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Host Defenses in Murine Malaria: Analysis of Plasmodial Infection-Caused Defects in Macrophage Microbicidal Capacities

JAMES R. MURPHY

Department of Microbiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

Macrophage-dependent killing of facultative intracellular bacteria was markedly impaired by overt erythrocytic *Plasmodium yoelii* or *Plasmodium berghei* infection of mice. *P. yoelii* infection was capable of ablating not only the macrophage microbicidal capacity of "normal" animals but also the bactericidal capacities of "activated" macrophages. The uptake by spleen and liver of an intravenous challenge of *Listeria monocytogenes* was not altered by plasmodial infection, but within hours of injection markedly enhanced bacterial growth was found in tissues of malarious mice. The evidence gives credence to the view that the uptake of bacteria by macrophages of malarious mice was normal but that malarious mice, unlike normal mice, were unable to kill the bacteria. The plasmodial infection-caused defect in macrophage microbicidal capacity could be partially mimicked by the intravenous injection of large numbers of nonreplicating heterologous particles (i.e., killed bacteria, sheep erythrocytes). This result suggests that the uptake of particles generated during overt erythrocytic malaria may be responsible for the malaria-associated defects in macrophage bactericidal capacity.

It is clear that the murine response to plasmodial infections includes the production of an enlarged population of phagocytic cells (30, 50, 56). It seems reasonable, therefore, to suggest that these cells might be produced in order to aid in the defense against malaria. However, with the exception of the recent demonstration of a cytophilic antibody (16) which might work in concert with macrophages in clearing *Plasmodium berghei* infection of rats, there is no direct evidence of a phagocytic cell-mediated restriction of plasmodial infection. There is indirect evidence which might be interpreted to support a protective role for these cells in malaria. This evidence includes demonstration of enhanced phagocytic capacities (4, 21, 24, 47) during malaria, the finding by histological techniques of parasitized (and nonparasitized) erythrocytes (RBC) in phagocytic vacuoles of infected animals (3, 51, 58), and the demonstrations that in vitro macrophages from *P. berghei*-infected mice have an enhanced capacity to phagocytize reticulocytes (48) and that in vitro under certain conditions immune sera opsonize plasmodia (2, 6, 16, 17, 57). The demonstration that intravenous inoculations of carbon particles or thorium dioxide (15) (materials which may cause a reticuloendothelial cell blockade) cause a potentiation of *P. berghei* infection in mice and the demonstration that injections of agents such as *Corynebacterium parvum* (8) and *Mycobacterium bovis* which can cause activation of macrophages (7) may nonspecifically protect against some plasmodial parasites also indirectly indicate a role for the macrophages in the control of plasmodial infection.

However, a substantial body of evidence has been presented which demonstrates that the macrophages of malarious animals are defective with respect to certain functional capacities. Thus, fixed phagocytic cells appear to be functionally compromised by malaria in that endotoxin detoxification capacity is markedly reduced (25), test lipids are not effectively cleared from blood (21), and processing or presentation of thymus-dependent antigens is aberrant (53, 54); furthermore, in this laboratory (32) it was found recently that macrophage bactericidal capacities are lost. It appears, therefore, that the host may respond to plasmodial infection by generating an enlarged population of phagocytic cells but that the plasmodia may in turn cause disturbances in phagocyte function. The mechanism(s) of these plasmodia-induced disturbances in macrophage function and the significance of these defects to the quality of the host defense to malaria remain to be discovered.

The purpose of this study was to further characterize the plasmodia-caused defect in macrophage microbicidal activities described previously (32). The results show that this defect is of substantial magnitude, compromises the host's capacity to deal with at least three genera of facultative intracellular bacteria, and may be

mediated by malaria infection-caused bombardment of fixed phagocytes with particulate materials perhaps in a manner similar to the phenomenon of reticuloendothelial cell blockade.

MATERIALS AND METHODS

Animals. Female mice of the randomly bred ICR strain, syngeneic A strain, and syngeneic F_1 hybrid B6D2 (C57BL/6 × DBA/2) strain were obtained when they were 5 to 8 weeks old from colonies maintained at the Trudeau Institute or the University of Maryland School of Medicine. The mice were provided with water and a standard diet (4RF, Charles River formula; Agway, Waverly, N.Y.) ad libitum.

Plasmodia. *P. berghei* strain NYU-2, *Plasmodium yoelii* strain 17X, and *Plasmodium chabaudi* were used. The source and procedures used for maintenance of *P. berghei* (33) and *P. yoelii* (32) have been published. *P. chabaudi* was obtained from Robert McAlister, Department of Biology, Southern Methodist University, Dallas, Tex. The parasites were maintained as frozen (−70°C) stocks which were passaged once in mice before use as a challenge. Plasmodial infections were initiated by the injection of 10^3 parasitized RBC (PRBC) delivered in 0.2 ml of 0.15 M NaCl solution into the lateral tail vein.

Bacteria. *Listeria monocytogenes* (strain EGD), *Salmonella enteritidis* (strain 5694), and *Yersinia enterocolitica* (strain WA) were used. The *L. monocytogenes* was from the same frozen seed used for a previous study (32). The *S. enteritidis* and *Y. enterocolitica* were obtained from Philip B. Carter of the Trudeau Institute, Saranac Lake, N.Y.; they were grown in Trypticase soy broth, dispensed, and stored at −70°C. For each experiment a sample was thawed and diluted with 0.15 M NaCl. Numbers of bacterial colony-forming units (CFU) were determined by plating on Trypticase soy agar. Mice were infected, unless otherwise noted, by the injection of 0.2 ml containing 10^5 CFU into the lateral tail vein.

