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Role of Macrophages in Malaria: O_2 Metabolite Production and Phagocytosis by Splenic Macrophages During Lethal *Plasmodium berghei* and Self-Limiting *Plasmodium yoelii* Infection in Mice

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The role of splenic macrophages in resistance to lethal *Plasmodium berghei* or self-limiting *Plasmodium yoelii* was studied by testing their rate of phagocytosis and their production of O_2 metabolites (H_2O_2 and O_2^-) upon nonspecific stimulation with zymosan. It was found that, compared with *P. berghei*, infection of mice with *P. yoelii* resulted in an earlier appearance and in higher numbers of adherent cells in the spleen. Furthermore, the capacity of macrophages to generate O_2 metabolites was significantly higher in *P. yoelii*- than in *P. berghei*-infected mice. This difference in the production of O_2 metabolites was more pronounced when calculated on a per spleen basis. On the other hand, phagocytosis by macrophages was similar in both types of infection. The data suggest that lethal and nonlethal malaria species differ in their capacity to induce the production of O_2 metabolites by macrophages, thereby influencing the final course of disease.

During the last few years, several studies have emphasized the role of lymphocytes and macrophages in protective immunity to malaria parasites. The role of serum antibody in acquired resistance has been established in passive transfer experiments in monkey, human, and rodent infections (reviewed in references 8 and 14). A monoclonal antibody against a membrane protein of *Plasmodium berghei* sporozoites has been shown to protect mice against mosquito-transmitted infection and to prevent the entry of sporozoites into cultured cells (13, 21). Furthermore, antibodies have been shown to facilitate phagocytosis of parasitized erythrocytes (PRBC; reviewed in references 14 and 22). The participation of T cells in the humoral immune response to parasites was first demonstrated by Brown (6) using T cell-deprived mice. Although functional B cells can clearly promote protection, they are not essential for the maintenance of secondary immunity (14). The direct involvement of T cells in the mediation of protection against malaria is also well established (reviewed in reference 14). In addition to T and B lymphocytes, macrophages are involved in defense against malaria (3, 4, 11, 14, 19, 23). Although resistance evoked by macrophages can be induced either by antigen-specific T cells (14) or by nonspecific mechanisms (15), the expression of such immunity is generally nonspecific.

There is evidence that reactive oxygen intermediates (O_2^- and H_2O_2) produced by macrophages cause hemolysis and parasite death in malaria (reviewed in references 4 and 18). Recently, a rapid reduction in parasitemia after a single intravenous injection of alloxan (a promoter of O_2^- generation) was demonstrated (7). In addition, Dockrell and Playfair (9) presented evidence that H_2O_2 is effective against murine blood-stage malaria at concentrations which might naturally occur in vivo. It was shown that lethal *P. berghei* and nonlethal *Plasmodium yoelii* parasites are equally sensitive to H_2O_2 in vitro. However, after intravenous injection of H_2O_2, parasitemia was reduced in *P. yoelii*- but not in *P. berghei*-infected animals. Here we present evidence that in spleens of mice infected with nonlethal *P. yoelii* the capacity of macrophages to produce O_2 metabolites appears earlier and at significantly higher levels than in mice infected with lethal *P. berghei*.

P. yoelii 17XNL, which induces a self-resolving course, and *P. berghei* K173, which induces a fatal infection in C57BL/10 mice, were used. Animals were infected with 10^7 PRBC intraperitoneally, and parasitemia was determined in Giemsa-stained tail blood smears. To determine the O_2 metabolite–producing capacity of whole splenic macrophages, luminol-aided zymosan-induced chemiluminescence (CL), which is known to parallel O_2^- and H_2O_2 production (1, 2, 10), was determined. A total of 2.5×10^6 nucleated spleen cells were seeded to eight CL vials in 0.5 ml of Dulbecco modified Eagle medium plus 200 mM asparagin plus 10% fetal calf serum (complete medium). After incubation for 2 h at 37°C in 10% CO_2 in air, nonadherent cells were removed by extensive washing. Four of the eight vials containing the adherent cell fractions were treated with 0.5 ml of Türks solution to resuspend the nuclei of the adherent cell population. Macrophage nuclei could be clearly distinguished from the few contaminating lymphocyte nuclei when counted microscopically; granulocytes were not found. As a control, adherent cells were also stained by neutral red and by the Giemsa method. The percentage of adherent macrophages per spleen cell population increased from 1% in uninfected mice to 3.5% at day 3 postinfection (p.i.) in *P. yoelii* infection and decreased from about 2.5% at day 10 p.i. to 1% at day 20 p.i. In contrast, adherent macrophages of *P. berghei*-infected mice reached a maximum of 2% per spleen cell population at day 10 p.i. To the remaining four vials containing the adherent cell fraction, 0.5 ml of complete medium and 10 μl of luminol (2 mg/ml) were added, and background CL was recorded in a Biolumat LB 9505 (5, 12, 17). All CL recordings were done at 37°C. After 10 min, 10 μl of zymosan (50 mg/ml) was added, and CL was determined simultaneously and continuously until peak levels were reached ca. 10 min later. From these data, CL per 5×10^4 cells and CL per whole spleen were calculated.

