0019-9567/78/0019-0138$02.00/0
INFECTION AND IMMUNITY, Jan. 1978, p. 138-145

Vol. 19, No. 1
Printed in U.S.A.

# Demonstration of the Role of Cytophilic Antibody in Resistance to Malaria Parasites (*Plasmodium berghei*) in Rats†

THEODORE J. GREEN AND JULIUS P. KREIER*
*Department of Microbiology, The Ohio State University, Columbus, Ohio 43210*

Received for publication 6 June 1977

This paper reports the results of a study of the nature of the immune response against *Plasmodium berghei* parasites by inbred rats. A macrophage-cytophilic antibody specific for malarial antigens was identified and characterized. Detection of the antibody on the macrophage surface was accomplished by the parasite adherence tests and by the indirect fluorescent antibody technique. Isolation and purification of the macrophage-cytophilic and opsonic antibodies from hyperimmune rat serum was accomplished by QAE-Sephadex A-50 elution chromatography, and of the macrophage-cytophilic antibody by adsorption with and elution from syngeneic macrophages as well. Characterization of the cytophilic antibody as immunoglobulin $G_1$ was done by immunoelectrophoresis and by Ouchterlony-type double diffusion in gel. Passive protection tests in weanling inbred rats have demonstrated that the opsonizing antibody conferred some protection against *P. berghei*. The macrophage-cytophilic antibody, on the other hand, was not protective alone but acted synergistically with the opsonizing antibody.

It is well established that antimalarial antibodies are capable of protecting rodents against malarial infections (9, 32). Antimalarial serum antibodies have been shown to coat free parasites, and the suggestion has been made that protection is achieved at the merozoite stage (7, 20). Previous work (13, 16, 20) has also shown that free parasites, coated with immune serum in vitro, remain capable of initiating infection. However, Hamburger and Kreier (13) have additionally demonstrated that such antibody-coated parasites do not retain full infectivity if they are accompanied into the test animal by immune serum. Chow and Kreier (6) concluded that immune serum enhances the phagocytic capability of both normal and immune macrophages.

Immunoglobulins associated with macrophages were first reported in 1954 by Girard and Murray (12); then, in 1963, Boyden (4) introduced a rosette-forming procedure utilizing macrophage monolayers to conveniently demonstrate the presence of such antibodies. The definition of cytophilic antibody proposed by Boyden (4) and by Sorkin (28) stated explicitly that the binding of antigen to cytophilic antibody takes place subsequent to cellular fixation of the antibody, with the consequent implication that these antibodies are capable of cellular fixation before their combination with antigen (31). This usage of the term "cytophilic antibody" has been accepted by the present authors and will be the definition of the term as used herein.

Both Parrish (23) and Tizard (30) have reported that opsonizing antibodies are not cytophilic before combination with specific antigen and therefore are not identical to those antibodies that are detected by Boyden's macrophage rosette system.

According to Tizard (31), the amount of cytophilic antibody found in the serum will depend upon both the avidity with which the antibodies bind to the macrophage receptors and the availability of those receptors. High binding avidity and ample receptor availability may result in undetectable levels of cytophilic antibody in the serum, with those antibodies that are found being of perhaps the lowest binding avidity for the macrophage receptors. For these reasons, we have chosen to use hyperimmunized rats for our source of cytophilic antibody in an effort to effect a spillover of relatively high-avidity cytophilic antibodies into the serum.

In the studies related here, we investigated the possibility that immune serum might directly arm macrophages against *P. berghei* parasites by the agency of a macrophage-cytophilic antibody, and we undertook to demonstrate by various procedures the presence of a macrophage-cytophilic antibody on nonimmune mac-

† Contribution no. 1473 from the Army Malaria Project.

rophages that had been presensitized in immune serum. In vitro experiments that demonstrated the capacity of a specific macrophage-cytophilic antibody to promote adherence of free parasites to macrophages were performed first. After this, an attempt was made to adsorb the cytophilic antibody component out of hyperimmune antiserum by using normal macrophages. The effectiveness of the adsorption was judged by the blockade of subsequent fluorescent staining of the macrophages by fluorescein-conjugated hyperimmune rat serum (HIRS) globulins that contained macrophage-cytophilic antibodies. Physical separation of the cytophilic antibodies from the opsonic antibodies in HIRS was attempted both by column chromatography and by adsorption-elution with macrophages. Boyden (4) and Sorkin (28) have reported that heating a macrophage suspension to 56°C for 30 min is sufficient to detach cytophilic antibodies. This process presumably destroys the receptor sites on the cell membranes, releasing the antibodies, which remain reactive. HIRS, serum fractions, and eluates were tested in vivo for protective effects.

