Complement-mediated Defect in Clearance and Sequestration of Sensitized, Autologous Erythrocytes in Rodent Malaria

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ABSTRACT We investigated the ability of malaria-infected and normal mice to clear particulate immune complexes consisting of autologous erythrocytes sensitized with either IgG or complement.

Normal mice rapidly clear autologous erythrocytes optimally sensitized with IgG and the liver plays a major role in their sequestration. Clearance of optimally sensitized erythrocytes is complement-dependent because cobra venom factor-treated, normal mice failed to clear these cells rapidly, unless they had been pretreated with fresh mouse serum.

In the initial phase of *Plasmodium berghei* infection, clearance of the optimally sensitized erythrocytes was markedly increased over that observed in normal mice. 2 wk after infection, however, clearance was minimal. This defect was most likely the consequence of the hypocomplementemia observed at this stage of infection since sensitized erythrocytes were removed rapidly from the blood if they had been coated with C3 in vitro before injection into malarial mice.

The functional activity of the complement receptors of phagocytic cells was assayed in malarial mice by the injection of autologous erythrocytes coated with C3 and C4 in the absence of antibody. The complement-coated erythrocytes were rapidly removed from the blood, accumulated in the liver, and then gradually returned to the circulation. Similar patterns were observed in normal animals, but the degree of clearance was considerably higher in the malarial mice late in infection. It appears, therefore, that complement receptors remain functional throughout the infection.

Erythrocytes suboptimally sensitized with IgG were cleared minimally by normal mice. This clearance was not complement-dependent and was mediated mainly by the spleen. During malaria, clearance of these particles was initially enhanced but later it was abolished.

The association of hypocomplementemia with a major splenic defect in clearance late in malaria infection may explain the accumulation of immune complexes in pathological sites observed in this disease.

INTRODUCTION

Malaria is characterized by several pathological manifestations which include anemia, splenomegaly, and hepatomegaly, hypocomplementemia, and glomerulonephritis (reviewed in 1). In attempting to clarify the mechanisms involved in these multiple defects, we observed that during the course of *Plasmodium berghei* infection in mice, both parasitized and nonparasitized erythrocytes are coated with IgG and sometimes IgM (2). Further studies (3) demonstrated that early during this infection spleen macrophages become highly activated and can ingest the Ig-coated parasitized and nonparasitized erythrocytes. Later, however, phagocytosis of the sensitized erythrocytes is severely diminished, perhaps contributing to the lethal outcome of the infection.

The experiments described in the present paper were undertaken to analyze the nature of this defect, and to determine if it led to a generalized impairment of in vitro clearance of immune complexes.

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METHODS

Reagents and solutions. Phosphate-buffered saline with or without Ca^{++} and Mg^{++}, Dulbecco's modified Eagle medium and penicillin-streptomycin solution were obtained from Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.; EDTA, glucose, and sucrose, from Fisher Scientific Co., Pittsburgh, Pa.; cobra venom factor (CVF)[1] from Cordis Laboratories Inc., Miami, Fla.; sodium heparin from Abbott Diagnostics, Diagnostic Products Dept., North Chicago, Ill.; glutaraldehyde from Sigma Chemical Co., St. Louis, Mo.; and bovine serum albumin and fetal calf serum, lot 29101068, from Flow Laboratories, Inc., Rockville, Md. Acid citrate-dextrose solution, pH 6.8, was prepared as described (4). Veronal buffer containing Ca^{++} and Mg^{++} (VBS) was prepared as described (5). Low ionic strength sucrose solution (LISS) was prepared by dissolving 9.24 g of sucrose in 90.9 ml of 0.005 M NaH_2PO_4, 9.0 ml of 0.005 M Na_2HPO_4, and adding 0.1 ml of a solution of 1.5 M Mg^{++} and 0.15 M Ca^{++} [modified from (6)].

Animals. We used 7–10-wk-old female A/J mice from Jackson Memorial Laboratories, Bar Harbor, Maine.

Infection of mice with P. berghei. The NK 65 strain of *P. berghei* was used in all experiments. This strain has been maintained in hamsters by passage with malaria-infected blood, alternating with mosquito-induced infections (7). Mice were injected intravenously with 10^4 infected erythrocytes. At 3, 5, 7, 14, and 21 d after injection, the mean levels of parasitemia of the infected mice were determined from Giemsa-stained smears (2). For the clearance experiments performed at different stages of infection, we selected mice whose parasitemias did not deviate more than ±2 SD from these means.

Complement-depleted mice. Some groups of mice were depleted of complement before injection of sensitized erythrocytes by multiple intravenous injections of CVF. 3 U of CVF were given at −36 h, followed by injection of 1 U of CVF at −24, −12, and 0 h. Serum depletion of C3 to <5% of normal levels was confirmed by rocket immunoelectrophoresis using monospecific antisera.

Preparation of antiserum. A New Zealand rabbit was immunized by two intravenous injections of 1.0 ml washed, packed A/J erythrocytes (E) on days 0 and 3, followed by three intramuscular injections of the same amount of E on days 6, 14, and 21. The rabbit was exsanguinated 4 wk after the primary immunization and the serum was collected.