In parallel, phagocytosis of zymosan by the adherent spleen cell population was determined. A total of 2.5×10^6 nucleated cells from infected mice was plated in multidish trays, each containing a round cover slip, and cultivated for

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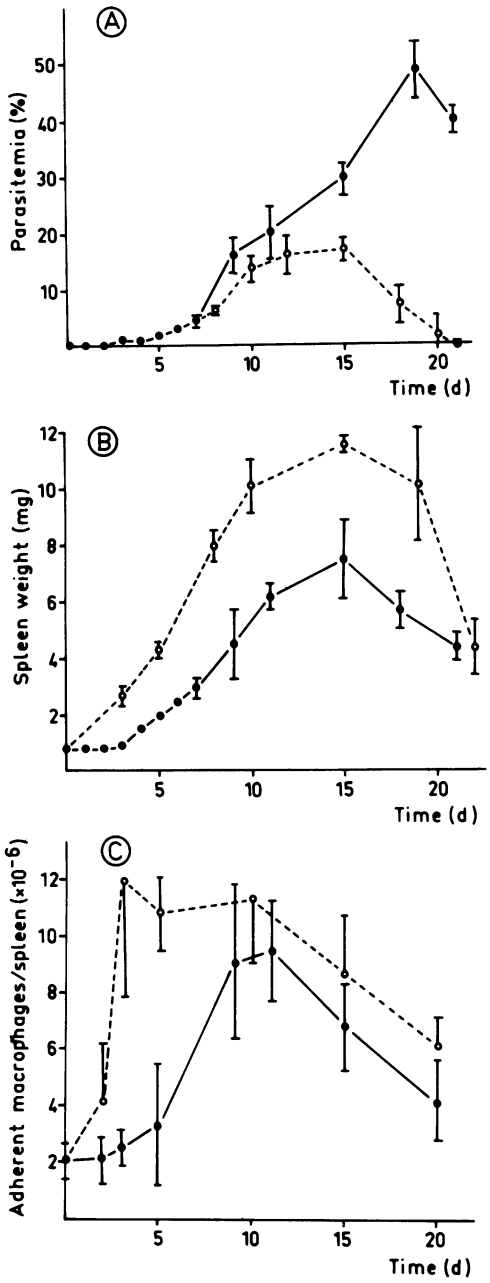

FIG. 1. Course of infection in C57BL/10 mice after intraperitoneal injection of 10^7 *P. yoelii* (○) or *P. berghei* (●) PRBCs. (A) Parasitemia as determined in Giemsa-stained tail blood smears. (B) Spleen weight as determined immediately after removal of the organ. (C) Number of adherent splenic macrophages. Pooled data from two independent experiments with three mice per experiment and means of six mice ± standard deviation are shown.

2 h at 37°C in 10% CO_2 in air. Afterwards, cover slips were washed in 37°C phosphate-buffered saline to remove nonadherent cells, overlaid with 1 ml of zymosan suspension (1 mg/ml of complete medium), and incubated for 1 h. Slips were washed once more in warm phosphate-buffered saline and immediately thereafter Giemsa stained. Phagocytized zymosan particles within phagosomes were counted microscopically.

