The Journal of Immunology

Plasticity of Immune Responses Suppressing Parasitemia During Acute *Plasmodium chabaudi* Malaria

William P. Weidanz, Justin R. Kemp, Joan M. Batchelder, Francine K. Cigel, Matyas Sandor and Henri C. van der Heyde

J Immunol 1999; 162:7383-7388; ;
http://www.jimmunol.org/content/162/12/7383

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Plasticity of Immune Responses Suppressing Parasitemia During Acute *Plasmodium chabaudi* Malaria[1]

William P. Weidanz,[2]* Justin R. Kemp,* Joan M. Batchelder,* Francine K. Cigel,* Matyas Sandor,† and Henri C. van der Heyde‡

γδ T cells have a crucial role in cell-mediated immunity (CMI) against *P. chabaudi* malaria, but δ-chain knockout (KO) ($\delta^{o/o}$) mice and mice depleted of γδ T cells with mAb cure this infection. To address the question of why mice deficient in γδ T cells resolve *P. chabaudi* infections, we immunized $\delta^{o/o}$ mice by infection with viable blood-stage parasites. Sera from infection-immunized mice were tested for their ability to protect $J_H^{o/o}$, $\delta^{o/o}$ double KO mice passively against *P. chabaudi* challenge infection. The onset of parasitemia was significantly delayed in mice receiving immune sera, compared with saline or uninfected serum controls. Immune sera were then fractionated into Ig-rich and Ig-depleted fractions by HPLC on a protein G column. Double KO mice were passively immunized with either fraction and challenged with *P. chabaudi*. The onset of parasitemia was significantly delayed in recipients of the Ig-rich fraction compared with recipients of the Ig-poor fraction of immune sera. We conclude that $\delta^{o/o}$ mice, which are unable to activate CMI against the parasite, suppress *P. chabaudi* infection by a redundant Ab-mediated process. ***The Journal of Immunology,*** **1999, 162: 7383–7388.**

Increased numbers of γδ T cells are found in the blood and spleens of human subjects and experimental animals with acute malaria (reviewed in Ref. 1). After cure, these cell counts remain elevated for prolonged periods of time. In human malaria, the expansion of the γδ T cell subset is polyclonal, involving the $V\gamma9^+$, $V\delta2^+$, and $V\delta1^+$ subsets (2, 3). Recent findings indicate that subjects living in areas of endemic malaria transmission either lack γδ T cells or have subnormal numbers of γδ T cells in their peripheral blood (4, 5). Whether parasitemic or not, these individuals, who are infected repeatedly or continuously with malarial parasites, may have down-regulated their γδ T cell response after developing more efficient mechanisms of immunity to control the low-grade parasitemia of chronic malaria.

Human γδ T cells proliferate in response to falciparum Ags in vitro (reviewed in Ref. 6). Their response is dependent upon $CD4^+$ T cells that supply help through the production of cytokines; the $CD4^+$ T cell requirement is replaced by cytokines that stimulate through components of the IL-2R (7). Similarly, the expansion of the splenic γδ T cell subset during murine malaria induced with *Plasmodium chabaudi* is also dependent upon $CD4^+$ T cells; treatment with anti-CD4 mAb prevents the expansion of the γδ T cell subset in infected mice (8). Human γδ T cells appear to recognize malarial Ags complexed to MHC class I, but not to MHC class II, molecules (9). In contrast, murine γδ T cells recognize malarial Ags independent of MHC class I molecules (10). Although the nature of the γδ T cell-stimulating Ags remains uncertain, human γδ T cells having the Vγ9, Vδ2 phenotype respond to nonpeptide pyrophosphate Ags similar to those extracted from mycobacterial species (11). When activated by malarial Ags, γδ T cells produce an array of cytokines, including IFN-γ and TNF-α, and less frequently, IL-4 (12). Thus, it has been suggested that these cells may function to activate other cells of both the innate and adaptive immune systems or function as a "first line of defense" (13).