The globulin fraction of the antiserum was obtained by precipitation with 50% NH_4SO_4. The precipitate was dissolved to one-half the original volume in distilled water, dialyzed for 24 h against 0.15 M NaCl containing 5 mM PO_4, pH 7.4 and filtered through a Sephadex G-200 column. The second optical density peak containing IgG was concentrated by ultrafiltration (Amicon Corp., Scientific Sys. Div., Lexington, Mass.) and rechromatographed on the same column.

Labeling with ^{51}Chromium. $Na(^{51}CrO_4)$ 1 mCi/ml in sterile isotonic saline, was obtained from New England Nuclear (Boston, Mass.). Fresh E, obtained by bleeding anaesthetized animals into 50 U of heparin, were washed three times in acid citrate-dextrose solution. 3 ml of washed E (5.5×10^8 cells/ml) in acid citrate-dextrose solution were incubated with 75 μCi sodium dichromate in a 37°C water bath for 45 min, with frequent mixing. The labeled E were washed three times in the cold and resuspended to 10^8 E/ml in Dulbecco's modified Eagle medium.

Preparation of IgG-sensitized E and complement-sensitized IgG-coated E. Equal volumes of different dilutions of rabbit antimouse erythrocyte IgG and suspensions of washed ^{51}Cr-labeled E at 10^8 cells/ml were incubated in a 37°C water bath for 30 min with frequent mixing. E sensitized with an equal volume of a 1:500 dilution of IgG (final dilution = 1:1,000, ~28,500 molecules/cell) are designated EIgG.10^{-3} and so on. All erythrocyte suspensions were centrifuged at 500 g, 4°C for 10 min, the supernates discarded, and the cells washed once more in Dulbecco's medium. No microscopic agglutination of the sensitized E was noted in any of the samples.

IgG-coated E (10^9/ml in VBS) were sensitized with complement by the addition of an equal volume of fresh normal mouse serum (8). The suspension was mixed and incubated for 2 min at 37°C in a water bath. The complement-sensitized E were then diluted 15-fold with ice cold VBS and washed twice more in VBS. Antibody-sensitized E in phosphate-buffered saline were incubated with fresh mouse serum containing 0.01 M EDTA as controls.

Preparation of complement-sensitized erythrocytes in low ionic strength sucrose solution (LISS). ^{51}Cr-labeled E were suspended in LISS at a concentration of 5×10^8 cells/ml. 0.1 ml of the cell suspension was added to 0.83 ml of LISS, followed by 66 μl of fresh mouse serum. The cell suspension was incubated in a 37°C water bath for 10 min with frequent mixing, then diluted 15-fold with ice cold phosphate-buffered saline and washed three times. Nonsensitized E were handled in a similar manner except that complement was not added to the cell suspensions.

Hemagglutination of the complement-sensitized cells (EC) with rabbit antimouse C3 and rabbit antimouse C4 was performed. Anti-C3 and anti-C4 were prepared as described (9, 10). These antisera reacted only with C3 or C4 by crossed immunoelectrophoresis against mouse serum. The EC were agglutinated by a 1:1024 dilution of rabbit anti-C3 and a 1:64 dilution of rabbit anti-C4.

Sensitization of E with complement was confirmed by their adherence to normal mouse peritoneal macrophages. Macrophages were prepared as in (11) and adherence determined (8). 92% of macrophages were heavily coated (>14 EC/macrophage) with EC prepared as described, but no ingestion was observed.

E clearance and spleen and liver sequestration. Mice were injected with 0.2 ml i.v. (4×10^7 cells) of ^{51}Cr-labeled non-sensitized, or sensitized E. The counts per minute in each preparation were determined before each experiment by counting 10-μl samples of the E suspension in a gamma scintillation counter. (Nuclear-Chicago Corp., Des Plaines, Ill.). Erythrocytic clearance was determined by serial 25-μl bleedings from the retro-orbital plexus with a calibrated, heparinized lambda pipette. These samples represent ~1/60th of the total blood volume of a normal 7–10-wk-old A/J mouse. The blood samples were suspended in 1.0 ml of cold phosphate-buffered saline, centrifuged at 500 *g* for 10 min at 4°C and the supernates removed from the E pellets. The counts per minute in the pellets and the supernates was determined after subtracting background counts per minute. The percentage of injected counts per minute in each pellet was plotted on semi-log graphs. In 10 randomly chosen experiments, the counts per minute in the supernates were minimal throughout the experiment, indicating that little lysis of E had occurred.

To determine spleen and liver sequestration of the labeled

[1] *Abbreviations used in this paper*: CVF, cobra venom factor; E, mouse (A/J) erythrocytes; EC, E sensitized with mouse complement components; EIgG.10^{-3}, E sensitized with a 1:1000 dilution of rabbit IgG anti E; EIgG.10^{-3}.C, EIgG.10^{-3} sensitized with mouse complement; EIgG.2×10^{-4}, E sensitized with a 1:5000 dilution of rabbit IgG anti E; LISS, low ionic strength sucrose; VBS, veronal-buffered saline.

E, mice were sacrificed by exsanguination at several time-points after injection of the ^{51}Cr-labeled E. Livers and spleens were removed and the radioactivity of the whole organs was determined. After subtracting background counts per minute, the percent retention of E was determined.

Statistical analysis. Clearance data were analyzed using least squares analysis and the paired *t* test. Liver and spleen sequestration data were interpreted using the group *t* test.

