Role of Endogenous Gamma Interferon in Host Response to Infection with Blood-Stage *Plasmodium chabaudi* AS

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The role of gamma interferon (IFN-γ), a pluripotent lymphokine capable of activating macrophages, in acquired immunity to blood-stage malaria was investigated. C57BL/6 mice, which were found to be resistant to intraperitoneal (i.p.) infection with 106 *Plasmodium chabaudi* AS parasitized erythrocytes, were treated with monoclonal anti-IFN-γ antibody (MAb). Two MAb were used: R4-6A2, a rat anti-mouse, neutralizing immunoglobulin G1, which was prepared against natural murine IFN-γ, and DB-1, a murine anti-rat immunoglobulin G1 prepared against recombinant rat IFN-γ, which can neutralize the murine molecule as well as the rat molecule. C57BL/10ScNH mice were injected i.p. with 200 μg of R4-6A2 1 day before infection and every 3 days through day 21. Control mice were treated with normal rat serum. In separate experiments, DB-1 (1.0 mg per week for 4 weeks) was administered i.p. to C57BL/10ScNH mice beginning on the day of infection; control mice were untreated. Control and MAB-treated mice were infected i.p. with 106 *P. chabaudi* AS parasitized erythrocytes, and the course and outcome of infection were determined. Control mice exhibited a course of infection that was characterized by a peak parasitemia between 30 and 40% parasitized erythrocytes and elimination of the parasite by 4 weeks. MAB-treated mice exhibited a significantly greater parasitemia 1 to 2 days before the peak parasitemia as well as a significantly greater peak parasitemia but also completely cleared the infection by 4 weeks. Thus, these results suggest that treatment with anti-IFN-γ MAb impairs but does not completely abrogate host resistance to *P. chabaudi* AS. We also examined the kinetics of IFN-γ production by spleen cells cultured in vitro with malaria antigen or concanavalin A. Spleen cells were recovered from individual C57BL/6 mice at various times after i.p. infection with 106 *P. chabaudi* AS parasitized erythrocytes. The amount of IFN-γ produced was quantitated by enzyme-linked immunosorbent assay. In each case, the peak of IFN-γ production occurred just before the peak parasitemia, followed by a decrease to little or no IFN-γ production through 42 days postinfection. There was thus a parallel between the kinetics of production of IFN-γ in vitro by spleen cells from infected animals and the requirement in vivo for the endogenous molecule just before and at the time of peak parasitemia. In conclusion, these results suggest that IFN-γ-dependent and -independent mechanisms contribute to host resistance to *P. chabaudi* AS.

Evidence from several experimental murine models supports the role of antibody-independent, cell-mediated immune mechanisms in acquired resistance to acute blood-stage malaria. This concept was demonstrated most convincingly by Grun and Weidanz (6), based on their observation of an identical course of acute infection with *Plasmodium chabaudi adami* in normal, intact mice and B cell-deprived mice. In contrast, nude mice were unable to resolve infection with this parasite. The significant role played by T lymphocytes, in particular CD4+ helper-inducer T cells, in the development of antibody-independent immunity to blood-stage malaria has been well documented (9, 34; and J. E. Podoba and M. M. Stevenson, submitted for publication).

It has been hypothesized that the primary role of CD4+ T cells in cell-mediated resistance to microorganisms is the production of gamma interferon (IFN-γ), which is capable of activating macrophages with enhanced microbialic activity (13). Experimental evidence from in vitro and in vivo studies suggests a role for this cytokine in acquired immunity to blood-stage malaria. Ockenhouse and Shear (16) demonstrated that macrophages recovered from normal mice could be activated in vitro to destroy intraerythrocytic *Plasmodium yoelii* by oxygen-dependent mechanisms after incubation in supernatants obtained from antigen-stimulated spleen cells from *P. yoelii*-immune mice. Such supernatants were presumed to contain IFN-γ. A role for IFN-γ was shown by these investigators in further studies demonstrating that the addition of anti-IFN-γ antibody to crude lymphokine supernatants blocked macrophage-mediated destruction of the parasites and that recombinant IFN-γ (rIFN-γ) activated human monocyte-derived macrophages to induce the appearance of crisis forms of *P. falciparum* in cultures of human erythrocytes (17).