Accumulating evidence suggests that γδ T cells function in protective immunity against malaria and are responsible for certain of the pathological changes associated with this disease (14–16). In addition to the characteristics described above, we have reported that cloned human γδ T cells are cytotoxic for blood-stage *P. falciparum* parasites (17). Moreover, we have observed that murine γδ T cells are a crucial component of cell-mediated immunity (CMI)[3] against *P. chabaudi* malaria (18); mAb depletion of γδ T cells from $J_H^{o/o}$ mice prevents the suppression of acute malaria that normally occurs in $J_H^{o/o}$ mice. In contrast, when $\delta^{o/o}$ mice deficient in γδ T cells, but otherwise intact, were infected with *P. chabaudi*, the course of infection was slightly prolonged and the recrudescent parasitemia was higher when compared with control $\delta^{+/+}$ mice (19). These authors suggest "... that γδ T cells can contribute, albeit in a minor way, to the clearance of the acute stage parasitemia of *P. chabaudi*." Preliminary studies in our laboratory confirmed this observation, i.e., *P. chabaudi* infections in $\delta^{o/o}$ mice were resolved in nearly the same time frame as control mice. Accordingly, the current study was undertaken to determine the mechanism by which acute *P. chabaudi* malaria is suppressed in mice deficient in γδ T cells. The results of passive immunization experiments with sera obtained from infection-immunized $\delta^{o/o}$ mice indicate that these mice suppress *P. chabaudi* malaria by mechanisms of Ab-mediated immunity (AMI). Moreover, they suggest a plasticity of immune responses that the host may activate to resolve infections caused by a single species of malarial parasite. Thus, in addition to suppressing the parasitemia of acute *P.*

Departments of *Medical Microbiology and Immunology and †Pathology, University of Wisconsin Medical School, Madison, WI 53706; and ‡Department of Microbiology and Immunology, Louisiana State University Medical Center, Shreveport, LA 71103

Received for publication November 2, 1998. Accepted for publication March 31, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work is supported by National Institutes of Health Grants AI12710 (to W.P.W.) and AI40667 (to H.C.H.)

[2] Address correspondence and reprint requests to Dr. William P. Weidanz, Department of Medical Microbiology and Immunology, 1300 University Avenue, Madison, WI 53706. E-mail address: wweidanz@facstaff.wisc.edu

[3] Abbreviations used in this paper: CMI, cell-mediated immunity; AMI, Ab-mediated immunity; KO, knockout; $J_H^{o/o}$, JHD (B cell-deficient mice); $\delta^{o/o}$, TCR δ-chain KO mice.

0022-1767/99/$02.00

chabaudi malaria by CMI, mice can suppress acute *P. chabaudi* infection by AMI in approximately the same time frame.

Materials and Methods

Mice

Female C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and used at 6–10 wks of age. $\delta^{o/o}$ and $J_H^{o/o}$ mice homozygous for targeted deletions of TCRδ genes (20) and J_H Ig genes (21), originally purchased from The Jackson Laboratory or kindly provided by Dr. D. Huszar (GenPharm International, Mountain View, CA), respectively, were maintained and bred at the University of Wisconsin Animal Care Unit (Madison, WI). Double knockout (KO) mice lacking both J_H and δ-chain genes were produced by crossing single KO mice to produce F_1 progeny heterozygous for both genes and then mating these back to the $J_H^{o/o}$ parent. KO mice homozygous for the mutated J_H gene but heterozygous for the δ-chain gene were then crossed to produce double KO ($J_H^{o/o}$, $\delta^{o/o}$) mice lacking B cells and $\gamma\delta$ T cells. Mice homozygous for mutated J_H genes and lacking serum Igs were identified by gel diffusion analysis. Mice homozygous or heterozygous for mutated δ-chain genes were identified by standard PCR-based analysis. Briefly, The Wizard Genomic DNA purification system (Promega, Fitchburg, WI) was used to extract DNA from ~100 μl of heparinized blood. Subsequently, 10 μl of the DNA sample was amplified in a 35-cycle PCR reaction with the GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT) and the following cycling conditions: 50 s at 94°C, 50 s at 60°C, and 1 min at 72°C. The primers used were as follows: CTATCTGCCCATTGATGAGA (D/Neo), CCATTTGTCACGTCCTG CACG (pgk $^-$286), CAAATGTTGCTTGTCTGGTG (TCR CD1), and GTCAGTCGAGTGCACAGTTT (TCR CD2). Amplicons were analyzed on a 1.8% ethidium bromide-stained agarose gel. Heterozygote mice yielded a 280-bp band (KO primers, D/Neo and pgk $^-$286) and a 200-bp band (wild-type primers, TCR CD1 and TCR CD2). Mice homozygous for the δ-chain deficiency produced only the 280-bp KO band. Age- and sex-matched mice of both sexes were used between 8 and 12 wk of age.

