Immune complexes in serum of rats during infection with *Plasmodium berghei*

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**Abstract.** Large amounts of immune complexes were present in the serum of infected rats early in infection when parasitemias were low. As the infection progressed and parasitemia increased and then decreased, the amounts of immune complexes in the serum also fell. This result suggests that increased efficiency of complex clearance was an important factor in determining the levels of immune complexes in the serum. In high performance liquid chromatography (HPLC), the complexes in the serum migrated as a peak with material of 350 kDa and greater in mass. They sedimented in a sucrose gradient as a band with a sedimentation coefficient of 22 s, which was calculated to yield a mass of approximately 1100 kDa. Immunoelectrophoresis and radial immunodiffusion showed that IgG was the major immunoglobulin in the complexes. As the IgG content of the complexes increased, the levels of complexes in the serum generally decreased. HPLC analysis of precipitated complexes suggested that they contained loosely bound albumin. Serum proteins were affected by the infection. A depletion of free immunoglobulin was observed during the initial period of immune complex formation.

Large amounts of circulating immune complexes (ICs) are a common feature of malaria. However, the clearance of these complexes and the resulting pathologic changes are not well understood. Malaria ICs collected during infection are immunosuppressive. Immune complexes, for example, collected from rats have inhibited the induction of immunity by a blood-stage vaccine (Alder et al. 1987), the in vitro antibody response to sheep red blood cells by sensitized spleen cells (Cox et al. 1983) and induction of immunity by a pertussis vaccine (Viens et al. 1974). These complexes have also inhibited in vitro phagocytosis of infected erythrocytes (Packer and Kreier 1985), and of free plasmodia (Brown and Kreier 1982; 1986). Inhibition of phagocytosis by IC is probably mediated by inhibition of Fc-receptor function (Shear 1984).

Ingestion of ICs initiates processes that activate macrophages (Pestel et al. 1981). Activated macrophages destroy ingested antigens rapidly and are thus less efficient for antigen presentation than resting macrophages (Biozzi et al. 1984). Some of the immunosuppressive effects of IC may therefore be a result of IC-mediated macrophage activation. Another method whereby ICs may affect immunity is by tying up antiparasitic antibody; both parasite antigens and antigen excess complexes may act by this mechanism. A study by Druilhe and Khumsith (1987), for example, linked immunity to malaria with a high level of antibodies that promote phagocytosis of merozoites. If antibody of the specificity needed for such phagocytosis were bound to complexes or free antigen, it would block protection. Antibody binding by immune complexes thus could shield the parasite from antibody and be a protective mechanism as stated in the smokescreen hypothesis advanced by Wilson (1974) and developed by Kreier et al. (1983).

In view of the importance of malaria ICs in the pathogenesis of malaria, we investigated the size and composition of the ICs that form and are cleared during the course of infection.

**Materials and methods**

*Parasites. Plasmodium berghei berghei* stabilates were prepared and maintained in liquid nitrogen by standard techniques.
Radial immunodiffusion. Radial immunodiffusion was used to determine the amounts of immunoglobulin present in PEG precipitates from rat serum collected at the various times during infections (Garvey et al. 1977).

Radioimmune precipitation assay. The radioimmune precipitation (RIP) assay was used to analyze the antigens in P. berghei immune complexes and to examine the relative amounts of antibody and antigen in the complexes. Plasmodia were labeled in culture by metabolic incorporation of [35S]methionine (Alder and Kreier 1984). Following the in vitro labeling, the infected red blood cells were disrupted by sonication. The supernatant fluid containing the labeled antigen was collected following centrifugation at 10000 g for 10 min.

The RIP assay was performed using two different procedures. The first analyzed the PEG precipitates following direct reaction with labeled antigen; the second analyzed the precipitates after reaction first with hyperimmune serum and then with labeled antigen. In the first procedure, the PEG precipitates were resuspended to the original volume of the serum from which they were precipitated (1 ml) and a 50 μl aliquot was mixed with 50 μl of labeled antigen along with 50 μl of 2% β-methionine. The mixture was incubated at room temperature for 2 h. Immune complexes and the bound labeled antigen were collected on 50 μl of protein-A coated latex beads (Sigma). In addition to the bead suspension, 150 μl of radiimmune precipitation assay (RIPA) buffer (0.05 M Tris, 0.15 M NaCl, 1% Na deoxycholate, 1% Triton-X 100) was added. The mixture was then incubated at room temperature for 30 min. The latex beads to which the immune complexes were bound were pelleted by centrifugation at 12800 g for 2 min. The pellets were washed 5 x with 150 μl RIPA buffer per wash. Next, the complexes bound to the pellets were solubilized in a SDS-B mercaptoethanol buffer, and polypeptides were separated by a discontinuous polyacrylamide gel electrophoresis (PAGE) system (Laemmli 1973). A 4% stacking and 10% separation gel was used, and the electrophoresis was carried out at 30 mA per gel (Allen et al. 1977). The gels, fixed in 10% acetic acid overnight, were processed for autoradiography by two 30-min washes in DMSO, followed by a 3-h immersion in DMSO+20% PPO, then by two 30-min washes in distilled water, as previously described (Alder and Kreier 1984). The gels were dried in a Bio-Rad gel drier and exposed to Kodak X-Omat x-ray film for two weeks at −70°C.

The second RIP assay procedure also utilized the PEG precipitates from 1-ml serum samples collected at various times during infection, but the ICs were first converted to antibody excess form by reaction with hyperimmune serum. The pellets were each resuspended to 0.5 ml (1/2 original volume) in saline, to which 0.5 ml of serum from immune rats was added. This mixture was incubated for 2 h at room temperature. Then, 50 μl of protein-A coated latex beads were added, followed by incubation at room temperature for 30 min. The latex beads were pelleted and washed as before, then resuspended in 50 μl of labeled antigen along with 50 μl of β-methionine and 150 μl RIPA buffer. The mixtures were incubated at room temperature for 2 h. The latex beads were again pelleted and washed five times. The immune complexes bound to the pellets were solubilized and the component polypeptides were separated by PAGE, followed by autoradiography as described above.

Results

Parasitemia in the course of infection

Serum was collected from rats during infection with Plasmodium berghei. Table 1 shows the par-