More recently, it has been demonstrated that treatment of mice with exogenous IFN-γ has a protective effect during blood-stage malaria. Clark and his colleagues (5) treated mice infected with *P. chabaudi adami* daily for 7 days with rIFN-γ and observed a dose-dependent delay in the onset of parasitemia. A significantly lower peak parasitemia was evident in mice treated daily for 17 days with 50,000 U of rIFN-γ. Shear and her colleagues (21) observed that daily treatment with a similar dose of rIFN-γ resulted in lower parasitemia and increased survival after infection with lethal *P. yoelii* 17x.
The studies described above to define the role of IFN-γ in antimalarial immunity have been performed either in vitro or by using infected animals treated with exogenous IFN-γ. Although the evidence for IFN-γ in immunity to blood-stage malaria derived from such studies is evocative, the in vivo importance of IFN-γ is still unknown. The availability of monoclonal antibodies (MAbs) generated against murine IFN-γ that can neutralize its ability to activate macrophages has proven useful to investigate the role of endogenous IFN-γ in cell-mediated immunity to microbial infections. Treatment of mice with anti-IFN-γ MAb has been found to exacerbate infection with a variety of microorganisms, including *Listeria monocytogenes* (3), *Toxoplasma gondii* (28), and *Leishmania major* (1, 20), *L. donovani* (24), *Rickettsia conorii* (11), and *Chlamydia trachomatis* (36).

In the present investigation, we used two different anti-murine IFN-γ MAbs, R4-6A2 (23) and DB-1 (31), to investigate the role of endogenous IFN-γ in the development of acquired immunity to acute, blood-stage *P. chabaudi* AS. Although in previous studies we could not demonstrate protection against *P. chabaudi* AS after treatment of resistant C57BL/6 or susceptible A/J mice with rIFN-γ (M. Stevenson and E. Ghadirian, unpublished observations), we show here that neutralization of endogenous IFN-γ impairs but does not completely abrogate host resistance to infection with *P. chabaudi* AS. In addition, we examined the kinetics of IFN-γ production by spleen cells in vitro in response to malaria antigen or concanavalin A (ConA) during the course of infection.

**MATERIALS AND METHODS**

**Mice.** C57BL/10scNHzd (C57BL/10ScN) mice 6 to 8 weeks old were purchased from Harlan Sprague Dawley, Inc., Indianapolis, Ind. Mice were aged and sex matched in all experiments. C57BL/6NCrlBR (C57BL/6) mice 6 to 8 weeks old were purchased from Charles River, Inc., St. Constant, Quebec. Female C57BL/6 mice were used in all experiments.

**Parasite.** The AS strain of *P. chabaudi* was obtained from D. Walliker (University of Edinburgh, Edinburgh, Scotland). The parasite preparation was screened for the presence of lactate dehydrogenase virus. Because it was found to be positive, it was freed of the virus by the methods of Parke et al. (18). Samples of parasitized blood were stored in liquid nitrogen. Parasites were kept viable by weekly passage in C57BL/6 mice. After 12 consecutive passages, a fresh inoculum was prepared from the frozen stock. For passage or infection, blood was collected from two C57BL/6 mice by bleeding via the retroorbital plexus and pooled. Total erythrocyte (RBC) counts were determined. The percent parasitemia was determined by counting the percentage of parasitized RBC (PRBC) per 100 RBC on duplicate, Dif-Quik (American Scientific Products, McGaw Park, Ill.)-stained thin blood smears. RBC, diluted in sterile phosphate-buffered saline (PBS), were adjusted to the desired concentration of PRBC and injected intraperitoneally (i.p.) into passage or experimental mice. Infection was initiated with a dose of $10^7$ PRBC for passage mice and $10^8$ PRBC for experimental mice. Intact PRBC collected from mice with >50% PRBC were used as the source of malarial antigen. PRBC were washed and adjusted to the desired concentration for use in spleen cell cultures.