M. bovis. A frozen seed of the Pasteur strain of *M. bovis* BCG was obtained from the Mycobacterium Culture Collection (TMC 1011), Trudeau Institute. Dosage was based upon colony counts on Middlebrook 7H-10 medium (Difco).

Particulate materials. Heat-killed *L. monocytogenes* cells (95°C for 90 min), dead *C. parvum* cells (Coparvax, supplied by Burroughs Wellcome Co., Research Triangle Park, N.C.), and sheep RBC (Animal Blood Center, Syracuse, N.Y.) were used to test the effects of heterologous particles on macrophage microbicidal capacities (see below). Numbers of bacteria were determined from direct counts in a Petroff-Hausser chamber; numbers of RBC were obtained from direct counts in a hemocytometer. Particle content was adjusted to the desired density with 0.15 M NaCl, and particles were inoculated in 0.2 ml delivered into the lateral tail vein.

Parasitemia. Thin smears were prepared from tail blood and stained by the Giemsa method. If the level of parasitemia was 1% or greater, the number of PRBC in 200 RBC was determined. In the case of parasitemia less than 1%, the number of PRBC in a number of microscopic fields containing 2×10^4 RBC was determined. Parasitemia is expressed as median PRBC per 10^4 RBC in $\log_{10}$ units for each group of mice. The lower limit of sensitivity of the parasitemia determinations was approximately 1 PRBC/10^4 RBC.

Statistics. The statistical significance of differences between treated and control groups was determined either by the *t* test or, when more than two groups were tested, by analysis of variance and Tukey's test.

Assay of systemic macrophage bactericidal activity. Changes in macrophage-mediated bactericidal activity were measured by comparing the growth of an intravenous challenge of facultative intracellular bacteria in experimentally manipulated mice with the growth of bacteria from the same inoculum delivered to normal mice. The recovery of fewer bacteria from treated than from control mice was evidence of activation of microbicidal capacity, whereas the recovery of greater numbers of bacteria from treated animals was evidence of a loss of macrophage bactericidal capacity. This is an established procedure for determining levels of macrophage microbicidal activities and is discussed elsewhere (32, 40, 41).

Data on microbicidal capacity of macrophages are expressed either directly as the $\log_{10}$ of the geometric mean number of bacteria per liver per group or indirectly as a resistance index which was obtained by subtracting the $\log_{10}$ geometric mean number of bacteria per liver of experimentally treated mice from the $\log_{10}$ geometric mean number recovered from control animals challenged with the same inoculum. In all experiments where resistance index was used, mice were sacrificed for bacterial enumeration 24 h after bacterial infection. A resistance index of +1 or −1, for example, meant that the bacteria grew 10-fold less or more, respectively, in treated animals than in controls. This convention causes groups with enhanced bactericidal capacities to appear in figures above the control value, which is normalized to 0, and animals with suppressed bactericidal capacities to appear below the control. The meaning of the resistance index in terms of macrophage activation has been discussed by North (38).

For one experiment it was found useful to calculate the number of particles required to cause 50% of mice to show a significant ($P \leq 0.01$) loss of bactericidal capacities. This was accomplished by determining the difference from the geometric mean number of bacteria per control liver which represented the $P \leq 0.01$ significance level (analysis of variance, Tukey's test) and assigning a + (plus) to each experimentally manipulated animal that met or exceeded this value. Animals that did not exceed this significance level were categorized as − (minus). The + and − values were then used to calculate a 50% suppressive dose by using the procedure of Reed and Muench (44).

RESULTS

Subversion of systemic macrophage microbicidal capacities by *P. yoelii* infection. Figure 1 presents the course in ICR mice of *P. yoelii* infection initiated by the intravenous inoculation of 10^3 PRBC and shows that during the course of patent infection the capacity of the malarious animals to resist an intravenous chal-

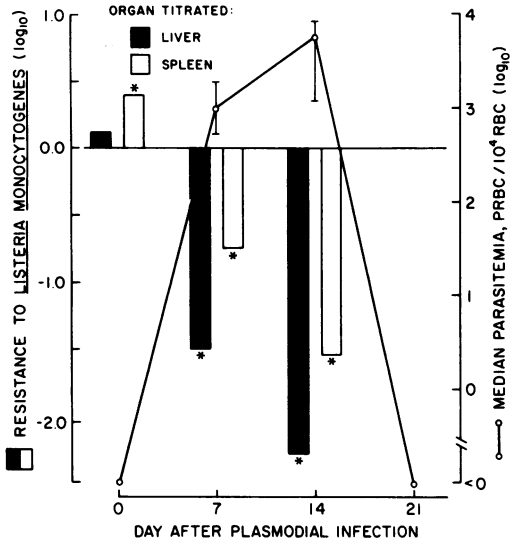

FIG. 1. *Development of suppressed macrophage microbicidal capacities during P. yoelii infection. The 24-h growth of a standard 10^5-CFU challenge of L. monocytogenes delivered either 10 min (0 days) or 7, 14, or 21 days after P. yoelii infection was compared with the growth of the same inoculum delivered to control mice. Greatly increased numbers of L. monocytogenes organisms were recovered from both livers and spleens of P. yoelii-infected mice during the patent blood infection. The solid line presents the course of parasitemia (± range). There were five mice per group per time point, and those groups of animals which showed titers of L. monocytogenes that differed significantly from controls ($P \leq 0.01$) are indicated with an asterisk* (*).

lenge with 10^5 CFU of *L. monocytogenes* was markedly reduced. This was evidenced on days 7 and 14 after plasmodial infection by a marked increase in the growth of bacteria in liver and spleen.