## MATERIALS AND METHODS

**Malarial parasites.** The *P. berghei* strain used in this study was obtained from M. Aikawa (Case Western Reserve University, Cleveland, Ohio) and originated from the Walter Reed Army Institute of Research. A pool of infected mouse blood in 10% glycerol, aliquoted and frozen in liquid nitrogen, served as the reference stable source for all infectious inocula used in this study. Thawed material was inoculated first in a mouse, then passed into a weanling rat, whose infected blood served as the infectious inocula for test animals and for animals harvested either for immune serum or parasites. Free parasites were used in some animals as the infectious inoculum.

Freed parasites were obtained by harvesting the blood of infected adult rats after parasitemia exceeded 50%. "Percentage of parasitemia," as used in this study, is the number of *P. berghei*-infected erythrocytes per 1,000 erythrocytes counted, ×100. These high levels of parasitemia were obtained by pretreatment of the rats with 30 mg of phenylhydrazine · HCl per kg, intraperitoneally, 5 and 3 days before intravenous infection with 0.5 ml of heavily infected blood. On day 3 after infection, the parasitemia exceeded 50%, and the animals were exsanguinated into Alsever solution for the parasite harvest. Parasites were isolated from the blood by a modified continuous-flow ultrasonic treatment procedure (24, 25). The only changes involved the filtration of the sonically treated blood material through a glass-wool pad before centrifugation for 10 min at 1,020 × *g* to remove unbroken erythrocytes, after which the supernatant was centrifuged for 10 min at 3,300 × *g* to pellet the parasites. The parasites were then washed twice, suspended in Hanks balanced salt solution (HBSS), and held at 1°C until used, no longer than 2 h. Centrifugations were done in a refrigerated Sorvall RC-2 centrifuge.

**In vitro macrophage-parasite adherence tests.** CDF rats were anesthetized with ether and killed by exsanguination. Using clean technique, the abdomen was opened and normal, unstimulated macrophages were removed by lavage with 8 to 10 ml of tissue culture medium 199 (M-199; Grand Island Biological Co., Grand Island, N.Y.) containing 10% heat-inactivated fetal calf serum and antibiotics. The cells were pelleted by centrifugation for 3 to 5 min at 1,000 × *g* and resuspended in tris(hydroxymethyl)aminomethane-ammonium chloride buffer [1 part 0.1 M tris(hydroxymethyl)aminomethane (pH 7.2) to 9 parts 0.83% ammonium chloride]. After 10 min in the tris(hydroxymethyl)aminomethane-ammonium chloride buffer at 37°C, the cells were pelleted by centrifugation as above and resuspended in M-199. Cell numbers and viability determinations were made in a standard hemacytometer by counting cells suspended in buffer containing 1% trypan blue stain.

Unstimulated peritoneal washout cells ($2 \times 10^6$) containing 80 to 85% macrophages, as determined by May-Gruenwald staining characteristics, were suspended in 2 ml of M-199 and incubated in plastic tissue culture dishes (35 by 10 mm) for 30 min at 37°C in an atmosphere of 5% $CO_2$. Nonadherent cells were then removed by three washes in HBSS, leaving primarily macrophages adherent to the culture dishes.

The macrophage monolayers were sensitized with 1 ml of either normal rat serum (NRS) or HIRS diluted with 2 ml of M-199 and incubated for 30 min at 37°C in an atmosphere containing 5% $CO_2$. After sensitization, the cells were washed three times in cold HBSS, and $2 \times 10^8$ free parasites in 1 ml of cold HBSS were layered over the macrophages and incubated for 30 min. The monolayers were washed three times by immersion in cold HBSS. The monolayers were then fixed with methanol and stained by the Giemsa technique. Two hundred cells showing typical macrophage morphology were counted for the presence and number of adherent parasites on each plate. Each experiment was performed in duplicate, and the data were averaged.

**Animals.** Male inbred CDF rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used throughout the study. Protection assays were done in weanling rats 4 to 5 weeks of age, and parasites and various sera were obtained from adult rats.

**Sera.** NRS was harvested from adult CDF rats, aliquoted, and stored at −20°C. HIRS was obtained by challenging rats newly recovered from *P. berghei* infection with $20 \times 10^6$ infected CDF rat erythrocytes at weekly intervals for 3 weeks. The animals were bled approximately 2 weeks later, and the sera were separated, pooled, and stored in 2-ml portions at −20°C until needed. Fluorescent serum globulins were prepared from hyperimmune anti-*P. berghei* serum by the method of Cherry et al. (5). Rabbit anti-rat immunoglobulin fluorescein-conjugated immunoglobulin G (IgG) was obtained from Microbiological Associates, Walkersville, Md. Rabbit anti-rat IgG serum for immunoelectrophoresis was obtained from Miles Laboratories, Inc., Elkhart, Ind.

