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Resolution of Acute Malarial Infections by T Cell-Dependent Non-Antibody-Mediated Mechanisms of Immunity

LISA A. CAVACINI,† LYDIA A. PARKE, AND WILLIAM P. WEIDANZ*

Malaria Research Group, Department of Microbiology and Immunology, Hahnemann University, Broad and Vine Streets, Philadelphia, Pennsylvania 19102

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While it is generally accepted that acute blood stage malarial infections are resolved through the actions of protective antibodies, we observed that resistance to acute infection with *Plasmodium chabaudi adami* was mediated by T cell-dependent cellular immune mechanisms independent of antibody. We now report that acute blood stage infections caused by three additional murine hemoprotozoan parasites, *Plasmodium vinckei petteri*, *Plasmodium chabaudi chabaudi*, and *Babesia microti*, appear to be controlled by similar T cell-dependent mechanisms of immunity. Mice rendered B cell deficient by lifelong treatment with goat anti-mouse immunoglobulin M (IgM) had IgM levels in serum of less than 0.6 μg/ml and contained precipitating amounts of goat anti-mouse IgM. When these B cell-deficient mice were infected with blood stage *P. vinckei petteri*, *P. chabaudi chabaudi*, or *B. microti*, they resolved their infections with kinetics similar to those seen in immunologically intact mice. Infected B cell-deficient mice did not produce antiparasite antibodies. As assayed by immunofluorescence, significant titers of parasite-specific antibody were present only in the sera of infected immunocompetent mice. In addition, only sera from infected immunocompetent mice immunoprecipitated metabolically labeled parasite antigens. In contrast to B cell-deficient mice, athymic nude mice failed to resolve acute *P. vinckei petteri* or *B. microti* infections. These data suggest that antibody-independent, T cell-mediated immune mechanisms play a more significant role in resisting acute blood stage infections caused by hemoprotozoa than was recognized previously.

In 1981, we reported that B cell-deficient mice acutely infected with the murine malarial parasite *Plasmodium chabaudi adami* resolved their infections spontaneously in the absence of chemotherapy (6). In contrast, nude mice infected with the same parasite failed to suppress parasitemia and eventually died with unremitting malaria. This was the first indication that immunity to acute blood stage malaria could be mediated by antibody-independent mechanisms of immunity. Subsequently, we showed that T cells but not B cells transferred immunity to *P. chabaudi adami* (5). In addition, the adoptive transfer of parasite-specific interleukin-2-expanded T cell lines (3) and, more recently, antigen-specific T cell clones (2) protected nude mice against lethal *P. chabaudi adami* malaria. In view of current vaccine research aimed at inducing protective antibodies, we questioned how commonplace the phenomenon of antibody-independent, T cell-mediated immunity against acute blood stage malaria is. Accordingly, we have investigated the ability of B cell-deficient mice to survive acute infection with three additional hemoprotozoan parasites: *Plasmodium chabaudi chabaudi*, *Plasmodium vinckei petteri*, and *Babesia microti*. Our data indicate that acute infections with these parasites are resisted by antibody-independent, T cell-mediated immune mechanisms.

MATERIALS AND METHODS

Experimental animals. BALB/c and C57BL/6 mice of both sexes were raised in our animal colony or purchased from Jackson Laboratory (Bar Harbor, Maine). Histocompatible BALB/c *nu/nu* and *nu/+* mice of both sexes were obtained from Walter Frost (Montana State University, Bozeman). All mice were housed and maintained in our Association for the Accreditation of Laboratory Animal Care-accredited animal facility under standard conditions. Mice ranged in age from 7 to 12 weeks when infections were initiated. Age- and sex-matched animals were used in all experiments. Sera from sentinel mice were routinely screened for antibodies to viral pathogens.

Parasites. *P. chabaudi chabaudi*, a clone derived from the CB isolate, and *P. vinckei petteri*, a clone derived from the CR isolate, were kindly provided by David Wallicker (University of Edinburgh, Scotland). *B. microti*, King strain, was obtained through the kindness of Anthony Allison. *B. microti* parasites were cloned as described previously (7), and all parasites were maintained as frozen stabilates in the vapor phase of a liquid-nitrogen freezer or at −70°C. Parasite material was tested for and found to be free of lactate dehydrogenase-elevating agent (15). All experimental infections were initiated with parasitized erythrocytes obtained from donor mice infected with stabilate material. Resulting parasitemias were estimated by enumerating parasitized erythrocytes on Giemsa-stained films of tail blood (7). Percent parasitemia was calculated as the number of parasitized erythrocytes per 100 erythrocytes.