Parasites and infection of mice

P. chabaudi adami 556KA, hereafter referred to as *P. chabaudi*, was maintained as frozen stabilate material and used as described previously (22). Briefly, malarial infections were initiated in mice that were not treated with Abs or sera by the i.p. injection of 1×10^6 parasitized erythrocytes obtained from a donor mouse. Mice treated i.p. with Abs or sera were injected i.v. with 1×10^5 parasitized erythrocytes. Comparison of parasitemia curves (23) suggests a similarity in the course of infection initiated by the injections of 10^5 parasites given by the i.v. route vs 10^6 parasites injected i.p. We have found that the injection of both parasites and serum i.p. can kill the parasites, thereby preventing infection. Therefore, we routinely use 10^5 parasites i.v. to give a course of infection comparable to 10^6 parasites i.p.

Parasitemia was assessed by enumerating 200-1000 erythrocytes in Giemsa-stained films of tail blood. ANOVA was performed with the Minitab (State College, PA) program and with SAS (Cary, NC) statistical software. With the exception of the passive immunization experiment with fractionated immune serum, all experiments were replicated at least once and gave essentially identical results. Because of the similarity of the data from passive immunization with immune sera presented in Fig. 4 and the results of the passive immunization experiment with fractionated immune sera (Fig. 5), we did not believe it necessary to repeat the experiment with fractionated immune sera.

Ab depletion

HPLC-purified anti-TCR$\gamma\delta$ (GL3) was kindly provided by Dr. C. Czuprynski (University of Wisconsin, Madison, WI). Purified hamster IgG was obtained commercially (Accurate Chemicals, Westbury, NY). Anti-TCR$\gamma\delta$ mAb and hamster Ig were injected (i.p.) into mice (four mice per treatment group) with 0.5 mg/mouse on days -1, 0, and 1. Mice were infected with 1×10^5 *P. chabaudi*-parasitized erythrocytes i.v. on day 0, and parasitemia was estimated subsequently, as above.

Immune sera

Immune sera were obtained from $\delta^{o/o}$ mice following the suppression of parasitemia in mice inoculated i.p. 4 wk previously with 1×10^6 *P. chabaudi*-parasitized erythrocytes. Infection-immunized and uninfected control $\delta^{o/o}$ mice were bled by cardiac puncture under metofane anesthesia; the sera were pooled as immune or control sera, respectively, and stored at -80°C. *P. chabaudi* immune sera were fractionated into Ig-rich and Ig-poor components as follows: immune sera were diluted 1:5 in running buffer (25 mM Tris, 0.1 M NaCl (pH 8)) and subjected to HPLC on a Poros protein G column (Perceptive Biosystems, Framingham, MA). The Ig-poor pass-through eluate was collected and pooled. Bound Ig was subsequently eluted stepwise with 0.3 M $MgCl_2$ in 3% acetic acid. The pH was immediately adjusted to ~7.2, and the Ig-rich fractions were pooled. Both fractions were dialyzed against PBS (pH 7.2) and concentrated to the original serum volume by means of ultrafiltration through an ULTRAFREE-20 filter (Millipore, Bedford, MA) having a 10-kDa cutoff. Before injection into mice, the fractions were sterilized by passage through a 0.22-μM filter (Costar, Cambridge, MA).

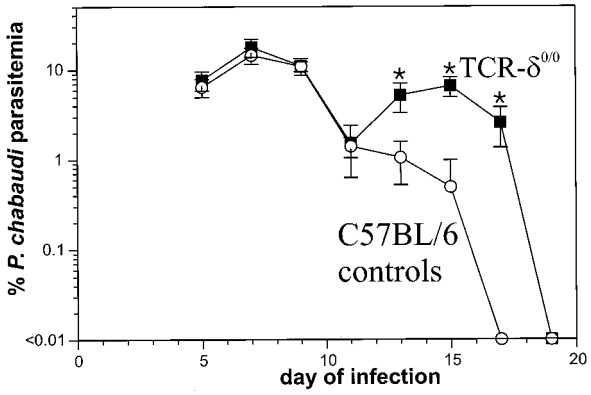

FIGURE 1. Time course of parasitemia during *P. chabaudi* infection in $\delta^{o/o}$ and C57BL/6 mice. The parasitemia (mean $\pm$ SD) was determined in groups of five $\delta^{o/o}$ and C57BL/6 mice. $*$, Statistical differences ($p < 0.05$) in parasitemia between KO and control groups of mice.