**Chromatographic separation of HIRS.** Pooled CDF rat anti-*P. berghei* HIRS (4 ml) was dialyzed for 3 days against ethylenediamine acetate buffer and then separated on a QAE-Sephadex A-50 column that

had been swollen in the same buffer. The buffer was prepared as follows: 2.88 g of ethylenediamine acetate was dissolved in 73 ml of glacial acetic acid, and the volume was adjusted to 1 liter with distilled water to give an ionic strength of 0.1 (pH 7.2). Elutions of serum fractions from the column were made with ethylenediamine acetate buffer adjusted to pH 5.0 by adding acetate buffer, and with acetate buffer at pH 4.0. Acetate buffer was made by adding 435 ml of 0.6 M acetic acid to 130 ml of 0.6 M sodium acetate and adjusting the volume to 1 liter with distilled water to give an ionic strength of 0.1 (pH 4.0). Serum fractions were examined and identified by immunoelectrophoresis and by indirect fluorescent antibody technique.

**Immunoelectrophoresis.** Immunoelectrophoresis was accomplished using 0.65% Difco agar (Difco Laboratories, Detroit, Mich.) in Gelman high-resolution buffer (pH 8.8; Gelman Instrument Co., Ann Arbor, Mich.). The test samples were subjected to electrophoresis at 200 V, 20 mA, for 60 min and then reacted with rabbit anti-rat IgG serum at 25°C in a humidity chamber for 24 h.

**Fluorescent antibody techniques.** Indirect fluorescent antibody assay for the opsonic antibody was conducted by incubating fixed films of parasitized blood for 30 min at 37°C in HIRS, followed by two washes of 10-min duration in phosphate-buffered saline (pH 7.2). The film was then incubated with rabbit anti-rat immunoglobulin fluorescein-conjugated IgG for 30 min at 37°C. The slides were again washed as above and examined for fluorescence. Indirect fluorescent antibody assay for the macrophage-cytophilic antibody was conducted by incubating macrophage monolayers for 30 min at 4°C in HIRS, followed by two rapid washes in phosphate-buffered saline (pH 7.2). The cells were then incubated with rabbit anti-rat immunoglobulin fluorescein-conjugated IgG for 30 min at 4°C. The cells were washed as before and examined for fluorescence.

**Adsorption of HIRS with macrophages.** Peritoneal washout cells consisting of at least 80% macrophages were collected from normal CDF rats in M-199, centrifuged at 1,000 × *g* for 2 min, suspended in tris(hydroxymethyl)aminomethane-ammonium chloride buffer for 10 min at 37°C to lyse erythrocytes, centrifuged at 1,000 × *g* for 2 min, and resuspended in M-199. The cells were then plated out in plastic tissue culture dishes (35 by 10 mm) and incubated for 1 h at 37°C in an atmosphere containing 5% $CO_2$ to promote adherence. Nonadherent cells were washed away after this incubation, and the M-199 was replaced. The plated cells were then held at 4°C until used. After the plate was washed in ice-cold HBSS and the excess was drained, serum was adsorbed by transferring a 1-ml serum sample to the plate, which was then incubated at 4°C for 30 min. At this time, another plate was rinsed in ice-cold HBSS and received the 1 ml of serum from the preceding. The serum was serially adsorbed six times in this manner. Each plate, after use, was washed twice in ice-cold HBSS and then incubated with a 1:10 dilution of fluorescein-conjugated anti-*P. berghei* CDF rat serum globulin for 30 min at 4°C. Each plate was then rinsed three times and examined for specific macrophage fluorescent staining. A negative test indicated blockade of specific macrophage receptors by cytophilic antibody bound during the serial HIRS adsorption. A positive test indicated that no cytophilic antibody remained in the serially adsorbed sample.

**Cytophilic antibody elutions.** Macrophage-cytophilic antibody was purified by serially adsorbing a 3-ml portion of HIRS six times with $15 \times 10^6$ peritoneal washout cells in suspension for 30 min at 4°C. The peritoneal washout cells were then incubated at 56°C for 30 min to effect the elution of bound antibody. The supernatant containing the eluted antibodies was adjusted to a 3-ml volume. Both the adsorbed serum and the eluate were tested by indirect fluorescence microscopy for specific activity. The eluate was compared to QAE-Sephadex A-50 fractions 1 and 2 for determination of composition by double diffusion in gel against rabbit anti-rat IgG antiserum.

**In vivo protection test no. 1: adsorbed serum.** HIRS that had been adsorbed six times by $5 \times 10^6$ peritoneal washout cells was compared with both NRS and unadsorbed HIRS in 5-week-old male CDF rats. Each test group contained five rats, and each rat received $5 \times 10^6$ freed parasites in 0.1 ml of HBSS mixed with 0.1 ml of either NRS, HIRS, or adsorbed HIRS and immediately injected intravenously. Percentage-of-parasitemia values were determined by counting 1,000 erythrocytes at random on a Giemsa-stained thin blood film each day. Data were plotted as the daily mean percentage of parasitemia for the group.