No relationship between level of suppression of macrophage microbicidal capacities as measured in liver and spleen and the ultimate outcome of the malaria infection was established. Rather, overt *P. yoelii* infection essentially ablated macrophage microbicidal capacity in these organs.

To establish that the changes in macrophage function recorded in Fig. 1 resulted from *P. yoelii* infection and not from the introduction of heterogeneic cells or plasma, RBC were collected from a normal ICR mouse, and a number of these cells equivalent to the number delivered at the initiation of *P. yoelii* infection was inoculated into naive recipients. These animals were challenged with *L. monocytogenes* at 10 min or 7 or 14 days. No evidence of defective macrophage bactericidal function was found.

Most of the studies presented below were conducted on day 14 of *P. yoelii* infection at about the peak of parasitemia and at the time of the maximum defect in macrophage microbicidal function. No attempt is made to separately present data generated from mice which would die of *P. yoelii*, because these could not be identified at day 14. Mice died of *P. yoelii* infection from as early as day 12 through as late as day 28 (most died between 17 and 21 days).

To determine whether the capacity of macrophages to kill bacteria other than *L. monocytogenes* was suppressed by murine malaria, mice were challenged intravenously with 10^5 CFU of either *L. monocytogenes*, *S. enteritidis*, or *Y. enterocolitica*. Twenty-four hours after bacterial challenge, these mice were sacrificed, and the number of organisms in liver was determined. These bacteria were chosen for this study because it has been established (5, 37, 41) that a large number of the intravenously inoculated organisms are apparently killed by liver macrophages.

Figure 2 shows that *P. yoelii* infection caused a loss in the capacity to kill nonspecifically bacteria of each of the genera tested. There were between 100 and 400 times as many organisms recovered from malarious mice as from normal

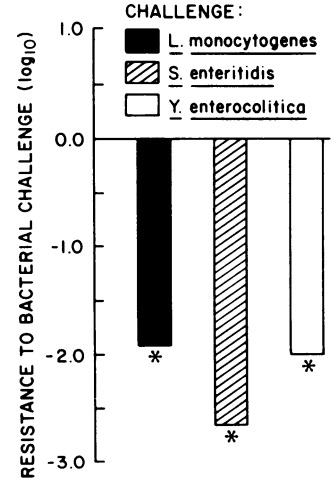

FIG. 2. *Evidence that P. yoelii infection suppresses resistance to intravenous infection with differing genera of facultative intracellular bacteria. Mice were inoculated intravenously with 10^3 PRBC infected with P. yoelii, and 14 days thereafter the mice were separately challenged by intravenous injection of 10^5 CFU of L. monocytogenes, S. enteritidis, or Y. enterocolitica. Significantly ($P \leq 0.01$, indicated by *) greater numbers of bacteria were recovered at 24 h after bacterial challenge from animals with concomitant malaria than from controls challenged with the same bacterial inoculum. Five mice were employed per point.*

mice challenged with the same bacterial preparations.

Defective microbicidal activities were demonstrated not only in malarious random-bred ICR mice but also in *P. yoelii*-infected (infection initiated with ICR PRBC) syngeneic parental strain A and syngeneic F_1 hybrid B6D2 animals (Fig. 3). The level of suppression of this macrophage function was similar for ICR and B6D2 animals, and strain A animals appeared somewhat more sensitive to the effect. The reason for the greater suppression in the A strain mice is not known. It is clear, however, that it did not result from markedly different levels of parasitemia.

To determine whether this subversion of microbicidal function was a property restricted to *P. yoelii* or more widely distributed among species of plasmodia, separate groups of ICR mice were infected intravenously with 10^3 PRBC infected with either *P. yoelii*, *P. berghei*, or *P. chabaudi*. The animals were challenged with *L. monocytogenes* at day 14. Figure 4 shows significant levels of suppression of microbicidal function in *P. yoelii*- and *P. berghei*-infected animals. *P. chabaudi* infection, however, did not cause a similar defect.

Changes in antibacterial resistance reflect a loss of preexisting macrophage bactericidal capacity. It is established that in vivo killing of *L. monocytogenes* is effected by macrophages (26, 27, 35). The level of bactericidal activities of these cells is in turn modulated by lymphoid cells (27, 29, 36). It is possible, therefore, that the loss of macrophage bactericidal capacities observed in the foregoing experiments might have resulted from a plasmodial infection-induced defect in macrophages or, alternatively, from a defect in the capacity of malarious mice to generate those lymphoid cells which modulate macrophage bactericidal activities.

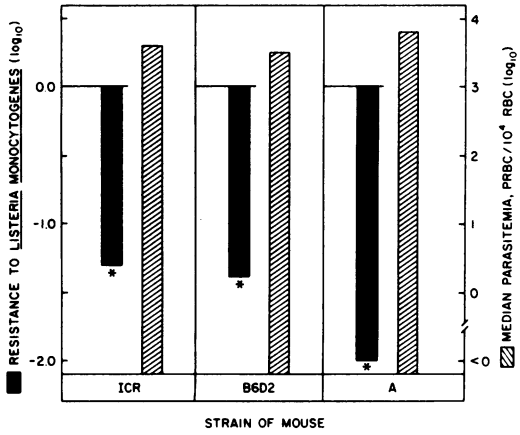

FIG. 3. *Evidence that P. yoelii infection of B6D2 or A strain mice caused suppressed resistance to L. monocytogenes. Significant ($P \leq 0.01$, indicated by *) suppression of microbicidal capacities was found for each of the three strains of mice challenged with L. monocytogenes on day 14 of P. yoelii infection. The level of parasitemia at day 14 is also presented. There were five mice per group.*

