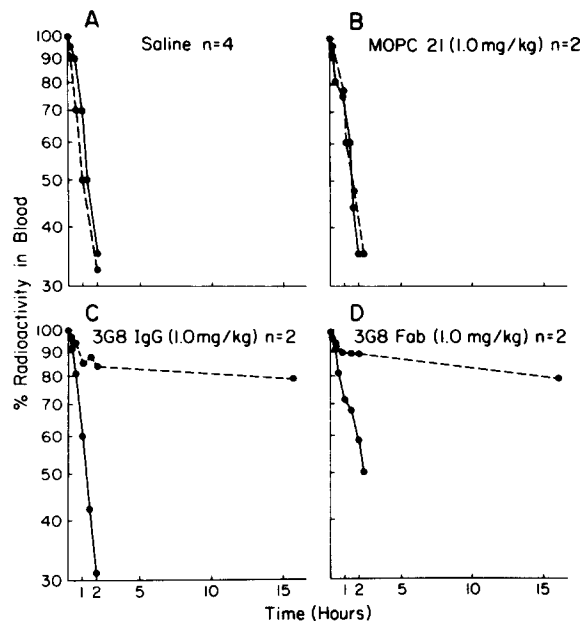


FIGURE 4. Effect of saline and antibody infusions on clearance of IgG-coated RBCs. Each figure represents clearance curves before (●—●) and after (●---●) the indicated infusions. The curves represent the average of the indicated numbers of experimental animals.

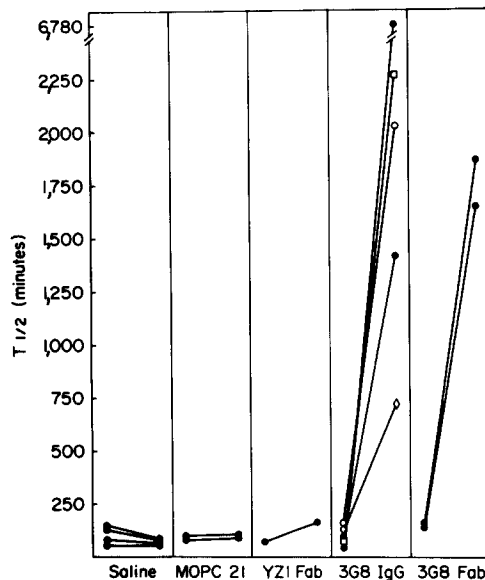


FIGURE 5. Effect on  $t_{1/2}$  (ordinate) of clearance before and after the indicated infusions. Points on left side of each panel represent  $t_{1/2}$  before infusions, and points on right,  $t_{1/2}$  after infusions. Doses of mAb 3G8 IgG are depicted in the fourth panel from the left: 1 mg/kg (●), 0.5 mg/kg (□), 0.25 mg/kg (○), and 0.125 mg/kg (◇).



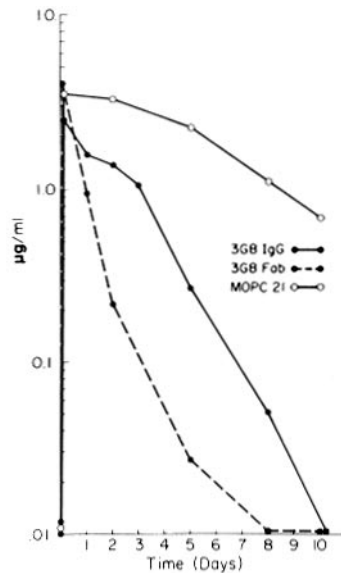


FIGURE 6. Serum levels of 3G8 IgG, 3G8 Fab, and MOPC 21. Infusions of 1 mg/kg were performed at time 0, and serial serum antibody levels were measured by solid-phase RIA. All three antibodies attained peak levels of  $>2 \mu\text{g/ml}$  immediately after infusion.

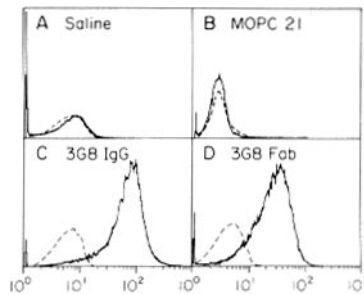


FIGURE 7. Flow-cytometric analysis of chimpanzee neutrophils after indicated infusions. Background staining of an irrelevant anti-rabbit Ig-FITC (dashed lines) is compared to the staining of an anti-mouse Ig-FITC (solid lines), which recognizes the infused antibodies.

should bind to these cells *in vivo*. We examined circulating neutrophils and mononuclear cells obtained after the infusions for bound murine IgG by immunofluorescent staining. Fig. 7 shows that, while there was no appreciable binding of saline (Fig. 7A) or MOPC 21 (Fig. 7B) to neutrophils, all neutrophils bound the infused mAb 3G8 IgG (Fig. 7C). 3G8 Fab showed comparable reactivity with neutrophils (Fig. 7D). By 8 d after infusion of mAb 3G8, there was no detectable mouse Ig on neutrophils (not shown).