RESULTS

To study the alterations in the fate of immune complexes during the course of malaria infection in rodents, we adapted to mice the techniques used in guinea pigs and humans (reviewed in 12) to study clearance of Ig-coated autologous erythrocytes. Normal and malarial mice were injected with autologous E coated with IgG antibody and their fate was determined as described in the Methods section. To determine the role of complement, clearance was studied in normal mice that had been injected with CVF to deplete C3. In addition, normal and infected mice were injected with E coated with fragments of autologous C3 and C4 (6), in the absence of antibody. In the sections below, we first describe the results in normal mice and then the alterations observed in malarial animals.

Clearance of IgG-sensitized E by normal mice. Normal mice were injected with autologous E sensitized with variable dilutions of IgG antibody prepared in rabbits. As shown in Fig. 1, E sensitized with a 1:1,000 dilution of IgG (EIgG.10^{-3}), were rapidly removed from the circulation. Further dilution of the antibody led to a very substantial decrease in clearance.

Sequestration of the E by the spleen and liver was determined 180 min after injection (Fig. 2A). At this time, the livers of normal mice contained 5% of non-sensitized E and about 60% of the injected EIgG.10^{-3}. Splenic sequestration of E and EIgG.10^{-3} was not significantly different. In contrast, when the E were sub-optimally sensitized (EIgG.2 × 10^{-4}), similar numbers of E were sequestered in the spleen and liver. Thus, the spleen was about 16 times more efficient than the liver by weight, in the removal of EIgG.2 × 10^{-4}.

Clearance of IgG-sensitized E in noninfected, complement-depleted mice. In vivo depletion of C3 by CVF affected both the patterns of clearance and organ sequestration of EIgG.10^{-3}. The disappearance of cells from the circulation was greatly retarded (compare Figs. 1 and 3). Splenic sequestration was increased more than five-fold as compared to nontreated mice, while liver uptake was greatly diminished (Table I).

When EIgG.10^{-3} were preincubated with fresh mouse serum as a source of complement, washed by centrifugation and then injected into CVF-treated mice, the clearance defect (Fig. 3) and the abnormal sequestration pattern (Table I) were not observed. As a control, EIgG.10^{-3} were incubated in EDTA-serum before in-

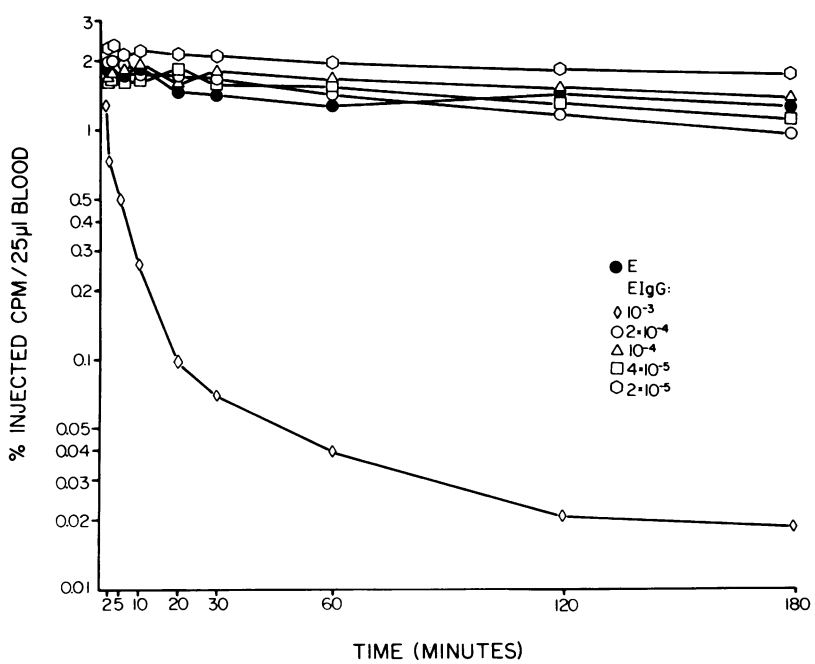

FIGURE 1 Clearance of ^{51}Cr-labeled E sensitized with varying amounts of IgG. Normal mice were injected intravenously with 4 × 10^7 nonsensitized cells or E sensitized with different dilutions of IgG. Data points represent the means of at least two mice.