**In vivo protection test no. 2: eluted antibody.** The antibody-containing eluate recovered after 3 ml of HIRS had been adsorbed six times with $15 \times 10^6$ peritoneal washout cells was compared for protective activity in vivo with the adsorbed serum as well as with both NRS and HIRS in 5-week-old male CDF rats. Each test group contained 5 rats, and each rat received $5 \times 10^2$ infected CDF erythrocytes suspended in 0.5 ml of HBSS, given intravenously 24 h after intravenous injection of 0.5 ml of either NRS, HIRS, macrophage-adsorbed HIRS, or macrophage eluate. Percentages of parasitemia were determined by counting 1,000 erythrocytes at random on a Giemsa-stained thin blood film each day. Data were plotted as the mean time required for the group to reach 1% parasitemia.

**In vivo protection test no. 3: HIRS fractions.** Fractions of HIRS that had been separated by elution chromatography on QAE-Sephadex A-50 were compared with both NRS and HIRS in 5-week-old male CDF rats. Each group contained 5 rats, and each rat received 0.1 ml of NRS, HIRS, fraction 1, 2, or 3, or 0.1 ml of fraction 1 plus 0.1 ml of fraction 2. The infectious inocula consisted of $5 \times 10^6$ freed parasites suspended in 0.1 ml of HBSS, which was mixed with the serum or serum fraction(s) and immediately injected intravenously via the lateral tail vein. Percentages of parasitemia were determined by counting 1,000 erythrocytes at random each day on a Giemsa-stained thin blood film. Data were plotted as the mean time required for the group to reach 1% parasitemia.

## RESULTS

**In vitro macrophage-parasite adherence tests.** The effects of arming the macrophages with macrophage-cytophilic antibody specific

for *P. berghei* parasites before their incubation with free parasites are presented in Fig. 1. Of the macrophages pretreated with HIRS, 25% were associated with adherent parasites, as opposed to only 9% of those pretreated with NRS. Of the actual parasites counted, more than three times as many parasites were found to be associated with macrophages pretreated with HIRS as with macrophages pretreated with NRS. Those macrophages pretreated with NRS that showed positive adherence results usually had one to three adherent parasites, whereas macrophages pretreated with HIRS that showed positive adherence results usually had two to six adherent parasites. Analysis by Student's *t* test showed a significant difference between NRS and HIRS for both the percentage of macrophages positive and the number of adherent parasites per 200 macrophages ($P < 0.025$).

**Chromatographic separation of HIRS.** Figure 2 is an optical density tracing at 280 nm recorded during the elution of fractions of HIRS from a QAE-Sephadex A-50 column at pH values of 7.2, 5.0, and 4.0 (fractions 1, 2, and 3, respectively). When examined by immunoelectrophoresis, fractions 1 and 2 were found to consist of $IgG_1$ and $IgG_2$, respectively, following the nomenclature of Bazin et al. (2), whereas fraction 3 was found to contain albumin and IgM.

**Adsorption of HIRS with macrophages.** HIRS (1 ml) adsorbed four times by $5 \times 10^6$ peritoneal washout cells was found to block subsequent direct fluorescent antibody staining of macrophages; after the fifth adsorption it partially blocked the fluorescent antibody staining of the macrophages, and after the sixth adsorption it did not block the direct fluorescent staining of the macrophages. Thus, the macrophage-cytophilic antibody was no longer present at inhibitory levels in 1 ml of HIRS after six adsorptions with $5 \times 10^6$ peritoneal washout cells.

**Cytophilic antibody elutions.** When the eluate from peritoneal washout cells that had been used to adsorb HIRS was compared by the indirect fluorescent antibody technique to the adsorbed HIRS, it was found that the eluate contained primarily macrophage-cytophilic antibodies, whereas the adsorbed HIRS contained primarily opsonic antibodies. When the same eluate was compared by double diffusion in gel to fractions 1 and 2 obtained by QAE-Sephadex A-50 chromatography of HIRS, fraction 1 was shown to be identical with the antibodies eluted from the macrophages, whereas fraction 2 was shown to contain antibodies distinct from both the eluate and fraction 1, although all were identified as belonging to the IgG class (Fig. 3). Thus, fraction 1 has been shown to provide