In accordance with published data (reviewed in reference 14), *P. yoelii* was cleared from infected C57BL/10 mice after 20 to 23 days, whereas *P. berghei* induced death within 3 weeks (Fig. 1A). *P. yoelii* infection resulted in a 12-fold increase of spleen weight as compared with an 8-fold increase in *P. berghei* infection (Fig. 1B). *P. yoelii* infection led to a sixfold increase in macrophage numbers in spleens as early as day 3 p.i. In contrast, *P. berghei* induced only a slight increase of adherent cells during this time, which increased to numbers comparable to those in *P. yoelii* infection by day 10 p.i. (Fig. 1C). With other models it has been shown that the numbers of macrophages per spleen are constantly lower in lethal than in nonlethal malaria (16). This may be due to varying amounts of chemotactic factors produced by splenic mononuclear cells upon stimulation with plasmodia (24). When the rate of zymosan phagocytosis by spleen macrophages during infection was compared between the two strains, no significant differences could be detected during the first 10 days p.i. (Fig. 2). In the later stages of infection, phagocytosis was more pronounced in lethal than in self-limiting malaria.

When the capacity of macrophages to produce O_2 metabolites was assessed on a per cell basis, *P. yoelii* showed significantly higher responses than *P. berghei* (Fig. 3A). In both cases CL decreased to control level by day 5 p.i. During the later course of infection, CL of adherent spleen cells from *P. yoelii*-infected mice was comparable to that of normal animals, whereas the capacity of adherent cells from *P. berghei*-infected mice to emit CL was markedly reduced. The data therefore suggest that infection with *P. berghei* leads, after initial enhancement, to the inhibition of O_2 metabolite production. Wyler et al. (23) found that supernatants obtained from adherent cells of mice early in infection with either *P. yoelii* or *P. berghei* (days 1 to 3 p.i.) contained elevated levels of lymphocyte-activating factor, whereas later in infection (days 4 to 5 p.i.), these supernatants contained inhibitory activity. This is reminiscent of the biphasic activity of the oxidative burst of macrophages.

When the capacity of macrophages to emit CL was calculated on a per spleen basis (Fig. 3B), the difference between the two parasites became more obvious. This was mainly due to the considerable increase in the number of macrophages in spleens of *P. yoelii*- as compared with *P. berghei*-infected mice on day 3 p.i. (see Fig. 1C). In addition,

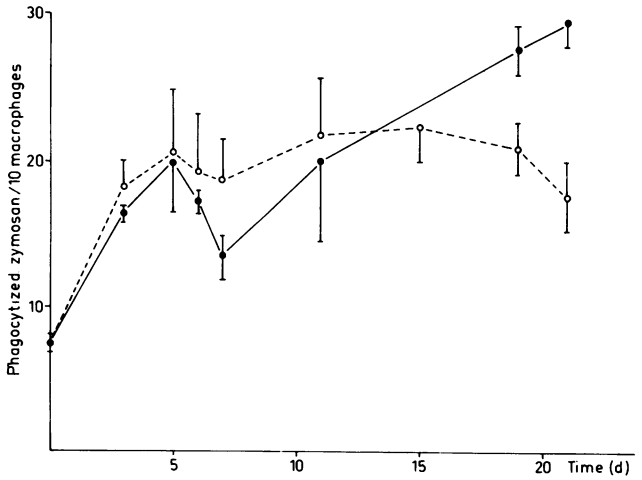

FIG. 2. Phagocytic capacity of splenic adherent macrophages after infection with 10^7 *P. yoelii* (○) or *P. berghei* (●) PRBCs. Phagocytosis of macrophages from noninfected mice never exceeded 10 zymosan particles per 10 macrophages.