Preparation and administration of goat anti-mouse IgM. Goat serum containing anti-mouse immunoglobulin M (IgM) (anti-μ) was prepared as described previously (6). Mice were rendered B cell deficient with a suppressive regimen of anti-μ. Within 8 h of birth, they were treated with 50 μl of anti-μ intraperitoneally and then injected daily with the same volume of anti-μ for 5 to 7 days. Thereafter, the mice received thrice-weekly injections of 100 μl of anti-μ intraperitoneally.

Assessment of immunological competence. Serum samples from mice treated with anti-μ were analyzed by immunodiffusion, both prior to and after infection, for IgM and circu-

* Corresponding author.
† Present address: Centocor, Inc., Malvern, PA 19355.

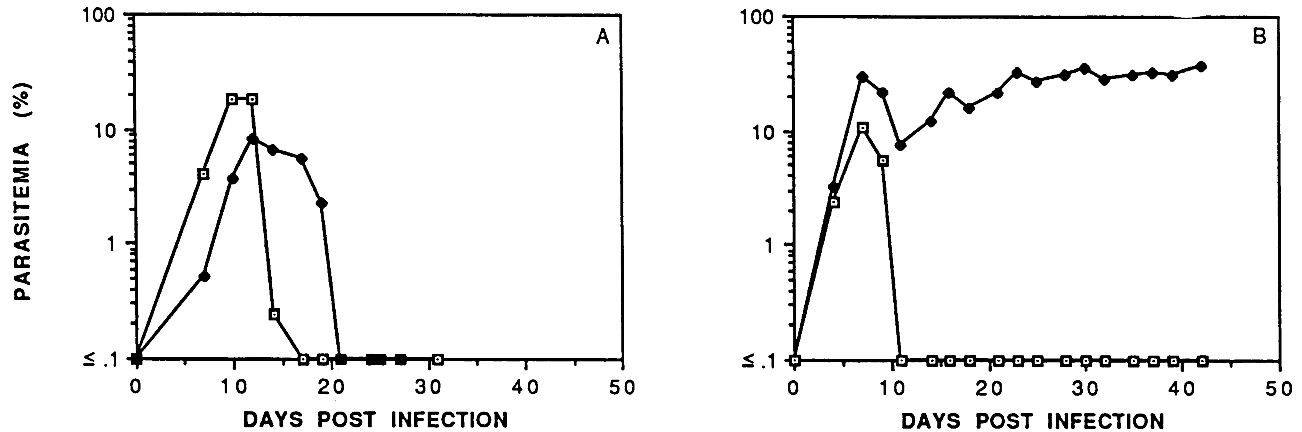

FIG. 1. Kinetics of *P. vinckei petteri* infection in immunodeficient and immunocompetent mice. (A) B cell-deficient (■) or immunologically intact (□) BALB/c mice were infected with 5×10^4 *P. vinckei petteri*-parasitized erythrocytes intravenously. (B) Athymic BALB/c nude mice (■) and immunologically intact *nu*/+ BALB/c mice (□) were infected with 10^5 parasitized erythrocytes intraperitoneally. Three or four animals were used per group.

lating goat anti-μ, as described previously (6). In addition, IgM levels in serum were measured by enzyme-linked immunosorbent assay by using a biotinylated goat anti-mouse IgM and alkaline phosphatase-conjugated avidin system. Test results were compared with a standard curve by using known concentrations of MOPC 104E. Only those animals found to be B cell deficient, as indicated by the presence of circulating goat anti-μ and IgM levels in serum of less than 0.01 mg/ml, were used in our experiments. After infection, parasite-specific antibody was measured by immunofluorescence with fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin (6). To measure anti-parasite IgG (12), sera from infected mice were also used to immunoprecipitate parasite antigens metabolically labeled with [^{35}S]methionine.

RESULTS

Kinetics of parasitemia in immunologically deficient and competent mice. When B cell-deficient mice were infected intravenously with erythrocytes parasitized with *P. vinckei petteri*, *P. chabaudi chabaudi*, or *B. microti*, they developed acute infections which they were able to resolve with parasitemia kinetics similar to those seen in immunologically intact mice of the same strain. In contrast to B cell-deficient mice, athymic nude mice failed to resolve acute infections induced with either *P. vinckei petteri* or *B. microti*.