**Course of infection.** To determine the course of infection, blood samples were collected from experimental mice by bleeding via the tail vein at the times indicated. Duplicate thin blood smears were prepared and stained with Dif-Quik. Parasitemias were determined by counting the percentage of infected cells per 100 RBC per slide. The parasitemia was expressed as mean percent PRBC ± standard error of the mean for each group of mice. The outcome of infection was determined by daily observation and, in the case of death, calculation of the mean survival time ± standard error of the mean.

**MAbs.** The rat hybridoma R4-6A2 (a kind gift of F. Nestel, McGill University, Montreal, Quebec), which produces rat anti-murine IFN-γ MAb, was cultured in RPMI 1640 (Flow Laboratories, Inc., Mississauga, Ontario) supplemented with 10% heat-inactivated fetal calf serum (Hyclone Laboratories, Inc., Logan, Utah), 2% HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (Flow), and 0.12% gentamicin. MAb was purified from crude supernatants by ammonium sulfate precipitation and protein A (Pharmacia, Montreal, Quebec) filtration. The immunoglobulin G (IgG) fraction was dialyzed against PBS and concentrated with a Centriprep (Amicon Corp., Danvers, Mass.) ultrafiltration device. The protein concentration was determined by standard absorbance measurements (Biotrack, Inc., Ontario). The concentration of IgG was determined by using a direct enzyme-linked immunosorbent assay (ELISA) with specificity for rat IgG and calculated with a commercially available standard (Becton Dickinson and Co., Canada, Montreal, Quebec). C57BL/10scN mice were infected i.p. with 200 μg of R4-6A2 1 day before infection and every 3 days for 8 weeks. Control mice were treated with normal rat serum containing an equivalent concentration of IgG.

The murine anti-rat IFN-γ MAb, DB-1, was produced and prepared as previously described (1, 31). C57BL/10scN mice were treated i.p. with DB-1 at a dose of 1.0 mg per week for 4 weeks beginning on the day of infection; control mice were untreated. In one experiment, mice were treated with 0.5 mg of DB-1 once per week for 4 weeks.

**L. monocytogenes.** To test the efficacy of the MAb treatment in impairing host resistance, MAb-treated and control mice were tested for their resistance to the bacterium *L. monocytogenes*. *L. monocytogenes* EDG, originally obtained from G. B. Mackaness, Trudeau Institute, Saranac Lake, N.Y., was kept virulent by regular passage through mice. A small portion of the stock culture, stored at −70°C, was thawed. A fresh culture was seeded in Trypticine broth for each experiment. The culture was grown overnight, and the number of organisms in the culture was determined by spectrophotometry with a nephelometric curve. In each experiment, mice were infected intravenously with approximately $2 \times 10^8$ CFU of *L. monocytogenes*. The dose of the infective inoculum was checked retrospectively by plating out appropriate dilutions of the bacteria, diluted in saline, on tryptose agar and counting the number of CFU after 24 h of incubation at 37°C. Bacterial growth in the spleens and livers of control and MAb-treated mice was determined 4 days after *Listeria* infection. The number of viable organisms in the spleens and livers was established by plating 10-fold dilutions of organ homogenates on tryptose agar. The number of *Listeria* CFU per organ was determined after incubation at 37°C for 24 h.

**Spleen cell preparation.** Spleens were aseptically removed and computerized with 10 ml of RPMI 1640 (Flow) supplemented with 5% heat-inactivated fetal calf serum, 2% HEPES buffer, and 0.12% gentamicin. Cell suspensions were centrifuged at 350 × g for 10 min. RBC were lysed with cold NH₄Cl (0.17 M), and the cells were washed two times in fresh medium. Membrane debris was removed by filtering the cell suspen-
sion through sterile gauze. The viability was determined by trypan blue exclusion and was always greater than 90%. Spleen cells were adjusted to a final concentration of 5 x 10⁶ cells per ml in RPMI 1640 supplemented with 5% heat-inactivated fetal calf serum, 2% HEPES buffer, and 0.12% gentamicin. Samples of 3.0 ml in triplicate were incubated for the number of hours indicated in 6-well flat-bottom tissue culture plates (Flow) with ConA (5 μg/ml; Calbiochem, La Jolla, Calif.) or with malarial antigen (10⁶ PRBC/ml). Control cultures were unstimulated. At the indicated times, supernatants were collected and centrifuged at 350 x g for 10 min. The supernatants were stored at -20°C until they were assayed for IFN-γ.