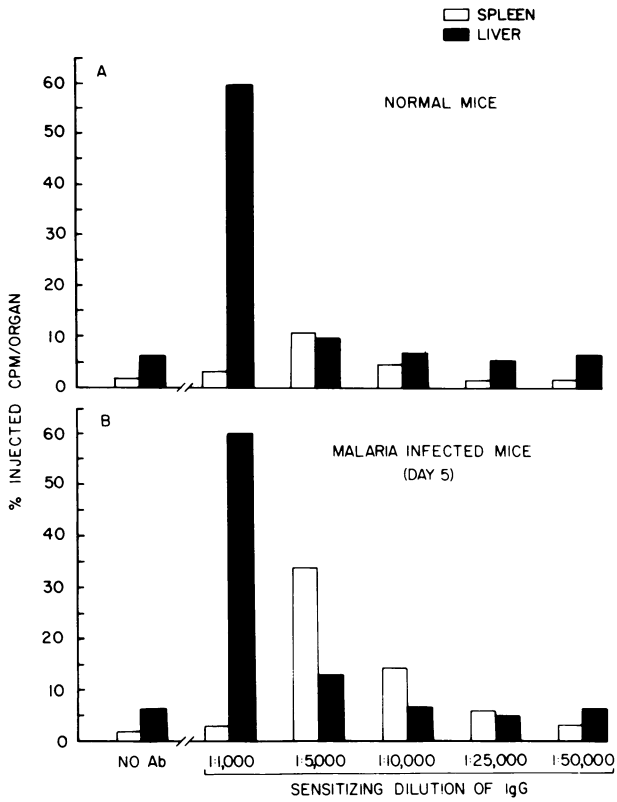

FIGURE 2 Sequestration in the spleens and livers of ^{51}Cr-labeled E sensitized with varying dilutions of IgG. (A) Normal mice. (B) Mice infected for 5 d with *P. berghei*. Mice were injected intravenously with 4×10^7 E sensitized with dilutions of IgG varying from 10^{-3} to 2×10^{-5}. 3 h after injection, the animals were exsanguinated. The spleens and livers were excised and counted in a gamma scintillation counter.

jection. Clearance of these cells (which may bear C1q) was similar to that of nontreated EIgG.10^{-3}, except that splenic sequestration was significantly enhanced. The reason for this increse is not known.

Changes in clearance and sequestration patterns of IgG-sensitized E during malaria. The patterns of clearance of EIgG.10^{-3} changed progressively during the course of *P. berghei* infection. During the 1st wk it was enhanced, but after 14 d a very sharp decrease in clearance was observed (Fig. 4). In fact, at late stages of infection, the patterns of clearance of sensitized and normal E were very similar.

In normal mice and in mice infected for 3, 5 and 7 d, 50–60% of the sensitized E were sequestered in the livers, 3 h after injection, while less than 10% were found in the spleens. During the 3rd wk of infection, however, similar to what had been observed in CVF-treated mice, liver uptake greatly diminished while splenic localization increased (Fig. 5A).

Evidence that the defect in the clearance of EIgG.10^{-3} late in infection is complement-mediated. During *P. berghei* infection, levels of C3 antigen in serum increase during the 1st wk of infection but decrease to about 25% of normal by the 3rd wk (13). The present results indicate that the rise and fall of C3 levels are accompanied by analogous changes in the efficiency of clearance of EIgG.10^{-3} and that the defect in clearance might be a consequence of the hypocomplementemia. The experiment illustrated in Fig. 6 supports this hypothesis. Clearance of EIgG.10^{-3} by malarial mice late in infection is greatly accelerated if the erythrocytes are presensitized with complement before injection. In fact, EIgG.10^{-3}.C were cleared significantly faster in malarial than in normal mice.

Most of the EIgG.10^3.C cells were retained in the livers of the infected mice. 3 h after injection, the number of E was greater in livers of mice infected for 21 d than in normal mice ($P < 0.02$). However, the clearance of EIgG.10^{-3} that had been preincubated in EDTA-serum was not significantly different from that of EIgG.10^{-3} (data not shown).

Clearance and sequestration of EC in malaria-infected mice. Additional direct evidence that late in malaria infection mice can clear EC, is shown in Fig. 7. In these experiments animals were injected with autologous E sensitized with autologous complement in the absence of antibody. This was achieved by incubating the E in a low ionic strength sucrose solution (6). Fragments of complement components C4 and C3 were detected on the E surface by direct hemagglutination using specific antisera. In normal mice, the EC were initially cleared from the blood, but gradually returned to the periphery. The release was maximal after ~30 min.

The clearance curves of EC in the infected animals are also shown in Fig. 7. The patterns are similar to those of normal mice but both the initial removal of the cells and their subsequent release into the circulation were more pronounced, particularly in animals infected for 21 d ($P < 0.05$).

5 min after injection, most of the EC were found in the liver. Sequestration in the spleen was minimal and not significantly different in malarial as compared to normal mice (data not shown).

Evidence for a defect in splenic clearance of suboptimally sensitized E late in malaria infection. E sensitized with the lower dose of IgG, (EIgG.2×10^{-4}, ~5,700 molecules/cell) were cleared at an increased rate during the early phase of malaria, i.e., up to the 7th day of infection (Fig. 8). Late in infection, these cells were not cleared differently than nonsensitized erythrocytes. Preincubation of EIgG.2×10^{-4} in fresh serum did not affect the pattern of clearance in normal or malarial mice (not shown).