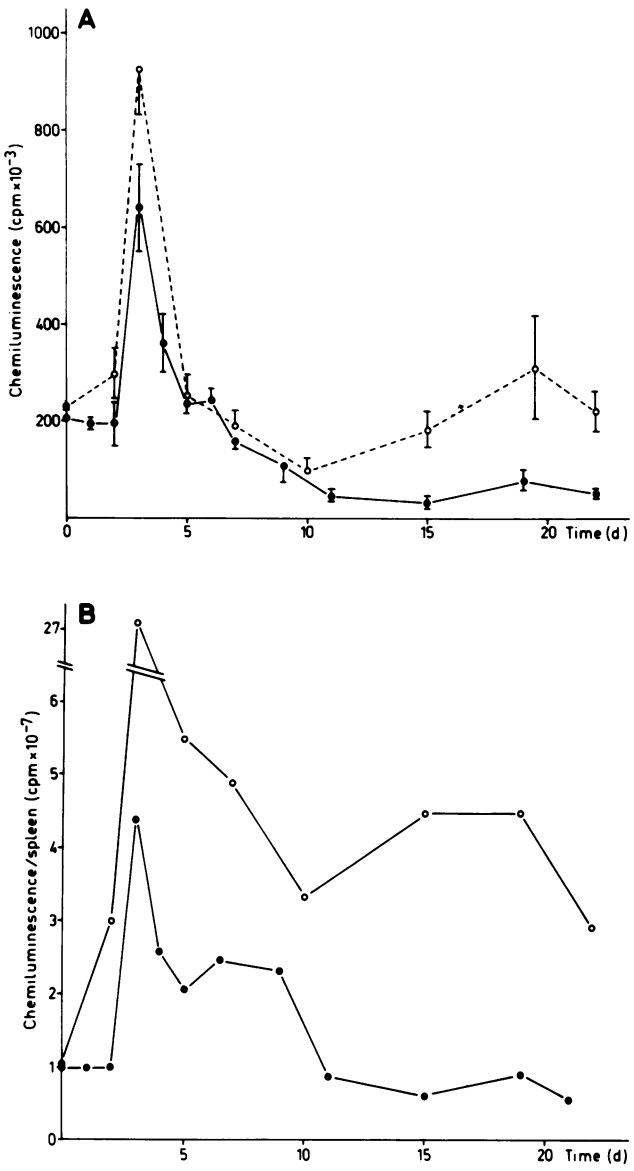

FIG. 3. Zymosan-induced luminol-aided CL response of splenic adherent macrophages from *P. yoelii* (○) or *P. berghei* (●)-infected mice. (A) CL responses per 5×10^4 adherent splenic macrophages. (B) CL responses per spleen. CL responses of macrophages from noninfected mice never exceeded 2×10^5 cpm/5×10^4 adherent cells. Pooled data from two independent experiments with three mice per experiment and means of 6 mice ± standard deviation are shown in (A).

during the whole course of infection the capacity to emit CL was significantly higher in nonlethal than in lethal malaria.

The data demonstrate that the capacity of macrophages to produce O_2 metabolites does not correlate with phagocytosis of zymosan particles. This is in line with recent observations showing that O_2 metabolites can also be induced by various nonphagocytic stimuli, thus indicating that phagocytosis is not essential for production of the O_2 metabolites (20).

Dockrell and Playfair (9) provided evidence that, under in vivo conditions, *P. yoelii* is more susceptible to exogenous H_2O_2 than is *P. berghei*. Our findings add the information that the capacity of the adherent spleen cell fraction to produce O_2 metabolites (H_2O_2 and O_2^-) is significantly higher in nonlethal *P. yoelii* than in lethal *P. berghei* infection. Taken together, the data suggest that, compared with *P. berghei*, *P. yoelii* parasites are more readily eliminated by the host because they are more sensitive to O_2 metabolites (9) and because they induce higher amounts of O_2^- and H_2O_2 in splenic macrophages (Fig. 3). The latter potential may be the result of the particular property of *P. yoelii* to recruit and stimulate T lymphocytes capable of activating macrophages. The question why the parasites are not eliminated at the time of peak O_2 metabolite production in vivo is still not answered. However, it may be due to the fact that the parasites rapidly multiply in the blood, where high concentrations of O_2 metabolites may not be achieved during the first days of infection. It is also possible that, as proposed by Allison and Eugui (3), specific antibodies which are absent during the first week p.i. facilitate the binding of effector cells to PRBC, reinforcing the effects of O_2 metabolites on the targets. Although the actual role of O_2 metabolites in the elimination of malaria parasites in vivo is still unclear, it can be envisioned that this mechanism is operative at sites at which effector cells come into close contact with PRBC, as is the case in the postarteriolar region of the splenic red pulp (4).

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