IFN-γ ELISA. A double-sandwich ELISA was performed to quantitate IFN-γ in the spleen cell supernatants. Flat-bottom 96-well microdilution plates (Immulon II; Dynatech Laboratories, Inc., Chantilly, Va.) were coated with 50 μl (3 μg/ml) of DB-1. The plates were incubated for 1 h at 25°C and then washed three times with PBS containing 0.1% Tween 20 (Sigma Chemical Co., St. Louis, Mo.). The wells, filled with a blocking buffer consisting of PBS with 0.1% Tween 20 and 1.0% bovine serum albumin (GIBCO Laboratories, Burlington, Ontario, Canada) that was filtered (0.22-μm pore size) before use, were incubated for 1 h at 25°C and then washed three times with PBS-0.1% Tween 20. Samples were diluted in blocking buffer, and 50 μl was added per well. The plates were incubated for 1 h at 25°C. The wells were washed three times with PBS-0.1% Tween 20, and 50 μl of a 1/500 dilution of rabbit polyclonal IFN-γ in blocking buffer was added to each well. The plates were incubated for 1 h at 25°C. After three washes with PBS-0.1% Tween 20, 50 μl of a 1/500 dilution of horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad) diluted in blocking buffer was added per well. The plates were incubated for 1 h at 25°C and washed three times, and 150 μl of the substrate, 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) (Bio-Rad), was added per well. The plates were incubated for 20 min in the dark at 25°C and then read in an ELISA reader at a wavelength of 405 nm. Units of IFN-γ were calculated from a standard curve ranging from 1 to 50 U of murine rIFN-γ (Genzyme, Boston, Mass.).

Statistical analysis. Differences between control and experimental groups were analyzed by the Student t test. A probability of less than 0.05 was considered significant.

RESULTS
Course of infection with P. chabaudi AS in C57BL/10ScN mice. To determine the role of endogenous IFN-γ in host resistance to P. chabaudi AS, in vivo treatment with two different neutralizing, anti-murine IFN-γ MAb's was used. In this series of experiments, lipopolysaccharide-resistant C57BL/10ScN mice were used to control for the possible contamination of the MAb preparations with lipopolysaccharide, a potent activator of macrophages that appears to play a role in the resolution of a blood-stage infection with P. chabaudi AS (26). We have previously demonstrated that C57BL-derived inbred mouse strains, including C57BL/6, C57BL/10, and B10.A, are resistant to infection with P. chabaudi AS and exhibit a peak parasitemia level of 30 to 40% PRBC and 100% survival (27). To determine whether C57BL/10ScN mice are resistant to infection with this hemoprotezoan parasite, we compared the course and outcome of an i.p. infection with 10⁶ P. chabaudi AS PRBC in these mice with those characteristic of C57BL/6 mice. The course of infection was identical in the two strains of C57BL-derived mice (Fig. 1). In each case, a peak parasitemia of 35 to 40% PRBC occurred on day 10, followed by decreasing parasitemia and elimination of the parasite with complete clearance of the parasite from the peripheral blood by approximately 4 weeks postinfection. In each case, 100% (five of five) of the animals survived.