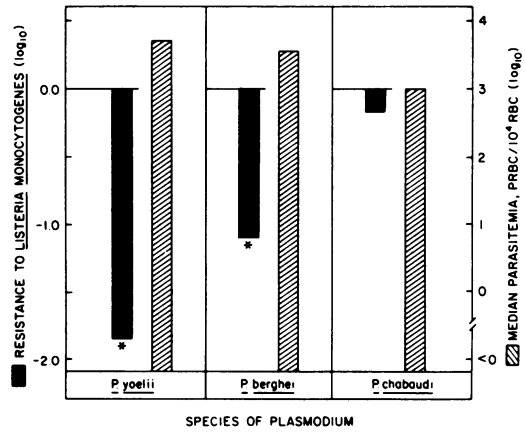

FIG. 4. *Evidence that differing species of plasmodia differ in their capacities to subvert antibacterial resistance. Although levels of parasitemia did not differ markedly on day 14 after P. yoelii, P. berghei or P. chabaudi infection, only P. yoelii and P. berghei caused significant ($P \leq 0.01$, indicated by *) suppression of anti-L. monocytogenes resistance. Five mice per group.*

To determine which of these groups of lesions was responsible for the loss of microbicidal capacities during *P. yoelii* infection, advantage was taken of the demonstration by North et al. (41) that macrophages of normal animals rapidly destroy the majority of the inoculated *L. monocytogenes* within 8 to 12 h after bacterial challenge. It was predicted that if *P. yoelii* directly modified macrophage function, then the fate of *L. monocytogenes* within the first 12 h would differ for malarious and normal mice. Alternatively, if *P. yoelii* caused a lesion in the generation of specifically sensitized anti-*L. monocytogenes* lymphoid cells, it might be expected that the fate of the bacteria during the first 12 h after inoculation would be similar for normal and malarious mice but that subsequently the failure of the malarious animals to generate or deploy specific lymphoid cells in response to *L. monocytogenes*, a process which requires days (36), would be reflected in differences in the later phase of the *L. monocytogenes* infection.

It can be seen in Fig. 5 that mice challenged with *L. monocytogenes* 10 min after *P. yoelii* infection, at a time when macrophage microbicidal capacities were previously shown to be near normal (see Fig. 1), showed for the first 24

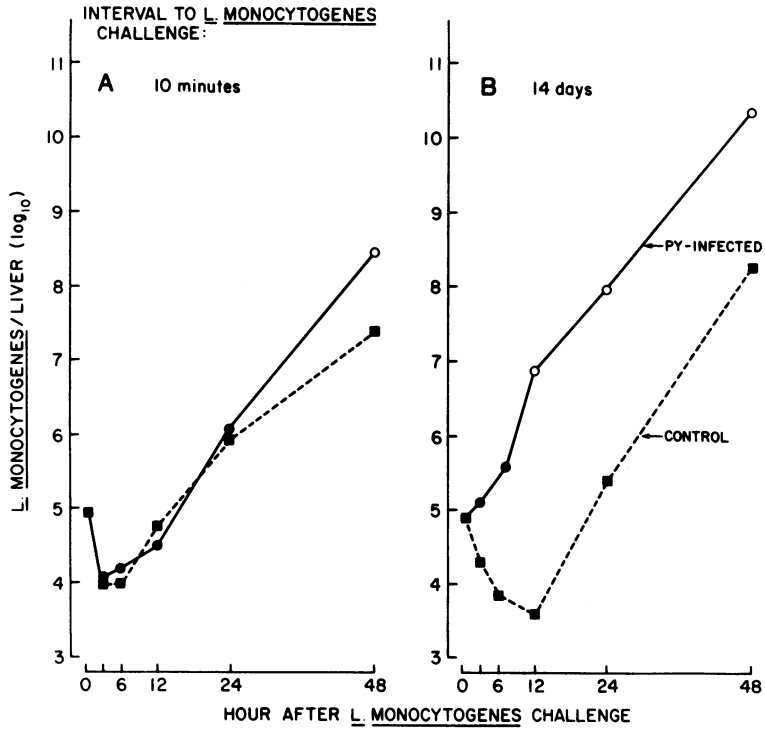

FIG. 5. *Evidence that suppressed anti-L. monocytogenes resistance seen at day 14 of P. yoelii (PY) infection of ICR mice is partly caused by a suppressed capacity of resident macrophages of liver to reduce the bacterial load in this organ during the first 12 h of bacterial infection. Five mice were employed per group per time point, and those groups of P. yoelii-infected mice which showed significantly ($P \leq 0.01$) more bacteria per liver than controls are indicated by open symbols.*

h of *L. monocytogenes* infection the same pattern of localization, killing, and subsequent growth of bacteria as did normal controls. By contrast, mice challenged with *L. monocytogenes* on day 14 of *P. yoelii* infection failed to kill bacteria during the first 12 h after challenge; it appeared that most of the inoculum survived to cause progressive infection.

Evidence that *P. yoelii* infection interferes with the microbicidal function of activated macrophages. To determine whether the disturbance in macrophage microbicidal function caused by *P. yoelii* infection could be expressed against activated macrophages, mice were infected intravenously with 10^7 CFU of *M. bovis* BCG, a strain which causes a protracted state of enhanced microbicidal activity (1, 23). Seven days after BCG infection, a subset of the mycobacterially infected mice and a group of normal mice were infected with *P. yoelii*, and on day 14 after the plasmodial infection (day 21 after BCG) these and control animals were challenged with *L. monocytogenes*.