P. vinckei petteri infections became patent in B cell-deficient and immunocompetent BALB/c mice within 5 days following infection with parasitized erythrocytes, with the exception of one mouse (Fig. 1A). These acute infections were resolved by both immunocompetent and B cell-deficient mice within 14 to 19 days postinfection, respectively. The apparent prolonged duration of infection in B cell-deficient mice is due to the inclusion of data from one mouse which developed a prolonged prepatent period lasting 9 days. In contrast, athymic nude mice developed unrelenting parasitemias and died when infected with *P. vinckei petteri*-parasitized erythrocytes.

Similar findings were observed when immunodeficient BALB/c mice and immunologically intact controls were infected with *B. microti*-parasitized erythrocytes, i.e., B cell-deficient mice resolved their acute infections while nude mice developed unrelenting parasitemias and eventually died (Fig. 2A).

In preliminary experiments, *P. chabaudi chabaudi* was found to be lethal for BALB/c but not C57BL/6 mice.

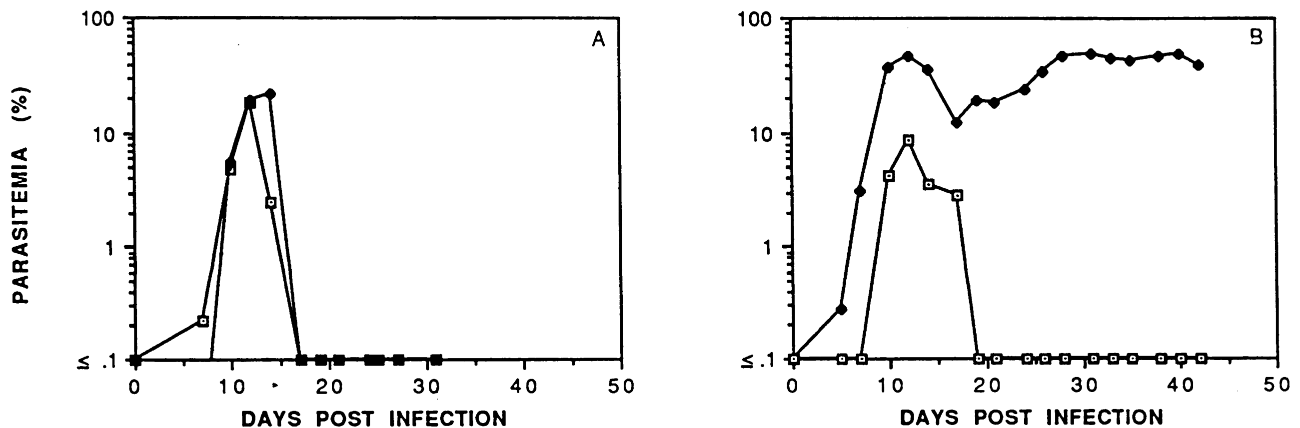

FIG. 2. *B. microti* infection in immunodeficient and immunocompetent mice. (A) Immunocompetent (□) or B cell-deficient (■) BALB/c mice were infected with 5×10^4 *B. microti*-parasitized erythrocytes intravenously. (B) Athymic nude mice (■) and immunocompetent *nu*/+ BALB/c mice (□) were infected with 10^5 parasitized erythrocytes intraperitoneally. Three or four animals were used per group.

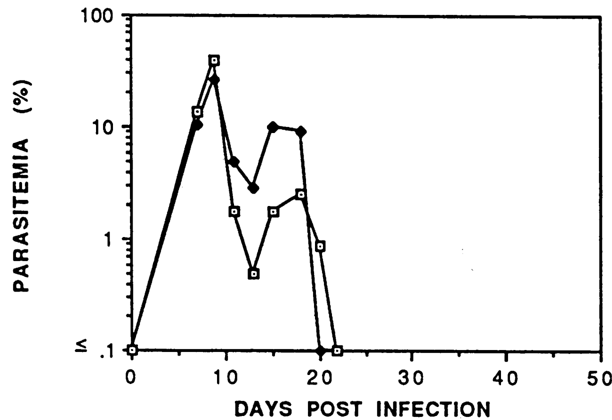

FIG. 3. Infection with *P. chabaudi chabaudi* in B cell-deficient and immunologically intact mice. B cell-deficient C57BL/6 mice (■) or immunologically intact littermates (□) were infected with 5×10^4 *P. chabaudi chabaudi* intravenously. Five mice were used per group.

Subsequently, we prepared B cell-deficient mice of the latter strain as described in Materials and Methods and challenged them at the same time as immunologically intact littermates were challenged with *P. chabaudi chabaudi*-parasitized erythrocytes. Both B cell-deficient and B cell-competent mice resolved their acute infections within 21 days (Fig. 3). Athymic C57BL/6 nude mice were not available for us to study the effects of T cell deficiency on the outcome of infection with *P. chabaudi chabaudi*.