There was no detectable mAb 3G8 reactivity on circulating monocytes by two-color flow-cytometric analysis using a phycoerythrin-conjugated antimonocyte mAb Leu-M3 and 3G8 (not shown). Lymphocytes were not examined for reactivity with mAb 3G8.

*Changes in Circulating Leukocyte Populations.* We monitored serial complete blood counts to study the effect of opsonization *in vivo* on circulating leukocytes

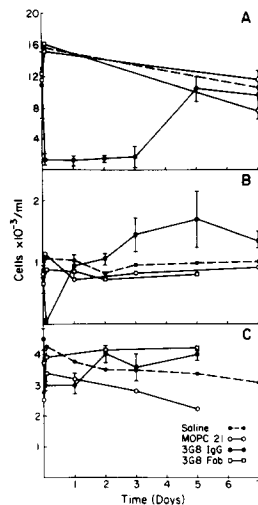


FIGURE 8. Effect of indicated antibody infusions on circulating leukocyte populations. Absolute neutrophil counts are represented in *A*, absolute monocyte counts are represented in *B*, and absolute lymphocyte counts are in *C*.

(Fig. 8). Absolute neutrophil counts (Fig. 8*A*) decreased precipitously immediately after infusion of mAb 3G8 IgG, and remained depressed until day 5, in close parallel to serum 3G8 levels. Although neutrophils displayed bright immunofluorescence with mAb 3G8 Fab, there was no decrease in absolute neutrophil counts after infusion of mAb 3G8 Fab. In fact, absolute neutrophil counts displayed only the small rise also seen with MOPC 21 and saline infusions, which is probably due to the stress of general anesthesia.

An immediate decrease in circulating monocytes was also seen only with mAb 3G8 IgG infusions (Fig. 8*B*), despite the absence of immunofluorescent staining of monocytes by mAb 3G8. The number of circulating monocytes returned to normal by 24 h, but by day 3 was increased as compared to saline controls. The number of circulating lymphocytes showed no significant differences following any type of infusion (Fig. 8*C*).

*Duration of Effect.* We repeated clearance studies at 10 and 42 d after infusion of mAb 3G8 IgG in two animals. By day 10, when the absolute neutrophil count was normal and there was no demonstrable mAb 3G8 IgG in serum or on neutrophil cell surfaces, clearance of opsonized RBC remained quite prolonged (Fig. 9). By day 42, however, clearance returned to values obtained before infusion of mAb 3G8. The chimpanzees have remained in good health 2–16 mo after mAb 3G8 infusion, without clinical evidence of immune complex-mediated diseases such as arthritis, serum sickness, or glomerulonephritis.

*Clearance of Soluble Immune Complexes.* We next examined clearance of soluble  $^{125}\text{I}$ -dsDNA-anti-DNA immune complexes. Soluble dsDNA-anti-DNA immune complexes behaved quite differently from opsonized RBCs. Both IgG-anti-dsDNA (IgG immune complex; IgG-IC) and IgM-anti-dsDNA (IgM-IC) quickly bound to RBCs after intravenous injection: 41–57% of IgG-IC and 57–78% of IgM-IC were RBC bound; the remaining counts were free in serum. In Exp. 1, both types of immune complexes were cleared rapidly from the circula-

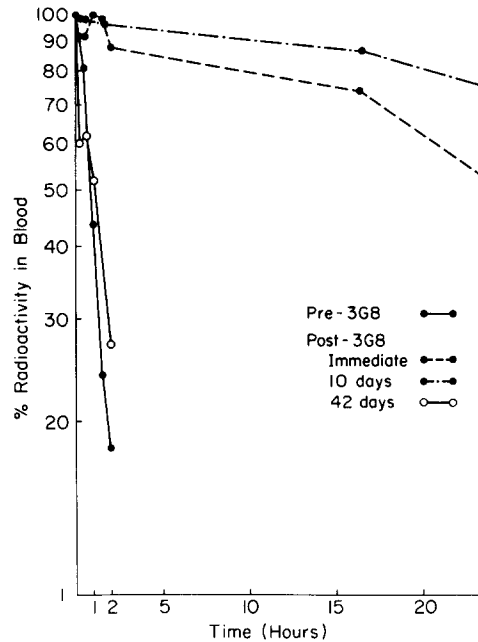


FIGURE 9. Duration of effect of mAb 3G8 IgG on the prolongation of clearance of IgG-coated RBCs.