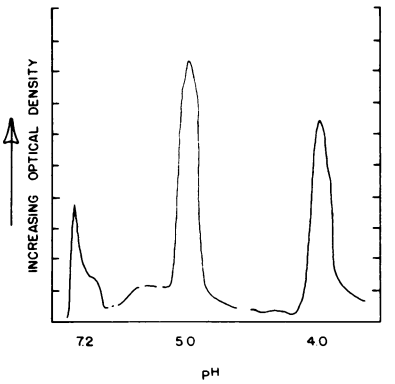


FIG. 2. *pH elution pattern of HIRS from QAE-Sephadex A-50.*

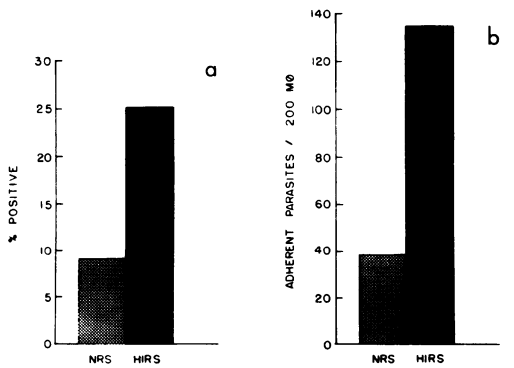


FIG. 1. *Effects of cytophilic antibodies upon the ability of macrophages (Mφ) to retain free P. berghei parasites. (A) Parasite-macrophage adherence (percentage of macrophages showing adherence). (B) Number of parasites adherent per 200 macrophages.*

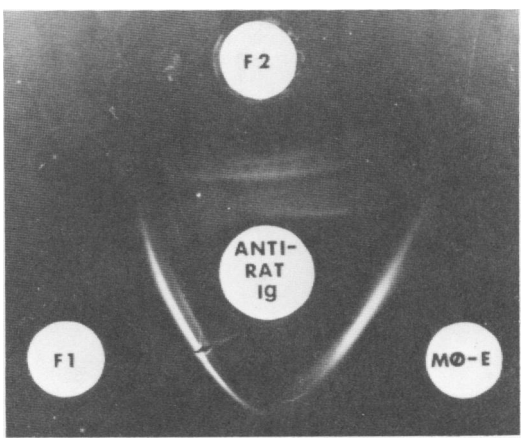


FIG. 3. *Precipitin pattern formed in gel by eluted macrophage-cytophilic antibodies and QAE-Sephadex HIRS fractions when reacted against anti-IgG antiserum.*

separation of those antibodies that are capable of macrophage-cytophilic activity from the opsonizing antibodies that are contained in fraction 2. Indirect immunofluorescent tests have verified that macrophage-cytophilic antibodies are contained only in fraction 1, and that parasite opsonizing antibodies are found in fraction 2, but not in fraction 1.

**In vivo protection test no. 1: adsorbed serum.** The protective effects of HIRS that had been depleted of macrophage-cytophilic antibodies was shown to be reduced from those exhibited by unadsorbed HIRS (Fig. 4), as demonstrated by the earlier onset and rise of parasitemia after the challenge infection was initiated. Analysis by Student's $t$ test showed a significant difference between the adsorbed and the unadsorbed HIRS groups on day 10 ($P < 0.05$).

**In vivo protection test no. 2: eluted antibody.** When eluted macrophage-cytophilic antibodies were compared with HIRS depleted of macrophage-cytophilic antibodies, it was found that the protective capacity of HIRS depleted of macrophage-cytophilic antibodies was diminished, and that the eluate which contained only the eluted macrophage-cytophilic antibodies had no protective capacity (Fig. 5). Data obtained subsequent to these experiments indicated that recovery of macrophage-cytophilic antibody was at an efficiency of approximately 58%; therefore, a quantitative reduction of cytophilic antibody occurred in the eluate as compared with unadsorbed serum. This would be expected to dimish any observed effects of cytophilic antibody when compared to the original volume. However, the magnitude of the reduction in activity of the adsorbed serum is such that it would be reasonable to expect some effect from 58% of the original amount if it were capable of independent protective activity. No significant difference was seen between the eluate and NRS, but Student's $t$ test showed a significant difference between the macrophage-adsorbed HIRS and unadsorbed HIRS ($P < 0.025$).

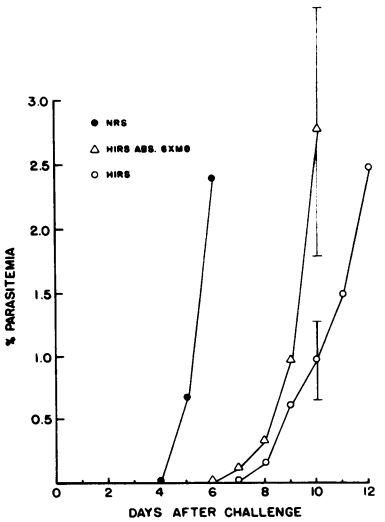


FIG. 4. *Effect of adsorption with macrophages upon the protective capacity of P. berghei immune serum in rats. Values plotted are means (± standard error of the mean) obtained from counts of 1,000 cells on each film (n = 5).*

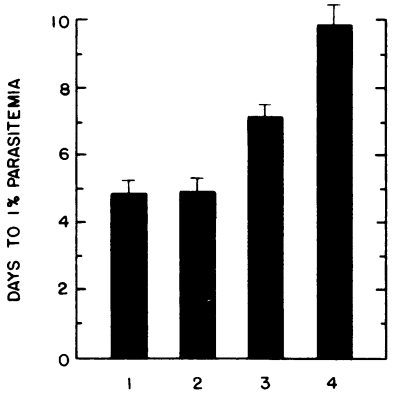


FIG. 5. *Protective effects against P. berghei of macrophage-adsorbed and macrophage-eluted HIRS fractions in rats. Values plotted are means (± standard error of the mean) obtained from counts of 1,000 cells on each film (n = 5). Rats received 0.5 ml of (1) NRS, (2) macrophage eluate, (3) macrophage-adsorbed HIRS, or (4) HIRS intravenously 24 h before infection.*