Spleen sequestration was also greatly increased during

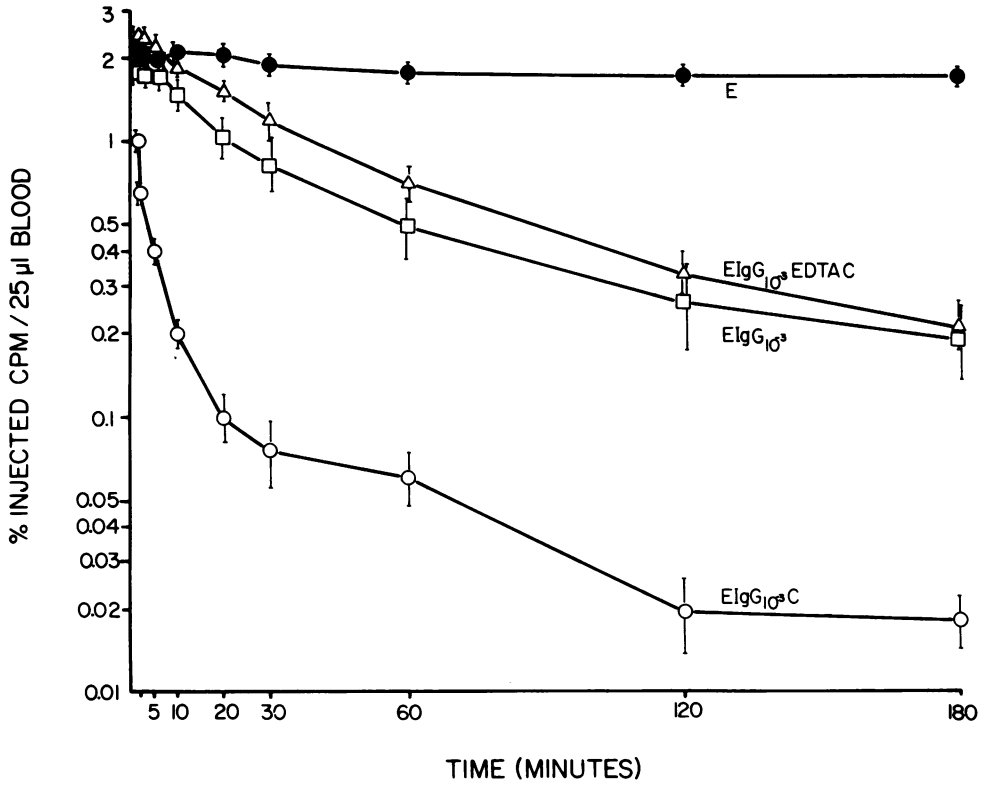

FIGURE 3 Clearance of 4×10^7 ^{51}Cr-labeled E in CVF-treated mice. Clearance was determined from 25-µl blood samples taken at the time points indicated. (●) E, (□) EIgG.10^{-3}, (○) EIgG.10^{-3}. C, and (△) EIgG.10^{-3} incubated with serum containing 10 mM EDTA. Data points represent the mean±1 SEM of three to seven mice in two to six experiments.

TABLE I
Sequestration of ^{51}Cr-Labeled EIgG.10^{-3} in the Spleens and Livers of Normal and CVF-treated Mice

E injected	Sequestration	
	Spleen	Liver
	% injected cpm	
CVF-treated mice		
E	1.4±0.03 (3)	6.8±0.20 (3)
EIgG	16.3±3.57[a] (7)*	37.2±5.03[b] (7)
EIgG incubated with fresh mouse serum	1.7±0.15[c] (7)	64.5±3.80[d] (7)
EIgG incubated with EDTA-mouse serum	30.7±4.03[e] (5)	29.5±3.27[f] (5)
Normal mice		
EIgG	3.1±0.76[g] (15)	59.6±1.76[h] (15)

All data represent the mean±1 SEM at 180 min after injection. The number of mice is in parentheses.
* Statistical analyses: a vs. g, $0.02 > P > 0.01$; b vs. h, $0.01 > P > 0.01$; c vs. g, d vs. h, b vs. f, nonsignificant; a vs. e, $0.05 > P > 0.01$.

the 1st week of infection. For example, on the 5th d of infection the sensitized E were found in much greater amounts in spleens (36%) than in livers (12%), despite the considerable difference in size of these organs (Fig. 5B). This corresponds to more than a threefold increase in spleen sequestration over that observed in normal mice. In fact, mice infected for 5 d with *P. berghei* showed significantly increased ($P < 0.05$) spleen sequestration of E sensitized with as little as 1:10,000 dilution of IgG (Fig. 2B).

In sharp contrast, on the 14th and 21st d of infection, we observed a nearly complete failure in splenic sequestration of EIgG.2×10^{-4}, much below that observed in noninfected mice ($P < 0.05$) [Fig. 5B]. The number of sensitized E found in the greatly enlarged spleens of the malarial animals was not significantly different from the number of nonsensitized E sequestered in spleens of normal mice.

DISCUSSION

In the experiments described in this paper we have used sensitized, autologous E to study clearance and