Figure 6 shows, as expected (1, 23), that mice infected with BCG alone had activated macrophages, as evidenced by enhanced levels of listericidal capacity as compared to normal animals. By contrast, BCG-infected animals subsequently infected with *P. yoelii* showed a marked suppression of microbicidal ability. The magnitude of the defect was demonstrated by the recovery of 10,000-fold more *L. monocytogenes* from BCG + *P. yoelii*-infected animals than from mice infected with BCG alone. Figure 6B shows that levels of *P. yoelii* infection were similar in BCG-infected and in otherwise normal mice.

Figure 6C presents the numbers of BCG recovered from the liver of *P. yoelii*-infected and control mice which 22 days previously had been challenged with the same inoculum of BCG. Approximately 10-fold more mycobacteria were recovered from the mice infected with *P. yoelii*.

Evidence that *P. yoelii* can subvert an established state of cellular immunity. The demonstration in the previous experiment of increased numbers of BCG per liver of malarious animals suggested that *P. yoelii* infection might interfere not only with the expression of nonspecific macrophage bactericidal capacities but also

with specific cellular immunity. However, because it requires 10 to 15 days for mice to acquire, after intravenous injection of BCG, a capacity to control bacterial growth in liver and spleen (1) and because in the previous experiment *P. yoelii* infections were initiated on day 7 of BCG infection, it was not possible to determine whether the plasmodial infection interfered with the expression of an established state of cellular immunity. Alternatively, *P. yoelii* might have prevented the development of the cellular defense.

To determine whether *P. yoelii* infection could interfere with the expression of an established state of cellular immunity, advantage was taken of the demonstration by Mackaness (26) that an immunizing infection with *L. monocytogenes* is followed by a long-lived state of heightened resistance to reinfection. Recently, North (37) and North and Deissler (39) have presented evidence which supports the view that this long-lived heightened resistance represents a state of T-cell immunological memory.

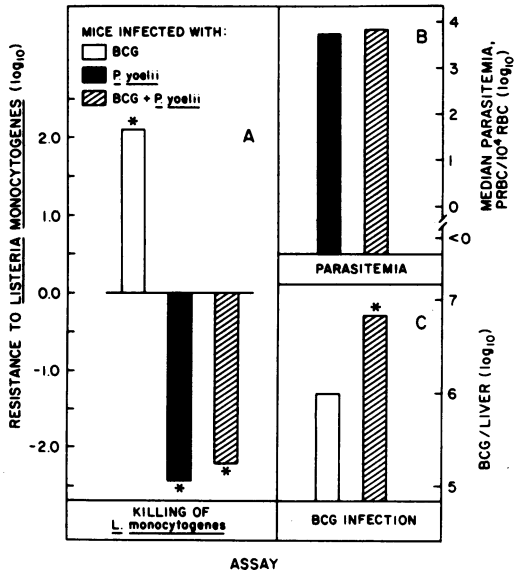

FIG. 6. *Demonstration that P. yoelii infection can subvert nonspecific microbicidal capacities in the face of conditions which would otherwise lead to activation of macrophage microbicidal capacities. (A) Greatly increased anti-L. monocytogenes resistance, expressed nonspecifically by liver macrophages activated as the result of BCG infection initiated 21 days before L. monocytogenes challenge. P. yoelii infection of either normal or BCG-infected mice resulted in a loss of microbicidal capacities of both normal macrophages and macrophages subjected to BCG activation. (B) Day 14 after plasmodial infection: the level of P. yoelii parasitemia was similar for control and previously BCG-infected mice. (C) P. yoelii infection interfered with the capacity of the mouse to control BCG infection in liver. Those experimental groups which differed significantly ($P \leq 0.01$) from controls are indicated by an asterisk (*).*

To establish a long-lived state of cellular resistance to *L. monocytogenes*, mice were inoculated intravenously with 10^3 CFU. Twenty-eight days later, when the bacteria had been cleared and, as has been shown by others, a population of nondividing T cells with anti-*L. monocytogenes* potential had been established (26, 37, 39), a subset of the *L. monocytogenes*-immune mice were infected with *P. yoelii*. Either 10 min or 14 days later, groups of these mice and controls were challenged with *L. monocytogenes*.

Mice immune to *L. monocytogenes* were better able to resist bacterial challenge than nonimmune controls (Fig. 7A). This increased resistance was manifested as an accelerated capacity to generate a defense and not as an enhanced level of bacterial killing during the first 6 to 12 h after challenge. Therefore, this enhanced resistance has characteristics of immunological memory (37, 39).

Mice infected with *P. yoelii* 10 min before *L. monocytogenes* challenge showed the same pattern of bacterial infection as the respective controls. Thus, it is apparent that levels of malaria infection that do not cause a loss of nonspecific macrophage bactericidal capacities (Fig. 1) do not interfere with the expression of cellular immunological memory.

In contrast, *L. monocytogenes*-immune mice challenged with *L. monocytogenes* on day 14 of *P. yoelii* infection (day 42 after immunization with *L. monocytogenes*) were completely unable to control the bacterial infection. Malarious mice with an established state of cellular immunity to *L. monocytogenes* fared no better than nonimmune controls in combating bacterial infection. Figure 7 also shows that the level of the cellular defense to *L. monocytogenes* waned between days 28 and 42 after the immunizing infection.

Evidence that the intravenous introduction of heterologous particles causes a suppression of macrophage microbicidal function similar to that caused by *P. yoelii* infection. Figure 8 shows the result of an experiment in which mice were inoculated with a variety of nonliving particles and challenged 3 h later with *L. monocytogenes*. It can be seen in this figure that recipients of particles were substantially compromised in their capacity to kill *L. monocytogenes* as compared with normal mice or recipients of the diluent (0.15 M NaCl). Figure 9 shows that the level of suppression of microbicidal activities was directly related to the number of heterologous particles inoculated.