Course of P. chabaudi AS infection in C57BL/10ScN mice treated with MAbs against IFN-γ. C57BL/10ScN mice were treated with two different neutralizing anti-IFN-γ MAb's. Groups of five mice in three replicate experiments were treated i.p. 1 day before infection and every 3 days through day 21 with 200 μg of R4-6A2, a rat anti-mouse IgG1 prepared against natural, murine IFN-γ, or with normal rat serum containing an equivalent concentration of IgG (22). The course of parasitemia and outcome of infection after i.p. infection with 10⁶ P. chabaudi AS PRBC were determined. The results of a representative experiment are shown in Fig. 2A. Control animals treated with normal rat serum exhibited a course of infection characterized by a peak parasitemia of approximately 40% PRBC followed by decreasing parasitemia and elimination of the parasite by 4 weeks postinfection. In contrast, mice treated with R4-6A2 had a significantly higher parasitemia on day 7 (46.8 ± 2.2% versus 26.2 ± 2.1% PRBC; P < 0.005), 1 day before the peak parasitemia, which in this experiment occurred on day 8. R4-6A2-treated mice also had a significantly higher peak parasitemia (58.2 ± 2.2% versus 44.2 ± 2.8% PRBC; P < 0.005). Similar to control mice, R4-6A2-treated mice exhibited decreasing parasitemia during the elimination phase and, in spite of significantly higher levels of parasitemia in this experiment on day 9 (P < 0.001) and day 17 (P < 0.001), resolved the infection at the same time as control animals. One hundred percent (15 of 15) of the R4-6A2-treated C57BL/10ScN survived the infection.

In two separate experiments, groups of five C57BL/10ScN mice were injected i.p. with 1.0 mg of DB-1, a murine anti-rat IgG1 prepared against recombinant rat IFN-γ which
TABLE 1. Effect of treatment with R4-6A2 or DB-1 on host resistance to *L. monocytogenes*

<table>
<thead>
<tr>
<th>Treatment* (n)</th>
<th>Log$_{10}$ Listeria CFU (mean ± SEM)</th>
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<tbody>
<tr>
<td></td>
<td>Spleen</td>
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<tr>
<td>None (5)</td>
<td>5.14 ± 0.22</td>
</tr>
<tr>
<td>R4-6A2 (6)</td>
<td>7.57 ± 0.42a</td>
</tr>
<tr>
<td>DB-1 (6)</td>
<td>7.41 ± 0.55b</td>
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* C57BL/10ScNHsd mice were either untreated, injected i.p. with 100 µg of R4-6A2 on days −4, −1, 0, and +3, or injected with 1.0 mg of DB-1 on days −4, 0, and +3 of infection. All mice were infected intravenously with 3 × 10⁵ *Listeria* CFU. Mice were killed on day 4 of infection, and the number of bacteria in the spleens and livers was determined as described in Materials and Methods.

**P < 0.05.

**P < 0.001.

A week for 4 weeks, beginning on the day of infection; control mice were untreated. The same protocol of treatment with DB-1 has been found to impair resistance of mice to infection with *L. major* (1). In these studies, a control MAb of the same IgG1 isotype prepared against an unrelated antigen did not alter host resistance to *L. major* (1). The course and outcome of i.p. infection with 10⁶ *P. chabaudi* AS PRBC were determined in treated and control animals.

Similar to mice treated with R4-6A2, DB-1-treated mice had a significantly higher parasitemia on day 8 or 1 day before the peak parasitemia (36.7 ± 1.8% versus 29.8 ± 0.8% PRBC; *P < 0.001) and a significantly higher peak parasitemia on day 9 (47.4 ± 2.0% versus 34.1 ± 0.9% PRBC; *P < 0.001) in comparison to control, untreated animals (Fig. 2B). Mice treated weekly with 1.0 mg of DB-1 were able to control the infection and eliminate the parasite by 4 weeks postinfection; 100% (10 of 10) of the DB-1-treated mice survived. Weekly treatment of C57BL/10Sc mice with 0.5 mg of DB-1 resulted in a course and outcome of *P. chabaudi* AS infection similar to that resulting from treatment with 1.0 mg per week (data not shown).