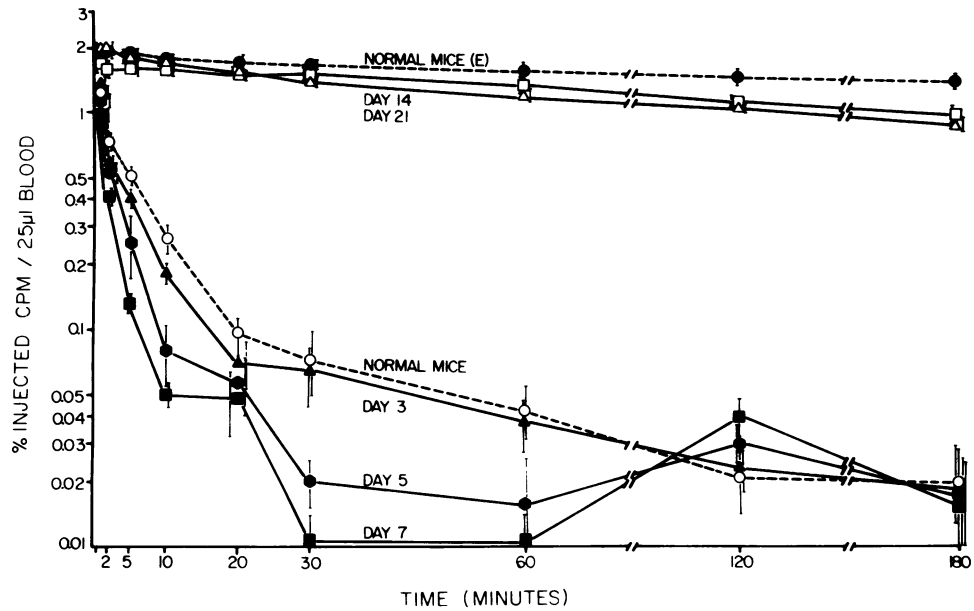

FIGURE 4 Clearance of ^{51}Cr-labeled 4×10^7 E or EIgG.10^{-3} in normal and *P. berghei*-infected mice. Clearance was determined from 25-µl blood samples taken at the time points indicated. (●) E injected into normal mice, (○) EIgG.10^{-3} injected into normal mice, and in mice 3 d (▲), 5 d (●), 7 d (■), 14 d (□) and 21 d (△) after infection with *P. berghei*. Data points represent the mean values ± 1 SEM of 6–14 mice in at least three experiments.

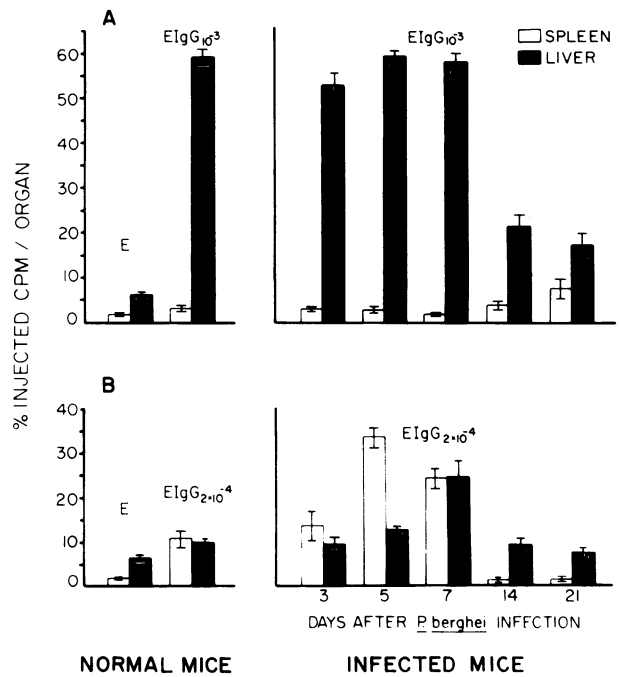

FIGURE 5 Sequestration of ^{51}Cr-labeled E sensitized with different dilutions of IgG in the spleens and livers of infected mice. (A) EIgG.2×10^{-4} (suboptimally sensitized). (B) EIgG.10^{-3} (optimally sensitized). Each mouse was injected intravenously with 4×10^7 nonsensitized or IgG-sensitized E. 3 h after injection, the animals were exsanguinated. The spleens and livers were excised and counted in a gamma scintillation counter.

organ sequestration of immune complexes in normal mice and in the course of malaria infection.

Clearance of sensitized E in normal mice. The observations on the clearance of EIgG and EC in normal mice confirm previous findings of Frank et al. (12) in humans and guinea pigs, that the liver is the major site of clearance of complement-coated particles, presumably as a consequence of their interaction with immunoadherence receptors found on the membranes of Kupffer cells that line the sinusoids. If both complement and IgG are present on the surface of the E, they are phagocytosed and destroyed. However, in the absence of IgG, the red cells only adhere to the phagocytes and eventually return to the circulation after further cleavage of the membrane-bound C3b and C4b by C3b/C4b inactivator. The interpretation of the in vivo findings has been substantiated by in vitro experiments indicating that the Fc and C3 receptors have different and synergistic roles in phagocytosis. The main role of C3 receptors is to bind the sensitized E while the Fc receptor-IgG interaction effectively triggers the ingestion process (14, 15).

Suboptimally sensitized erythrocytes were removed from the circulation much less efficiently and were localized to a greater extent in the spleen (Figs. 1 and 2B). Identical results were observed when these red cells were injected in CVF-treated mice (not shown); thus, it appears that clearance under these conditions is complement-independent. Atkinson et al. (16) also found that in C4-deficient guinea pigs injected with