**In vivo protection test no. 3: HIRS fractions.** Fraction 1, containing the macrophage-cytophilic antibodies, had no protective capacity against *P. berghei* infection in vivo, and fraction 2 contained only moderate protective capacity. However, when fractions 1 and 2 were recombined in vivo, a synergistic effect was seen, resulting in a greater protective capacity than would be indicated by the expected additive effects of the individual fractions. Fraction 3 contained minimal protective capacity, which can be ascribed to the presence of IgM antibodies (Fig. 6). Analysis by Student's $t$ test showed a significant difference between the time required for the group that received fraction 2 and the group that received fraction 1 and 2 combined to reach 1% parasitemia ($P < 0.05$).

## DISCUSSION

Our findings indicate that there are two major and distinct types of IgG antibody that provide protection against *P. berghei* in rats. The first of these is an opsonic antibody, which has as its specific ligand some site on the parasite sur-

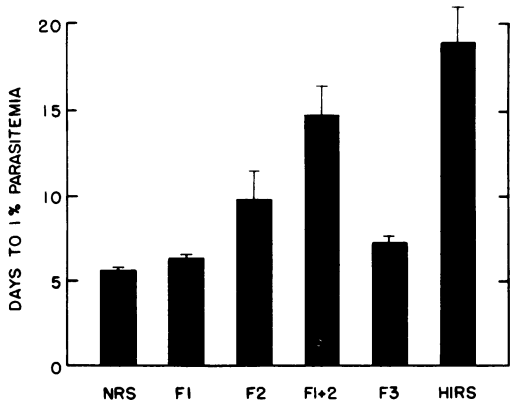


FIG. 6. *Protective effects against P. berghei in rats of QAE-Sephadex HIRS fractions. Values plotted are means (± standard error of the mean) obtained from counts of 1,000 cells on each film (n = 5). F1, Fraction 1 (cytophilic); F2, fraction 2 (coating); F1+2, 0.1 ml of each; F3, fraction 3 (albumin with or without coating).*

face. The second is a macrophage-cytophilic antibody that is unable to bind to parasites until after it has become attached to the surface of a macrophage. Sodoman and Haferkamp (27) reported finding in rats such macrophage-cytophilic antibodies to tuberculoprotein after BCG immunization and after appropriate immunization to group A *Streptococcus* carbohydrate also. Binding of the cytophilic antibody to the macrophage surface is probably by an Fc receptor (8, 10). Davey and Asherson (8) concluded that the macrophage receptor for macrophage-cytophilic antibodies against sheep erythrocytes in guinea pigs might be a phospholipid or phospholipoprotein, and Arend and Mannik (1) found that cytophilic antibody receptors on macrophages were destroyed by phospholipase. We have not yet attempted to determine the nature of the receptors in our system.

Upon attachment to the macrophage surface membrane, the antibodies presumably undergo steric rearrangement, whereupon they become capable of specifically binding parasite receptors. However, the protective nature of this interaction is not manifested in the absence of specific opsonic antibody. Indeed, it is well established that some cytophilic antibodies fix antigens to macrophages without inevitably stimulating phagocytosis (31). Levenson and Braude (17) observed that anti-*S. typhi* Vi polysaccharide obtained early in immunization could induce rosette formation of Vi-coated erythrocytes around macrophages, and that prolonged incubation of these rosettes at 37°C did not lead to phagocytosis. Internalization may be dependent upon the presence of complement-fixing antiparasite antibodies at the macrophage-parasite interface. Ehlenberger and Nussenzweig (11) have recently demonstrated synergy between Fc and C3 receptors in the phagocytic process. Bianco et al. (3) have concluded that the process of phagocytosis is accompanied by a change in the macrophage C3b receptor that allows a shift in function from binding to both binding and internalization.

Miller et al. (21) have recently reported a lack of correlation in monkeys between functional immunity in vivo and the presence of antibodies capable of inhibiting merozoite invasion in vitro. In the immune response of rats to *P. berghei*, a synergistic relationship exists between the macrophage-cytophilic and the opsonic antibodies wherein the combined protective effect is greater than the sum of the individual protective capacities. The exact nature of this interaction remains unclear. It has been proposed (7, 20) that the antibody coating the parasites may interfere with penetration of the erythrocyte by the merozoite form of the parasite. We have observed in this study that the opsonic antibody is necessary for the manifestation of the protective effect of the macrophage-cytophilic antibodies. Perhaps the macrophage-cytophilic antibodies promote only the adherence of parasites to the phagocytic cells, and not their subsequent ingestion. Stossel (29) has observed that, under differing conditions, "some particles stick tightly to phagocytes but are not ingested, some stick and are partly ingested, and still others stick and are completely ingested." The opsonic antibodies may trigger the actual ingestion of the parasites by the macrophages, but may be less efficient alone in promoting adherence. It has been stated that internalization of particles attached to macrophages is triggered by IgG, and that different roles are assigned for the macrophage receptor sites for complement (C3) and for immunoglobulin (19, 26).