tion, the IgG-IC with a  $t_{1/2}$  of total counts of 155 s, and IgM-IC with a  $t_{1/2}$  of 75 s. After infusion of 1 mg/kg of mAb 3G8 Fab, the clearance of both types of particles was slowed a small, and equal amount (not shown). This effect, therefore, was not specific for IgG-IC.

In Exp. 2, again 42–57% of IgG-IC were quickly bound to RBCs after injection. Neither mAb 3G8 Fab nor a control saline infusion significantly slowed clearance of either RBC-bound or free immune complexes. Interestingly, clearance rates of free immune complexes were slower than that of RBC-bound fractions, and approximated the rate of clearance of  $^{125}\text{I}$ -dsDNA that was not complexed with anti-DNA antibodies. It appeared that mAb 3G8 Fab infusions had little effect on clearance of these soluble  $^{125}\text{I}$ -dsDNA-anti-DNA complexes.

### Discussion

This paper presents both immunohistological and in vivo functional data which suggest that 51–73 kD Fc $\gamma$ R are important in clearance of immune complexes. This Fc $\gamma$ R is present in high concentration in human liver and spleen, and in chimpanzee liver, tissues important for clearance of immune complexes. We showed that intravenous infusion of an mAb that blocks ligand binding by 51–73 kD Fc $\gamma$ R dramatically prolongs clearance of IgG-sensitized RBCs in primates. This effect was seen with doses of mAb as low as 0.125 mg/kg, and persisted for at least 10 d.

Studies by Atkinson and Frank (30), and Schreiber and Frank (31) showed that rates of clearance and sites of deposition of opsonized RBCs depend upon the nature of the opsonin. RBCs sparsely coated with IgG are removed primarily

by the spleen, and the rate of clearance is relatively constant (31). RBCs opsonized with IgM and C3bi are rapidly sequestered in the liver, and then are equally rapidly released back into the circulation. A slow component of clearance follows their re-release (30). Analyses of rates of clearance after complement depletion (31) and in complement-deficient animal models (30) show that CRs probably mediate the early sequestration of IgM- and C3bi-coated RBCs (30), and possibly, the early phase of IgG-sensitized RBC clearance (31, 32). However, the relative importance of FcR and CR in later phases of clearance is unknown.

The liver is the predominant site of clearance of antibody-protein immune complexes in antibody excess (33). Work by Cornacoff, et al. (33) and Waxman, et al. (34) suggested that primate RBCs bind very large antibody-protein immune complexes via CRs for C3b and C4b (CR1) (33), and that the RBCs are stripped of immune complexes in the liver and returned to the circulation. After complement depletion, rates of clearance from the circulation increase (34), which suggests that tissue FcRs or other phagocytic mechanisms play a more important role than CR in the actual removal of soluble immune complexes from the circulation. Reduction and alkylation of internal disulfide bonds of IgG prolonged clearance of small soluble immune complexes in rhesus monkeys (35). However, the specific contribution of the Fc $\gamma$ R has not been examined, as reduction and alkylation of IgG interferes with both fixation of complement to IgG (36) and binding of IgG to Fc $\gamma$ Rs (37).

In these experiments we took advantage of the specificity of an mAb against a defined antigen, the 51–73 kD Fc $\gamma$ R, and demonstrated dramatic blockade of clearance of sensitized RBCs by small doses of 3G8. There are, however, at least three possible mechanisms to explain effects of infusion of mAb 3G8 IgG on clearance of opsonized RBCs. The first is that 3G8 Fab mediates specific blockade of 51–73 kD Fc $\gamma$ Rs. We believe that the dramatic effect of infusion of mAb 3G8 Fab on clearance of opsonized RBCs, without apparent removal of circulating neutrophils or monocytes, strongly supports this hypothesis.