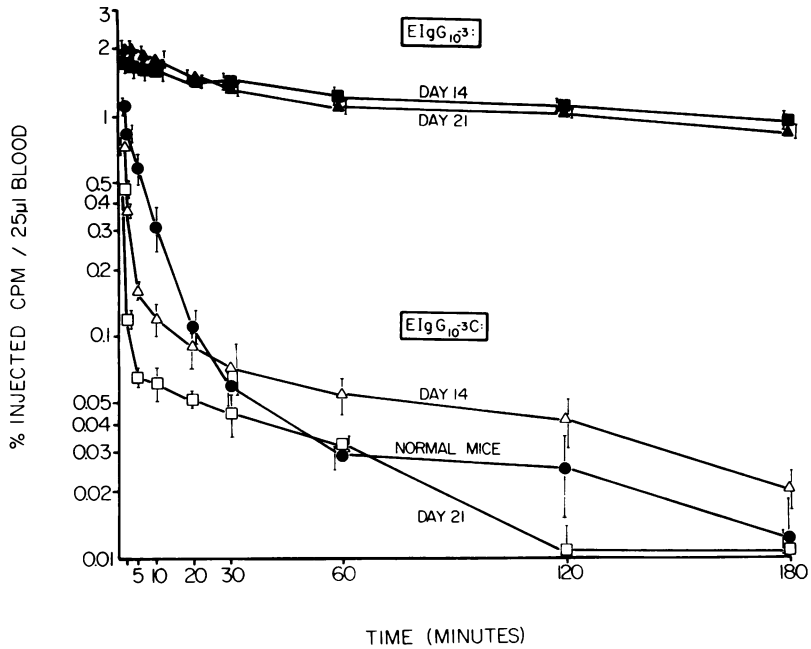

FIGURE 6 Clearance of 4×10^7 ^{51}Cr-labeled EIgG.10^{-3} and of EIgG.10^{-3}.C in normal and *P. berghei*-infected mice. Clearance was determined from 25-µl blood samples taken at the time points indicated. EIgG.10^{-3} injected into malarial mice 14 (■) or 21 (▲) d after injection. EIgG.10^{-3}.C injected into normal mice (●), and mice infected for 14 d (△) and 21 d (□) with *P. berghei*. Data points represent the mean values±1 SEM of four mice in two experiments.

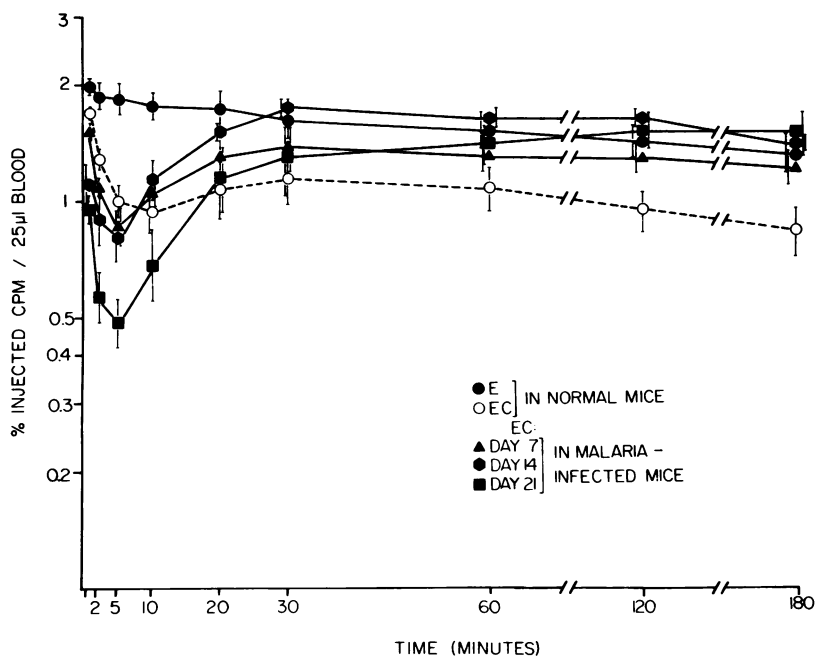

FIGURE 7 Clearance of 4×10^7 ^{51}Cr-labeled EC in normal and *P. berghei*-infected mice. Clearance was determined from 25-µl blood samples taken at the times indicated. Nonsensitized E injected into normal mice (●), EC injected into normal mice (○), and mice infected for 7 d (▲), 14 d (●) and 21 d (■) with *P. berghei*. All data points are the mean values±1 SEM of four mice in two experiments.

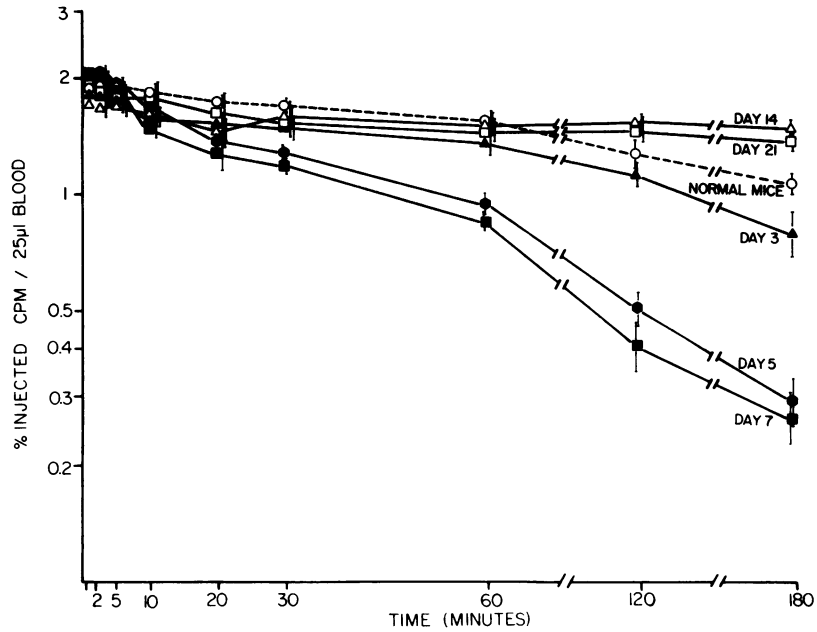

FIGURE 8 Clearance of 4×10^7 ^{51}Cr-labeled, EIgG.2 $\times 10^{-4}$ in normal and *P. berghei*-infected mice. Clearance was determined from 25-µl blood samples taken at the time points indicated in normal mice (○) and in malarial mice 3 d (▲), 5 d (●), 7 d (■), 14 d (△), and 21 d (□) after infection with *P. berghei*. Data points represent the mean values±1 SEM of 6–15 mice in at least three experiments.

autologous E sensitized with low doses of IgG, the E were cleared mainly by the spleen. When the concentration of IgG used to sensitize the E was increased, leading to complement fixation, splenectomy became less effective because the liver was better able to remove the cells from circulation. Some of the possible reasons to explain the high efficiency of the spleen as a filter for the Ig-coated, noncomplement-sensitized particles have been discussed elsewhere (12).