Treatment with a hamster anti-murine IFN-γ neutralizing MAb has been demonstrated to result in significantly greater bacterial burdens in the organs of mice infected with *L. monocytogenes* (3, 12). To evaluate the efficacy of in vivo treatment with R4-6A2 or DB-1 to neutralize endogenous IFN-γ, we examined the ability of treatment with these MAbs to impart host resistance to *Listeria* infection. On day 4 after intravenous infection with 2 × 10⁵ *Listeria* CFU, C57BL/10ScNH mice treated with either R4-6A2 or DB-1 exhibited significantly greater bacterial burdens in their spleens and livers in comparison with control, untreated mice (Table 1). In addition, R4-6A2 neutralized the ability of both recombinant and natural murine IFN-γ present in the supernatants of mitogen-stimulated spleen cells to activate macrophages in vitro to kill LS178Y tumor target cells (data not shown).

**In vitro production of IFN-γ during the course of *P. chabaudi* AS infection.** As a second experimental approach to defining the role of IFN-γ in the control and elimination of infection with blood-stage *P. chabaudi* AS, we examined the in vitro production of IFN-γ by spleen cells recovered from C57BL/6 mice at various times after i.p. infection with 10⁹ *P. chabaudi* AS PRBC. Before this series of experiments was initiated, we defined the culture conditions optimal for the production of IFN-γ by splenocytes recovered from infected mice. The amount of IFN-γ in the supernatants was quantitated by a double-sandwich ELISA as described above. IFN-γ production in response to ConA or malarial antigen at

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24, 48, 72, or 144 h by spleen cells recovered from normal and *P. chabaudi* AS-infected mice is presented in Fig. 3. Spleen cells recovered from normal animals or from mice infected 5 days previously with 10^6 *P. chabaudi* AS PRBC produced 684 ± 85 and 1,388 ± 95 U of IFN-γ per ml, respectively, in response to ConA by 48 h; in each case, this represented maximal levels. Spleen cells from infected mice produced significant levels of IFN-γ in response to malarial antigen at each time point, whereas spleen cells from normal mice did not respond to antigen by either IFN-γ production or proliferation (data not shown).

Single-cell suspensions of spleen cells recovered from individual C57BL/6 mice at various times after i.p. infection with 10^6 *P. chabaudi* AS PRBC were cultured for 48 h in vitro with malarial antigen, and IFN-γ production was quantitated by ELISA. Production of IFN-γ in response to malarial antigen was maximal on day 5 postinfection, which in this experiment was 2 days before the peak parasitemia occurred (Fig. 4). Significant IFN-γ production was still evident on day 7 postinfection when the peak parasitemia occurred. After this time, production of IFN-γ in vitro in response to malarial antigen declined, and less than 10 U/ml was produced through day 42, when the infection had been resolved. Thus, the kinetics of IFN-γ production in vitro in response to malarial antigen by spleen cells recovered from *P. chabaudi* AS-infected mice paralleled the requirement for endogenous IFN-γ just before and at the time of peak parasitemia in infected animals.

To determine whether aberrations in production of IFN-γ occur during infection, we examined the in vitro production of IFN-γ by spleen cells recovered from infected animals at various times during infection in comparison with spleen cells from normal mice in response to ConA. Spleen cells recovered from mice on day 5 postinfection produced two-fold higher levels of IFN-γ (*P < 0.001*) than did spleen cells from normal mice. The ability of spleen cells from mice infected on day 5 postinfection to produce significant levels of IFN-γ in response to ConA correlated with enhanced IFN-γ production in response to parasite antigen at this time. IFN-γ production decreased on day 7 and, except for day 28 when there was significant production (382 ± 20 U/ml), was significantly depressed through day 42 postinfection (Fig. 5). This observation demonstrates that production of IFN-γ is defective during blood-stage malaria infection in response to a nonspecific mitogen.