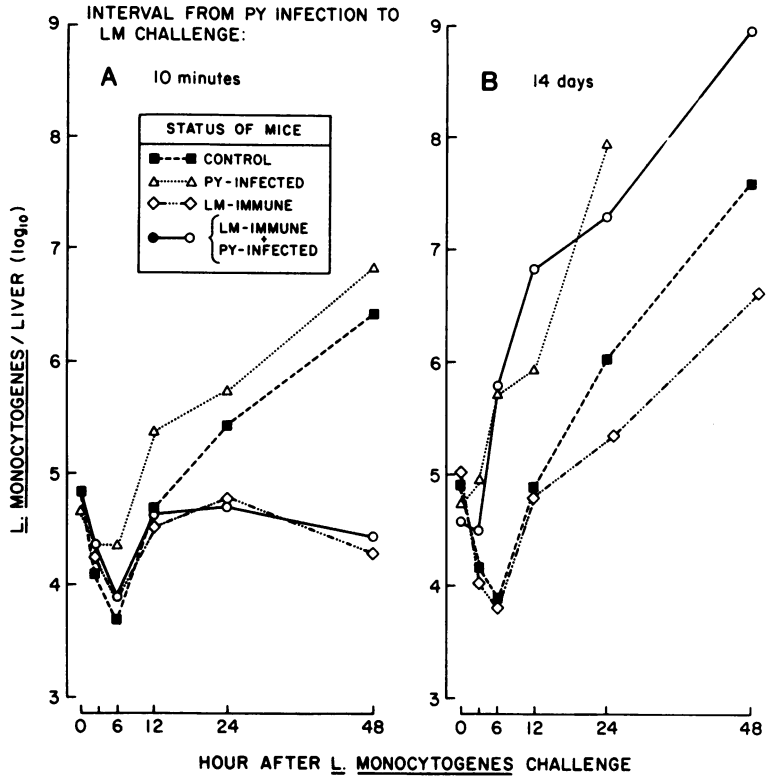

FIG. 7. *Demonstration that P. yoelii (PY) infection can subvert an established state of cellular immunity. The liver growth curves show that mice immunized against L. monocytogenes (LM) were better able to resist subsequent homologous challenge than nonimmune mice and, further, that fully developed but not incipient P. yoelii infection destroys the antibacterial advantage provided by the established cellular defense. Five mice were employed per group per time point, and those groups which showed numbers of bacteria per liver significantly ($P \leq 0.01$) different from controls are indicated by open symbols.*

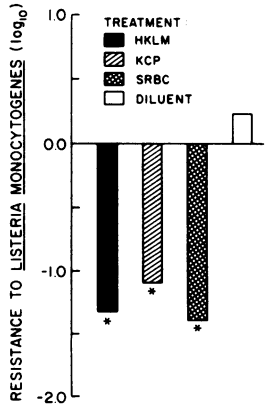

FIG. 8. *Demonstration that intravenous injection of particulate materials can subvert the capacity of macrophages to kill a subsequent challenge of L. monocytogenes. Separate groups of five mice were inoculated intravenously with 10^{10} heat-killed L. monocytogenes organisms (HKLM), 10^{10} killed C. parvum organisms (KCP), 10^9 sheep RBC (SRBC), or an equivalent volume of diluent (0.15 M NaCl) 3 h before an intravenous challenge with 10^5 CFU of L. monocytogenes. Livers were collected 24 h later from each of the groups and from otherwise untreated L. monocytogenes-infected mice. Each of the groups pretreated with particulate materials showed significantly more ($P \leq 0.01$, indicated by *) L. monocytogenes cells per liver than control mice which received L. monocytogenes infection only.*

Mice inoculated with either sheep RBC or killed *L. monocytogenes* and challenged with live *L. monocytogenes* 3 h later failed to kill the bacteria during the first 12 h after challenge to the extent that control animals did (Fig. 10).

To determine the duration of the particle-induced lesion in bactericidal activities, mice were inoculated with 10^{10} killed *L. monocytogenes* cells or 10^9 sheep RBC and challenged at intervals from 3 h through 48 h thereafter with live *L. monocytogenes*. Suppression of this capacity was transient, with the maximal defect occurring at 6 h and normal macrophage bactericidal activity returning by day 2 (Fig. 11).

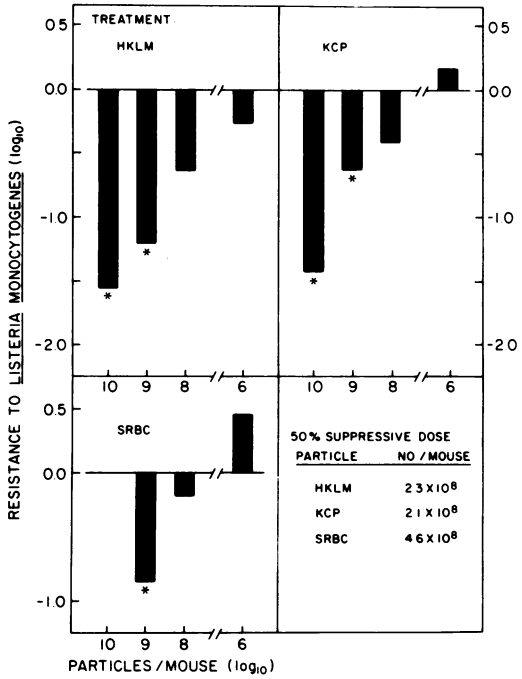

FIG. 9. *Demonstration that particle-mediated suppression of macrophage bactericidal capacities is directly related to the number of particles injected. The experimental protocol differed only in the number of particles injected from that presented for the experiment described in Fig. 8. Treated mice which showed significantly ($P \leq 0.01$) more L. monocytogenes organisms per liver than controls are indicated (*). The number of particles calculated, on the basis of these results, to cause 50% of mice to show significant ($P \leq 0.01$) suppression of bactericidal capacities is presented. Five mice per group.*