In light of the information obtained in normal mice, we next analyzed the alterations in clearance during malaria infection. Different pictures were observed at different stages of the disease.

Enhancement in clearance during the early phases of infection. During the early stages of the infection clearance was markedly enhanced (Figs. 4 and 8) and, similarly to what had been observed in normal mice, the spleen played a prominent role in retaining the lightly sensitized autologous E (Fig. 5B). Moreover, at this time malarial mice were capable of recognizing and sequestering E sensitized with relatively small amounts of antibody, as compared to normal animals (Figs. 2B and 8). These findings, taken together with previous observations that during infection of rodents with *P. berghei* (2) or humans with *P. falciparum* (17) parasitized and normal E are coated with IgG, suggest that the enhanced erythrophagocytosis associated with this disease may be, at least in part, immunologically mediated. Woodruff et al. (18) also recently showed in humans that even after complete eradication of malarial parasites, E have immunoglobulin and complement on their membranes and suggested that this may be an important factor causing anemia.

Of particular relevance to the hypothesis that immunological factors play an important role in the sequestration of E in this infection, are the recent observations that splenic macrophages are activated in malarial mice (3) and that at the initial stages of the disease, complement levels in serum are significantly increased (13). However, it should be pointed out that other nonspecific factors such as erythrostasis (19), splenomegaly, as well as modification in the membrane (20) and deformability (21) of infected E most likely also contribute to enhanced erythrophagocytosis.

Defects in clearance in the late stages of malaria. During the late stages of the disease, however, we observed profound deficiencies in clearance. The defects appear to be caused by two abnormalities, splenic malfunction and complement deficiency.

The splenic defect was demonstrated when EIgG.2 $\times 10^{-4}$ were injected into malarial mice. 2–3 wk after infection, the spleen did not recognize these particles (Fig. 5B) although it was grossly enlarged. That hypocomplementemia plays a role in the pathogenesis of this defect is improbable since normal mice injected with CVF show, on the contrary, a marked increase

in the uptake of sensitized E by the spleen (Table I). It is more likely that the splenic malfunction is due to the saturation of the macrophage Fc-receptors by circulating immune complexes. Indeed, recent in vitro experiments have shown that serum obtained late during murine malaria infection, drastically reduced the phagocytosis of sensitized E by normal spleen macrophages. Although the inhibitory factor has not been fully characterized, it is likely to consist, at least in part, of Ig, since it can be removed with immobilized protein A (3). Functional defects in Fc-receptor handling of immune complexes have also been reported in patients with systemic lupus erythematosus (22).

An additional defect was observed late in infection; that is, the malarial mice removed EIgG.10^{-3} from circulation very slowly (Fig. 6). This defect was a consequence of the hypocomplementemia characteristic of this phase of the disease. Indeed, malarial mice cleared EIgG.10^{-3}.C normally (Fig. 6) and these cells were sequestered in the liver. These findings suggested that the complement receptors of liver macrophages were functional during the later stages of the disease. This was confirmed by injecting normal and malarial mice with E sensitized with complement in the absence of antibody. An even larger proportion of the EC disappeared from the circulation of malarial mice than in normal mice (Fig. 7) and accumulated in their livers. The return to circulation after hepatic sequestration was also more pronounced in malarial mice, perhaps because their serum contains high levels of C3b/c4b-inactivator (unpublished observations).

Although a complement-receptor mediated clearance deficiency has been detected in patients with primary biliary cirrhosis (23), to our knowledge this is the first demonstration of a disease in which a major defect in clearance can be directly shown to be caused by hypocomplementemia. The findings also suggest that in conditions in which circulating immune complexes do not interact fully with the complement system and are not removed effectively by the liver, the spleen can be overwhelmed and also fail to clear them. Recent observations indicate that the spleen plays a key role in host defense against poorly opsonized bacteria (24) and that this organ offers a particularly important protective advantage when C3 levels are low (25). It is therefore conceivable that the profound complement deficiency observed at later stages of rodent malaria in association with the splenic defect may lead to the accumulation of immune complexes in the circulation and enhance their deposition at pathological sites. The observations of Bartolotti et al. (26), also suggest that complement deficiency may delay removal of complexes from the kidney. In other words, the abnormal processing of complexes by complement may contribute to pathology (reviewed in 27).

Hypocomplementemia, the presence of circulating immune complexes, and glomerulonephritis are common features of human, monkey, and rodent malaria (reviewed in 1) as well as in other immune complex diseases. Perhaps the rodent malaria model, in which the degree of infection can be experimentally manipulated to produce different degrees of disease severity, will help to elucidate the important question regarding the role of complement in the mechanisms leading to renal deposition of immune complexes.

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