The second possibility is nonspecific blockade of all phagocytosis by the MPS due to massive uptake of opsonized neutrophils. The more pronounced blockade that resulted from infusions of intact mAb 3G8 IgG suggests that both the first and second mechanisms contribute. However, decreased binding affinity of monovalent Fab fragments compared to bivalent IgG may also account for some of the difference in effect. The third possibility is that interaction with any antimacrophage antibody nonspecifically paralyzes tissue macrophages. This is not likely, because infusion of YZ-1 Fab, an anti-CR1 mAb, had very little effect on clearance of sensitized RBCs. Additionally, *in vitro* work documents the independent action of many antimacrophage antibodies directed against other epitopes and Fc $\gamma$ R function (38).

Infusions of mAb 3G8 Fab had a small and inconsistent effect on clearance of soluble dsDNA–anti-DNA complexes. Given the large array of both Fc $\gamma$ Rs and CR on phagocytes, it is possible that other receptors are more important than 51–73 kD Fc $\gamma$ Rs in clearance of this type of immune complex. In fact, data obtained in both rhesus monkeys (35) and baboons (34) suggest that complexes formed in large antibody excess (such as ours) are handled predominantly by the complement system. Additionally, the relatively lower binding affinity of mon-

ovalent mAb 3G8 Fab compared to bivalent 3G8 IgG may not be sufficient for soluble immune complex blockade. Therefore, we concluded that the effect of mAb 3G8 was confined to blockade of IgG-sensitized RBCs in our system.

The prolonged effect seen with infusions of mAb 3G8 IgG, beyond the duration that 3G8 was detected in serum or on cells, is of particular interest. This phenomenon may be due simply to occupation of receptors by levels of antibody below the sensitivity of our RIA and immunofluorescence assay, or despite the absence of *in vitro* cytotoxicity, to *in vivo* cytotoxicity to macrophages by mAb 3G8. The return of receptor function after 10 d may be due to either macrophage repopulation or to new synthesis of Fc $\gamma$ Rs. The time course of effect of mAb 3G8 IgG agrees with the theoretical turnover time of resident macrophage of 10–14 d determined in rodents by Crofton, et al. (39). Another possibility is that mAb 3G8 IgG causes irreversible modulation of Fc $\gamma$ Rs from cell surfaces of resident macrophages. However, modulation without resynthesis of receptors within 24 h would be in contradistinction to the type of modulation of Fc $\gamma$ Rs reported (40) in murine macrophage cell lines, in which resynthesis of FcRs occurred by 24 h.

Significant side effects of mAb 3G8 appeared to be limited to transient neutropenia. There was no clinical evidence of immune complex-mediated disease, such as glomerulonephritis or arthritis. The profound effect of the mAb on the clearance of sensitized RBCs, coupled with the relative absence of side effects may lead to the better understanding of the pathogenesis, and possibly to new treatment modalities of immune-mediated hemolytic anemia and thrombocytopenic purpura.

Human low-affinity, 51–73 kD IgG FcR, found on macrophages, neutrophils, and natural killer cells, may play a significant role in clearance of sensitized RBCs. We showed that Fc $\gamma$ Rs are present in human liver and spleen, and in chimpanzee liver by immunoperoxidase staining using a murine IgG1 anti-human Fc $\gamma$ R mAb, 3G8. We then established a chimpanzee model in which to study 51–73 kD Fc $\gamma$ Rs. Inhibition by mAb 3G8 of immune complex binding to chimpanzee and human neutrophils *in vitro*, and the distribution of mAb 3G8 reactivity on circulating human and chimpanzee leukocytes was comparable.

We studied the role of Fc $\gamma$ Rs in clearance of immune complexes by infusing mAb 3G8 in chimpanzees. Intravenous infusion of mAb 3G8 IgG profoundly prolonged *in vivo* clearance of model immune complexes (IgG-opsinized, autologous, <sup>51</sup>Cr-labeled RBCs) from an initial clearance half-time of 90 min to 4,095 min immediately after infusion. Infusion of mAb 3G8 Fab had a similar effect, while saline, an anti-CR1 mAb, and murine IgG1 myeloma protein infusions had little effect. Transient neutropenia occurred at 24 h only with intact mAb 3G8 IgG infusions, and resolved by day 5. The duration of effect was at least 10 d, outlasting detection of 3G8 in serum and on neutrophils. Infusions of 3G8 Fab appeared to have little effect on clearance of double-stranded (ds) DNA–anti-dsDNA soluble immune complexes. mAb blockade of Fc $\gamma$ Rs prolongs *in vivo* clearance of IgG-sensitized RBCs, suggesting that Fc $\gamma$ Rs may play an important role in clearance of this type of immune complex.

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