DISCUSSION

Mice can be partially protected from erythrocytic-stage *P. berghei* (31, 55) or *P. yoelii* (32, 42, 46) infection by the passive transfer of immune serum. However, recipients of immune serum do not usually exhibit a quality of immunity similar to that exhibited by a mouse which has recently recovered from a plasmodial infection (31, 42). Thus, the possibility that a T-cell-mediated cellular defense may work in concert with antibody in clearing plasmodial infections must be considered. This possibility receives indirect support from (i) observations which show that delayed footpad swelling reactions to injections of plasmodial antigens, possibly a measure of cellular delayed-type hypersensitivity, at times develop as part of successful immune responses to plasmodia (10, 14) and (ii) the demonstrations that potent stimulators of T-cell activity such as BCG infection can at times protect from rodent malaria (7).

If a cellular defense is indeed generated and expressed against malaria, then it would seem reasonable to suggest that activation of heterologous macrophage microbicidal capacities would

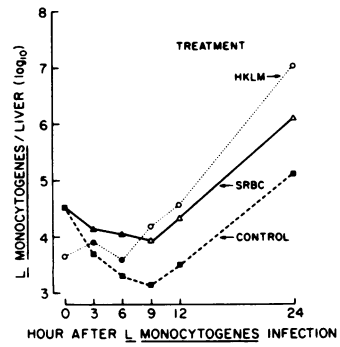

FIG. 10. *Evidence that the suppressed anti-L. monocytogenes resistance caused by intravenous injection of 10^{10} heat-killed L. monocytogenes (HKLM) cells or 10^9 sheep RBC (SRBC) 3 h before L. monocytogenes challenge is partly caused by a suppressed capacity of resident macrophages of liver to reduce the bacterial load in this origin during the first 9 h of bacterial infection. Five mice were employed per group per time point, and those recipients of particles which showed significantly ($P \leq 0.01$) different numbers of L. monocytogenes organisms per liver than controls are indicated by open symbols.*

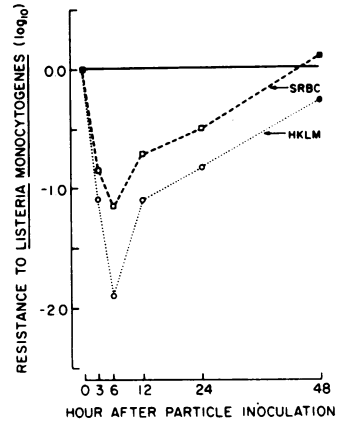

FIG. 11. *Course of particle-induced suppression of macrophage bactericidal capacities. Mice were injected intravenously with 10^{10} heat-killed L. monocytogenes cells (HKLM) or 10^9 sheep RBC (SRBC) and, at the indicated intervals, challenged intravenously with 10^5 CFU of L. monocytogenes. Twenty-four hours after the respective L. monocytogenes challenge, livers were collected from treated and control animals, and the number of L. monocytogenes organisms per organ was determined. Those groups of mice showing significantly ($P \leq 0.01$) more bacteria per liver than the respective control group are indicated by open symbols. There were five mice per group per time point.*

be demonstrable during the development or expression phase of a successful antiplasmodial defense because ongoing systemic T-cell responses generate a signal which in turn causes activation of this macrophage capacity (38, 40). In a previous study (32), this proposition was tested using avirulent *P. yoelii* infection of ICR mice as the experimental model. In contrast with what would be expected if a cellular defense were generated, it was found that animals which successfully eliminated malaria showed defective macrophage microbicidal capacities in liver, spleen, and peritoneal cavity. Thus, the present studies were conducted (i) to further characterize the defects in macrophage microbicidal capacities caused by murine malarial parasites, (ii) to determine the effects of the loss of this macrophage function on the expression of macrophage-dependent cellular defenses to bacterial pathogens, and (iii) to probe the mechanism through which malaria causes the loss of microbicidal capacities.

The present studies show that patent *P. yoelii* and *P. berghei* infections cause a loss of macrophage antimicrobial capacity and compromise macrophage-dependent cellular immunity to at least two genera of bacterium, *Mycobacterium* and *Listeria*. The demonstration that *Yersinia* and *Salmonella* were less well controlled by malarious mice than by normal mice suggests that the cellular defense to these agents might similarly be compromised, although direct evidence of this was not obtained. Although *P. yoelii* infection markedly affected the pattern of growth of *L. monocytogenes*, it did not cause a marked change in the localization of an intravenous challenge of *L. monocytogenes*. This suggests that malaria does not affect the capacity of *L. monocytogenes* to localize to macrophages, but rather affects the subsequent capacity of macrophages to kill *L. monocytogenes*. The demonstration that sheep RBC could similarly cause a loss of macrophage microbicidal capacities without altering the capacity of *L. monocytogenes* to locate in liver (presumably in Kupffer cells [38]) gives credence to the view that blood-borne heterologous particles can ablate antilisterial capacities of macrophages without affecting the capacity of *L. monocytogenes* to localize in these cells. Direct evidence of the cellular location of intravenously inoculated *L. monocytogenes* was not sought in the present studies of malarious mice, but the patterns of localization and growth observed are consistent with those seen by others (35).