Destruction of the infective merozoites is necessary to control an infection by malarial parasites. Preliminary electron microscopic studies under way in this laboratory by C. Brooks have indicated that, although trophozoites in a free-parasite preparation will adhere to macrophages in the presence of either normal or immune serum, merozoites are found to be adherent to macrophages only in the presence of immune serum. A capsule has been demonstrated as present on the infective merozoite stage of *Plasmodium* (20). This capsule may very well confer antiphagocytic properties upon the merozoite. Immune serum has been shown to contain protective antibodies which bind to this capsule (20), yet these antibodies are not capable of direct merozoite neutralization. However, these

antibodies may serve to reduce or eliminate the antiphagocytic nature of the capsular material by reduction of the hydrophilic characteristics of the capsular material, thereby favoring the physical processes of phagocytic engulfment (22); by stabilization of the amorphous capsular material, thereby facilitating adhesion and subsequent engulfment; or by both mechanisms.

Although the macrophage-cytophilic antibodies may facilitate the attachment of the merozoites to the macrophage, alteration of the capsular material (perhaps by complement fixation) may not occur in the absence of the opsonic antibody, and ingestion of the parasite would therefore be less likely. Furthermore, should the merozoite be swept loose, leaving some capsular material attached at the macrophage surface, the recognition capability of the cytophilic antibody-armed macrophage would be reduced. Thus it becomes apparent that cytophilic antibody-mediated attachment of infective merozoites would result in enhancement of the protective effects of the opsonic antibody, but would probably not be protective alone.

Previous work in this laboratory by Hamburger and Kreier (14) has demonstrated that passive immunity to malaria cannot be conferred to mice by rat-origin immune serum, with the implication that the site of protective action of antimalarial antibodies is species specific. This action is probably at the level of phagocytosis, i.e., the macrophage. Further work (13) suggested that the parasite opsonizing antibodies were of low avidity and quickly became dissociated from the parasites in vivo, or that a cytophilic antibody was involved in phagocytosis, or both. Low opsonizing-antibody avidity was demonstrated by these authors in a subsequent publication (15), and now we have additionally demonstrated the presence and role of a macrophage-cytophilic antibody.

## ACKNOWLEDGMENTS

This work was supported by a grant to J.P.K. from the U.S. Army Medical Research and Development Command, contract no. DADA 17-72-2072.

We thank Kathryn M. Brown for invaluable technical assistance and Harold Cooper for the procedure for the column separation of antibody. We also thank Linda K. Wange for the identification of the antibodies in the column fractions by indirect fluorescent antibody techniques.