Assessment of immunodeficiency in B cell-deficient mice. Sera from experimental animals collected approximately 35 days postinfection (a time when both B cell-deficient and immunocompetent mice had resolved their acute infections) were tested for IgM and circulating goat anti-mouse IgM. The sera of all B cell-deficient mice contained precipitating amounts of goat anti-μ (data not shown). Immunodiffusion studies using goat anti-mouse IgM failed to detect IgM in the sera of B cell-deficient mice. Levels of IgM in serum were subsequently quantitated by enzyme-linked immunosorbent assay. A 500- to 1,000-fold difference in the levels of IgM in infected immunocompetent control mice versus B cell-deficient mice was observed (Table 1). For all B cell-deficient BALB/c mice infected with *P. vinckei petteri* or *B. microti*, the level of IgM in serum was less than 0.003 mg/ml, whereas infected immunocompetent BALB/c mice had levels greater than 5 mg/ml. While two of eight B cell-deficient C57BL/6 mice had high levels of IgM in serum following infection with *P. chabaudi chabaudi*, the remaining six mice had levels of less than 0.0014 mg/ml, compared with levels greater than 3 mg/ml in immunocompetent C57BL/6 mice. Regardless of the levels of IgM in serum, all B cell-deficient mice resolved acute *P. chabaudi chabaudi* infections with parasitemia kinetics identical to those seen in immunocompetent mice.

When the sera from B cell-deficient and immunocompetent mice which had been infected with hemoprotozoa were assayed for parasite-specific antibody by indirect immunofluorescence, significant titers were observed only in the sera of immunocompetent mice (Table 2). Only one B cell-deficient C57BL/6 mouse infected with *P. chabaudi chabaudi* had a titer of 64. On the other hand, significant titers of parasite-specific antibody ranging from 256 to 4,096 were measured in the sera of immunologically intact mice.

When the sera of mice infected with *P. chabaudi chabaudi* were tested for their abilities to precipitate metabolically labeled parasite antigens, only sera from immunologically intact mice were found to do so (Fig. 4). Similar results were obtained when the sera of mice infected with *B. microti* or *P. vinckei petteri* were reacted with homologous-labeled parasite antigens, i.e., multiple precipitin bands were seen when the sera of immunologically intact mice were used, whereas no bands were detected with the sera of B cell-deficient mice (data not shown).

TABLE 1. IgM levels in sera of mice infected with assorted hemoprotozoa

Infecting parasite (mouse strain)	Concn of IgM[a] in sera of mice (no. of mice)	
	B cell deficient	Immuno-competent
B. microti (BALB/c)	0.0014 ± 0.0005 (4)	5.5 ± 0.7 (5)
P. vinckei petteri (BALB/c)	0.0028 ± 0.0010 (4)	7.0 ± 0.5 (5)
P. chabaudi chabaudi (C57BL/6	0.0132 ± 0.0259 (6) 5.545 ± 5.324 (2)	3.7 ± 1.0 (5)
None (pooled sera from uninfected BALB/c and C57BL/6 mice)		3.4 ± 0.8

[a] The amounts of IgM in sera collected 28 to 30 days postinfection were assessed by enzyme-linked immunosorbent assay. The concentrations are expressed as milligrams per milliliter and were derived from a standard curve of known quantities of MOPC 104E.

TABLE 2. Parasite-specific antibodies in sera of mice infected with assorted hemoprotozoa

Infecting parasite (mouse strain)	Antiparasite antibody titer[a] of sera from mice (no. of mice)	
	B cell deficient	Immuno-competent
B. microti (BALB/c)	<16 (4)	4,096 (5)
P. vinckei petteri (BALB/c)	<16 (4)	1,024 (3) 4,096 (2)
P. chabaudi chabaudi (C57BL/6)	≤16 (7) 64 (1)	256 (1) 1,024 (3) 4,096 (1)
None (pooled sera from uninfected BALB/c and C57BL/6 mice)		<16

[a] Sera from mice were examined 28 to 30 days postinfection for parasite-specific antibodies, as described in Materials and Methods.