It was found that BCG grew to higher titers in malarious mice than in normal mice and that malaria ablated the capacity of mice immunized against *L. monocytogenes* to clear a challenge of *L. monocytogenes* more rapidly than nonimmune controls. This demonstrates that *P. yoelii* infection can interfere with the expression of specific acquired cellular immunity. It is reasonable, therefore, to ask whether it is the immunologically specific T lymphocytes or the nonspecific macrophage activities which are compromised by malaria. Direct evidence for a compromise in the nonspecific macrophage functions is provided through the data presented in Fig. 5 and 7. The work of others (22) suggests that lymphocytes may also be adversely affected, but the present study does not address this point.

Direct evidence that macrophages are required for the successful acquisition or expression of immunity to malaria in vivo is not available. However, numerous roles could be or have been suggested. In turn, it could be envisioned that a malaria-caused defect in macrophage function might favor the persistence of the parasite. For example, it is established that immunoglobulin G antibodies are in part responsible for immunity to malaria (9, 12). The knowledge that some immunoglobulin G antibodies are thymus dependent (11, 28, 45) and require macrophages for antigen presentation (13, 49, 52) might be interpreted to suggest that macrophages play a role in the acquisition of antimalarial immunity. Evidence to prove this proposition is not available. However, Weidanz and his co-workers (53, 54) have recently shown that macrophages from spleens of malarious mice are deficient in the presentation of heterologous T-cell-dependent antigens (sheep and horse RBC). Thus, it can be further suggested that malaria might interfere with the process by which protective antimalarial antibody is generated. Again, direct evidence is lacking.

It might also be argued that antibody might act as an opsonin, thereby causing parasites to preferentially accumulate in macrophages. Evidence from in vitro systems (2, 6, 17, 57) supports the view that opsonins develop during the immune response to murine malaria and that these may be directed against not only parasites (6, 16) but also PRBC (17, 48) and normal RBC (48). In vivo evidence for a protective effect of opsonins, however, is scarce. Green and Kreier (16) have shown that a serum factor which binds to macrophages in vitro conveys to recipients which also receive an opsonizing serum a level of protection superior to that transferred with opsonizing serum alone. However, the precise identity of the humoral component with in vivo protective activity was not established, and, further, that the humoral factor caused parasites to accumulate in or on macrophages was not shown. Thus, the question of whether opsonins are active in vivo remains open. Recent studies

by Quinn and Wyler (43) suggest that antimalaria antibodies may not require macrophages to effect their antimalarial role in vivo.

This study was directed specifically to the question of whether or not *P. yoelii* infection in mice would alter the nonspecific microbicidal function of macrophages so that susceptibility was altered to infection with bacteria, such as *L. monocytogenes*, in which the microbicidal action of macrophages, normal or activated, is currently generally regarded to be the major mechanism of host defense and acquired immunity (34, 38, 40). The results were overwhelmingly in favor of malaria-caused nonspecific interference with macrophage function. Moreover, the results suggest some explanations for the failure of this immune mechanism in malaria itself.

Thus, in this study, it was clearly shown that some plasmodial infections may cause a mouse to lose its capacity to kill facultative intracellular bacteria. Because killing of *L. monocytogenes* in vivo is effected by macrophages (26, 27, 35), these results strongly support the view that malaria abrogates macrophage microbicidal capacities. Furthermore, the demonstration that *L. monocytogenes* was killed less effectively in malarious than in control mice during the first 6 to 12 h after bacterial challenge is in agreement with the suggestion that malaria causes macrophages to lose normal preexisting background levels of bactericidal capacity.

Kaye et al. (20) reported previously that mice concurrently infected with *P. berghei* (NYU-2) and *Salmonella typhimurium* (strain KK) died more rapidly than mice infected with but one species of parasite. They showed further that the decreased survival time corresponded to a more rapidly progressing *S. typhimurium* infection and not with a more fulminant malaria or more rapidly developing or severe anemia. It seems probable, therefore, that a malaria-caused defect in macrophage bactericidal capacities, such as that described in these studies, was in part responsible for enhanced *S. typhimurium* infection in malarious mice. Kaye and co-workers (18, 19) have also shown that hemolysis can cause increased susceptibility to bacterial infection.

The present studies show that the intravenous injection of any of a variety of heterologous particles may cause a loss of preexisting macrophage microbicidal capacities. Thus, it is suggested that, via a similar mechanism, parasites and debris released into the vascular compartment during malaria, and possibly PRBC, may in part cause the defect in macrophage bactericidal activity observed. The results suggest that a very large number of particles must be generated over a 2-week interval if this mechanism of suppression is to explain totally the loss of microbicidal capacities during *P. yoelii* infection. The possibility that suppression of macrophage bactericidal capacities occurs through mechanisms in addition to particle bombardment should be considered.

Although it is clear that the murine response to plasmodial infections includes the generation of an enlarged population of phagocytic cells (30, 50, 56), direct evidence that these cells contribute to the host defense against malaria is sparse. The demonstrations that (i) activation of some phagocyte-dependent functions occurs during malaria (3, 4, 21, 24, 47, 48, 51, 58), (ii) procedures which cause nonspecific activation of phagocytes may concomitantly cause nonspecific protection from plasmodial infection (7, 8), and (iii) manipulations which interfere with phagocyte function potentiate malaria (15) suggest that macrophages might play a protective role. However, other macrophage-dependent capacities are suppressed or lost during malaria (21, 25, 32, 53, 54). Thus, although it is tempting to speculate that plasmodial infection might subvert those macrophage functions which, if working properly, could contribute to elimination of the parasites, the possibility that macrophages do not directly contribute to the destruction of parasites must be considered. In this case, the changes in their functional capacities observed during malaria may simply reflect a consequence of their role as scavengers of debris.

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