**DISCUSSION**

Accumulating evidence from in vitro studies as well as studies in which malaria-infected mice were treated with exogenous rIFN-γ have convincingly but indirectly suggested a role for this lymphokine in acquired immunity to blood-stage malaria. The results of our study, with a more direct approach, demonstrate that IFN-γ is an important host defense mediator in antibody-independent, cell-mediated resistance to blood-stage malaria in vivo. The role of IFN-γ, a pluripotent lymphokine capable of activating macrophages, in acquired immunity leading to control and elimination of the intraerythrocytic parasites was investigated during infection with *P. chabaudi* AS. Acquired immunity to acute infection with *P. chabaudi* occurs by an antibody-independent, cell-mediated mechanism that requires T cells, macrophages, and an intact spleen (6, 9, 26,
Single-cell suspensions of spleen cells, adjusted to 5 x 10^6 cells per ml, were cultured for various times after i.p. infection with 10^6 P. chabaudi AS PRBC. Supernatants were recovered and assayed for IFN-γ by using a double-sandwich ELISA that was specific for IFN-γ. Symbols: (---) and (---) mean and standard error of the mean, respectively, for normal mice (n = 8); (-----) IFN-γ; (-----) percent PRBC.

34; D. Rae, M.S. thesis, McGill University, 1987). Lipopolysaccharide-resistant, C57BL-derived C57BL/10ScN mice, which were found to be resistant to infection with P. chabaudi AS and to exhibit a course of infection characterized by a peak parasitemia between 30 and 40% PRBC and resolution of acute infection by 4 weeks, were treated with anti-murine IFN-γ MAb. We used two different MAbs: R4-6A2, a rat anti-mouse neutralizing IgG1 that was prepared against natural murine IFN-γ, and DB1, a murine anti-rat IgG1 generated against recombinant rat IFN-γ that can neutralize the murine molecule as well as the rat molecule (22, 31). We found that treatment of C57BL/10ScN mice with either MAb impaired but did not completely abrogate host resistance to infection with blood-stage P. chabaudi AS. Anti-IFN-γ MAb-treated animals exhibited a significantly higher parasitemia 1 to 2 days before the peak parasitemia as well as a significantly higher peak parasitemia. Similar to control mice, however, MAb-treated mice resolved the infection by 4 weeks, and 100% of the MAb-treated mice survived. These results suggest that early in infection control of both parasite multiplication and the level of peak parasitemia occur by IFN-γ-dependent mechanisms, whereas later on in infection elimination of blood-stage P. chabaudi AS occurs by an IFN-γ-independent, cell-mediated mechanism.

A role for IFN-γ early in infection was confirmed in experiments designed to quantitate IFN-γ production in vitro by spleen cells recovered from mice at various times after infection. Single-cell suspensions of spleen cells from normal or infected animals were cultured with malaria antigen for 48 h, and IFN-γ was quantitated by an ELISA. In vitro production of IFN-γ in response to malaria antigen was maximal approximately 2 days before the peak of parasitemia and rapidly decreased to <10 U/ml by day 11 postinfection and remained at this level through day 15. The kinetics of in vitro production of IFN-γ in response to malaria antigen by spleen cells from infected mice, thus, corresponds to the in vivo requirement for this cytokine 1 to 2 days before the peak parasitemia. The absence of endogenous IFN-γ leads to significant differences in the percentage of infected erythrocytes in the peripheral blood between control and anti-IFN-γ MAb-treated mice apparent at this time and at the peak of parasitemia.

We also demonstrated defects in production of IFN-γ in vitro by spleen cells from infected mice in comparison with that of normal mice in response to ConA. After a peak in production at day 5, there was little or no IFN-γ produced through 42 days after infection with P. chabaudi AS. Although IFN-γ has been found to be produced by peripheral T cells from patients with P. falciparum in response to parasite antigen, IFN-γ production in response to mitogens or unrelated antigens has not been studied (30).