DISCUSSION

The data presented in this study show that mice rendered B cell deficient by lifelong treatment with anti-μ antibody were nevertheless able to resolve acute infections caused by three hemoprotozoan parasites: *P. vinckei petteri*, *P. chabaudi chabaudi*, and *B. microti*. These findings extend our previous observation that *P. chabaudi adami* infection in B cell-deficient mice healed spontaneously, despite the fact that such mice did not produce detectable antibodies in response to infection (6). It had been reported earlier that bursectomized chickens resolved acute *Plasmodium lophurae* (11) infection, indicating that immunity to this parasite was in all likelihood resolved by similar resistance mechanisms. Taken together, these data indicate that the resolution of acute infections with plasmodial and babesial species by antibody-independent mechanisms of immunity may be more commonplace than previously realized.

Acute blood stage infections caused by other species of

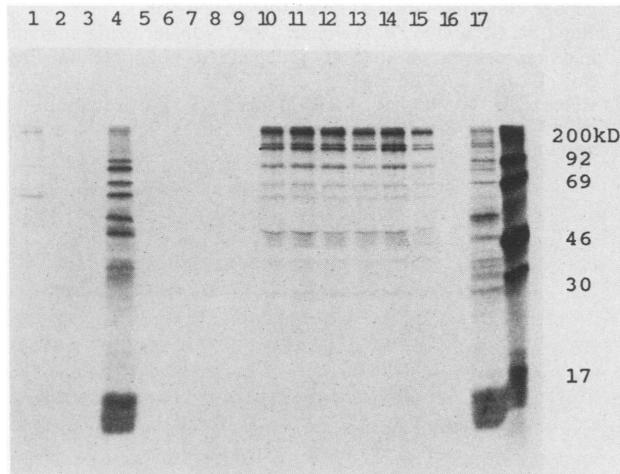

FIG. 4. Antiplasmodial IgG response in B cell-deficient mice and immunologically intact mice. Mice were infected intravenously with 5×10^4 *P. chabaudi chabaudi*-parasitized erythrocytes, and sera from these animals were examined at day 28 postinfection by immunoprecipitation with *P. chabaudi chabaudi* metabolically labeled antigens with [^{35}S]methionine, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lanes 1, 2, and 5 through 9, Sera obtained from B cell-deficient mice; lanes 10 through 15, sera from immunologically intact mice infected with the same parasite; lane 16, normal mouse serum; lanes 4 and 17, radiolabeled parasite antigen alone. Numbers at the right indicate molecular sizes in kilodaltons (kD).

avirulent plasmodia, including *Plasmodium yoelii* (17) and *Plasmodium gallinaceum* (16), terminate fatally in B cell-deficient mice and chickens, respectively. These findings suggest a compartmentalization of parasite species on the basis of the nature of the immune mechanisms predominantly responsible for the resolution of acute infection, i.e., antibody-independent versus antibody-dependent mechanisms. The fact that a monoclonal antibody has been reported to protect mice infected with *P. chabaudi adami* (10) suggests that under certain conditions infection with this parasite can be resolved by antibody-mediated mechanisms as well. Interestingly, the injection of this protective antibody into mice with patent parasitemia did not produce crisis forms, which are routinely observed in B cell-deficient and immunocompetent mice during the resolution of infection (I. A. Clark, personal communication). The significance of this observation is that blood stage parasites may be eliminated by distinct mechanisms of resistance which may be activated by the same or different parasite antigens. It is quite possible that certain of these mechanisms are not activated during the normal course of infection but could be primed through immunization.

The fact that nude mice infected with *P. vinckei petteri* or *B. microti* developed patent parasitemia which they were unable to resolve and eventually died suggests that T cells play an essential role in resisting these parasites. The question that remains to be answered is how T cell-mediated mechanisms effect killing or inhibition of the growth of hemoprotozoa. Release of gamma interferon by antigen-stimulated T cells, which results in the activation of macrophages to produce reactive oxygen species, may play an important role in parasite destruction, as suggested by several authors (1, 13). However, additional mechanisms of resistance may be involved, as indicated by the abilities of polymorphonuclear leukocytes (9) and macrophages (14) from patients with chronic granulomatous disease to kill *Plasmodium falciparum*, as well as our findings that P mice deficient in macrophage function spontaneously resolve infection with *P. chabaudi adami* (4). Preliminary experiments in our laboratory have shown that preparations rich in tumor necrosis factor β are toxic for *P. falciparum* in vitro. It is quite possible that tumor necrosis factor β produced by activated T cells plays an important role in parasite destruction, as originally proposed by the late A. Jayawardena (8). In any event, elucidation of the T cell-mediated immune mechanisms involved in the killing or growth inhibition of malarial parasites could provide new avenues for therapeutic intervention as well as prophylactic immunization.

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