## LITERATURE CITED

1. **Arend, W. P., and M. Mannik.** 1973. The macrophage receptor for IgG: number and affinity of binding sites. J. Immunol. **110**:1455–1463.
2. **Bazin, H., A. Beckers, and P. Querinjean.** 1974. Three classes and four (sub)classes of rat immunoglobulins: IgM, IgA, IgE and $IgG_1$, $IgG_{2a}$, $IgG_{2b}$, $IgG_{2c}$. Eur. J. Immunol. **4**:44–48.
3. **Bianco, C., F. M. Griffin, and S. C. Silverstein.** 1975. Studies on the macrophage complement receptor: alteration of receptor function upon macrophage activation. J. Exp. Med. **141**:1278.
4. **Boyden, S. V.** 1963. Cytophilic antibody, p. 7–17. *In* B. Amos and M. Koprowski (ed.), Cell-bound antibodies. Wistar Institute Press, Philadelphia.
5. **Cherry, N. B., M. Goldman, T. R. Carski, and M. D. Moody.** 1966. Fluorescent antibody techniques in the diagnosis of communicable diseases. U.S. Public Health Serv. Publ. no. 729.
6. **Chow, J. S., and J. P. Kreier.** 1972. *Plasmodium berghei*: adherence and phagocytosis by rat macrophages *in vitro*. Exp. Parasitol. **31**:13–18.
7. **Cohen, S., and G. A. Butcher.** 1970. Properties of protective malarial antibody. Immunology **14**:127–138.
8. **Davey, M. J., and G. L. Asherson.** 1967. Cytophilic antibody. I. Nature of the macrophage receptor. Immunology **12**:13–20.
9. **Diggs, C. L., and A. G. Osler.** 1969. Humoral immunity in rodent malaria. II. Inhibition of parasitemia by serum antibody. J. Immunol. **102**:298–305.
10. **Dissanayake, S., and F. C. Hay.** 1975. Investigation of the binding site of mouse IgG subclasses to homologous macrophages. Immunology **29**:1111–1118.
11. **Ehlenberger, A. G., and V. Nussenzweig.** 1977. The role of membrane receptors for C3b and C3d in phagocytosis. J. Exp. Med. **145**:357–371.
12. **Girard, K. F., and E. G. D. Murray.** 1954. The presence of antibody in macrophage extracts. Can. J. Biochem. Physiol. **32**:14–19.
13. **Hamburger, J., and J. P. Kreier.** 1975. Antibody-mediated elimination of malaria parasites (*Plasmodium berghei*) in vivo. Infect. Immun. **12**:339–345.
14. **Hamburger, J., and J. P. Kreier.** 1976. *Plasmodium berghei*: use of free blood stage parasites to demonstrate protective humoral activity in the serum of recovered rats. Exp. Parasitol. **40**:158–169.
15. **Hamburger, J., and J. P. Kreier.** 1976. Interaction between protective antibodies and malarial parasites (*Plasmodium berghei*): involvement of low avidity antibodies. Tropenmed. Parasitol. **27**:385–390.
16. **Jerusalem, C., M. L. Weiss, and L. Poels.** 1971. Immunologic enhancement in malaria infection (*Plasmodium berghei*). J. Immunol. **107**:260–268.
17. **Levenson, V. I., and N. I. Braude.** 1967. Different forms of interaction of macrophages with foreign antigens provided by "early" and "late" immune sera. Folia Biol. **13**:113–121.
18. **Liew, F. Y.** 1971. The binding of the Fc fragment of guinea pig cytophilic antibody to peritoneal macrophages. Immunology **20**:817–829.
19. **Mantovani, B., M. Rabinovitch, and V. Nussenzweig.** 1972. Phagocytosis of immune complexes by macrophages. Different roles of the macrophage receptor sites for complement (C3) and for immunoglobulins (IgG). J. Exp. Med. **135**:780.
20. **Miller, L. H., M. Aikawa, and J. A. Dvorak.** 1975. Malaria (*Plasmodium berghei*) merozoites: immunity and surface coat. J. Immunol. **114**:1237–1242.
21. **Miller, L. H., K. G. Powers, and T. Shiroishi.** 1977. *Plasmodium knowlesi*: functional immunity and antimerozoite antibodies in rhesus monkeys after repeated infection. Exp. Parasitol. **41**:105–111.
22. **Mudd, E. B. H., and S. Mudd.** 1933. The process of phagocytosis. The agreement between direct observation and deductions from theory. J. Gen. Physiol. **16**:625.
23. **Parrish, W. E.** 1965. Differentiation between cytophilic antibody and opsonins by a macrophage phagocytic system. Nature (London) **208**:594–595.
24. **Prior, R., and J. P. Kreier.** 1972. Isolation of *Plasmodium berghei* by use of a continuous flow ultrasonic

system: a morphological and immunological evaluation. Proc. Helminthol. Soc. Wash. **29**:563–574.

25. **Prior, R., and J. P. Kreier.** 1972. *Plasmodium berghei* freed from host erythrocytes by a continuous-flow ultrasonic system. Exp. Parasitol. **32**:239–243.
26. **Scribner, D. J., and D. Farhney.** 1976. Neutrophil receptors of IgG and complement: their roles in the attachment and ingestion phases of phagocytosis. J. Immunol. **116**:892–897.
27. **Sodoman, C. P., and O. Haferkamp.** 1969. Macrophage cytophilic antibodies to tuberculoprotein in rats immunized with Bacillus Calmette-Guérin. Int. Arch. Allergy Appl. Immunol. **36**:566–572.
28. **Sorkin, E.** 1963. Cytophilic antibody, p. 38–61. *In* G. E. W. Wolstenholme and M. P. Cameron (ed.), The immunologically competent cell. Ciba Foundation Symposium no. 16. Churchill, London.
29. **Stossel, T. P.** 1976. The mechanism of phagocytosis. J. Reticuloendthel. Soc. **19**:237–245.
30. **Tizard, I. R.** 1969. Macrophage cytophilic antibody in mice. Differentiation between antigen adherence due to these antibodies and opsonic adherence. Int. Arch. Allergy Appl. Immunol. **36**:332–346.
31. **Tizard, I. R.** 1971. Macrophage-cytophilic antibodies and the functions of macrophage-bound immunoglobulins. Bacteriol. Rev. **35**:365–378.
32. **Zuckerman, A.** 1970. Malaria of lower mammals, p. 793–829. *In* G. J. Jackson, R. Herman, and I. Singer (ed.), Immunity to parasitic animals, vol. 2. Appleton-Century-Crofts, New York.