Results from other laboratories also demonstrate that IFN-γ is produced several days before the peak parasitemia during infection with nonlethal and lethal P. yoelii 17x (21) and with P. chabaudi chabaudi (9, 22). Shear and her colleagues (21) found that in CBA/J mice, which are resistant to nonlethal P. yoelii 17x, the IFN-γ response was biphasic. The early peak was followed by a second peak, which occurred later as the infection was resolved. Susceptible BALB/c mice did not exhibit the second peak. They also found that CBA/J mice had a biphasic IFN-γ response during infection with lethal P. yoelii 17x, whereas BALB/c mice did not produce detectable levels at any time during infection. Other investigators demonstrated that the level of IFN-γ in the serum of P. chabaudi chabaudi-infected mice peaked 1 to 2 days before the peak parasitemia (22). In addition, no significant differences were found in either the level or kinetics of production of IFN-γ in the serum of P. chabaudi chabaudi-resistant BALB/c mice and susceptible DBA/2 mice (9). We are presently examining the kinetics of appearance of IFN-γ in the serum during infection with P. chabaudi AS in resistant C57BL/6 and susceptible A/J mice.

The finding of IFN-γ-dependent and -independent phases of host resistance to P. chabaudi AS is not unique to this microbial agent. Although treatment with anti-IFN-γ MAb impairs host resistance to a variety of microorganisms, such treatment does not result in the complete abrogation of resistance except in one instance. That is, there is death among all T. gondii-infected mice treated with hamster anti-murine IFN-γ MAb (28). Even under conditions of continued administration of MAb, which was the protocol used by us and several other investigators (1, 11, 24), there was survival among the treated animals. This suggests that other immune mechanisms that are IFN-γ independent and that may take longer to develop also contribute to host resistance to infection with P. chabaudi AS as well as with other pathogenic microorganisms.

One possibility for the role played by IFN-γ in acquired immunity to P. chabaudi AS is through its ability to activate macrophages, which are capable of destroying Plasmodium parasites in vitro via oxygen-dependent and -independent mechanisms (16, 17). Another possibility is that IFN-γ...
contributes to the development of acquired immunity through its ability to induce class II antigen expression on macrophages, which is necessary for antigen presentation to CD4+ T cells (33). Our results do not distinguish between these two possibilities, but we are currently investigating this point. Recent studies from our laboratory, however, convincingly demonstrate the requirement for some aspect of macrophage function in resistance to P. chabaudi AS. Resistant C57BL/6 mice depleted of macrophages by treatment on day 6 with macrophage poisons, either silica or liposome-encapsulated dichloromethylene diphosphonate (26, 32; M. M. Stevenson, J. E. Podoba, and N. van Rooijen, unpublished observation), succumb to infection within 14 days with fulminant parasitemia levels. On the other hand, recent studies by Cavacini and her colleagues (4) demonstrate that macrophage-defective P/J mice, which exhibit both defective oxidative activity by their splenic leukocytes and no significant tumor cytototoxicity by their splenic macrophages during P. chabaudi adami infection, can resolve an acute infection with this parasite. This observation suggests that an alternate, yet undefined mechanism can mediate control and elimination of blood-stage malaria. Our recent finding that C57BL/6 mice depleted of CD8+ T cells cannot eliminate P. chabaudi AS infection as efficiently as intact animals suggests that the cytotoxic-suppressor subset of T cells may be partially responsible for the alternate mechanism (Podoba and Stevenson, submitted).

It is also evident in other models of infection that IFN-γ-independent pathways result in macrophage activation (7, 8, 14, 15, 19, 29, 35). Among the molecules that can activate macrophages, perhaps the most extensively studied is the monokine tumor necrosis factor alpha (cachectin). Tumor necrosis factor alpha has been found to induce class II antigen expression on macrophages as well as to activate microbicidal macrophages, among its many immunomodulatory activities (2, 10). It is of interest to point out that we observed that treatment with recombinant tumor necrosis factor alpha protects susceptible A/J mice against an otherwise lethal infection with P. chabaudi AS, suggesting a role for this cytokine in resistance to infection (25).

In conclusion, it is becoming obvious that cell-mediated immunity to blood-stage malaria is a complex, multifactorial process to which several identified and unidentified cytokines and effector cells appear to contribute. In the present study, we investigated the role of one cytokine, IFN-γ, in acquired immunity to infection with P. chabaudi AS. Our results suggest that endogenous IFN-γ plays a role in the control of parasite multiplication and peak parasitemia early in infection but does not play a role later on in the elimination of the parasite and resolution of the infection.

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LITERATURE CITED