Passive immunization

The immune sera obtained above were injected in a dose of 0.45 ml i.p. on days -1, 0, and $+1$, relative to i.v. inoculation with 1×10^5 *P. chabaudi*-parasitized erythrocytes. Control mice were injected identically with sterile saline or control sera from uninfected $\delta^{o/o}$ mice and challenged with 1×10^5 *P. chabaudi*-parasitized erythrocytes. Passive immunization with fractionated immune sera was conducted in the same manner, except that the recipients were injected with 0.5 ml of Ig-rich or Ig-poor fractions of immune sera.

Flow cytometry

Two-color flow cytometry was performed on single cell suspensions of spleen cells as described previously (24). The biotinylated Abs were anti-CD3-ϵ (Boehringer Mannheim, Indianapolis, IN), anti-TCR-$\alpha\beta$, anti-TCR-$\gamma\delta$, anti-Vγ3, and hamster IgG isotype control (PharMingen, San Diego, CA). The streptavidin-PE was obtained from Southern Biotechnology Associates (Birmingham, AL). FITC-conjugated Abs were: anti-CD3, anti-CD4, anti-CD8 (Boehringer Mannheim), and rat IgG isotype control (PharMingen). Hybridomas producing anti-Vγ1.1 (25), anti-Vγ2 (26), and anti-Vγ5 (27) were kindly provided by Dr. Jeffrey Bluestone (Ben May Institute for Cancer Research, University of Chicago, Chicago, IL). The hybridomas were grown in serum-free medium, and the resulting mAbs were conjugated with FITC using standard procedures. Propidium iodide was added 5 min before data acquisition to allow exclusion of dead cells. Data acquisition and analysis were performed on a FACScan (Becton Dickinson, Mountain View, CA) with the use of Cellquest and Attractors (Becton Dickinson) programs, respectively.

Results

Time course of acute P. chabaudi *malaria in $\delta^{o/o}$ mice*

Previously, we reported that *P. chabaudi* infections are prolonged and display higher parasitemia in C57BL/6 mice depleted of $\gamma\delta$ T cells by treatment with anti-TCR$\gamma\delta$ mAb in comparison with TCR$\gamma\delta$-intact controls (18). To determine whether the time course of *P. chabaudi* infection is prolonged similarly in gene KO mice lacking $\gamma\delta$ T cells, we infected $\delta^{o/o}$ mice and $\gamma\delta$ T cell-intact C57BL/6 control mice with 1×10^6 parasitized erythrocytes i.p.; the resulting parasitemia was monitored as described above. The results (Fig. 1) reveal that the parasitemia in $\delta^{o/o}$ mice was significantly greater during the latter part of the acute infection and the

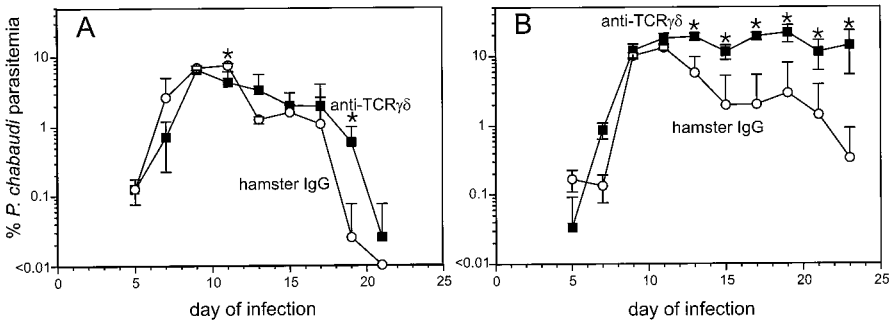

FIGURE 2. Time course of *P. chabaudi* infection in $\delta^{o/o}$ mice treated with anti-TCR$\gamma\delta$ mAb or hamster IgG (*A*) and $J_H^{o/o}$ mice treated with anti-TCR$\gamma\delta$ or hamster IgG (*B*). The parasitemia (mean ± SD) was determined in groups of four (*A*) or three (*B*) mAb or hamster IgG-treated mice on the days shown. *, Statistical differences ($p < 0.05$) in parasitemia between group test and control mice.

course of infection prolonged by several days compared with control mice. However, for the most part, the time course was similar in both groups of mice.

Effects of treating $\delta^{o/o}$ mice with anti-TCR$\gamma\delta$ mAb on the course of P. chabaudi *malaria*

To make a functional check for the depletion of minor subpopulations of cells, due to the possible toxicity of the mAb, we treated $\delta^{o/o}$ and $J_H^{o/o}$ mice with the same regimen of anti-TCR$\gamma\delta$ mAb injected i.p., as described above. Control $\delta^{o/o}$ and $J_H^{o/o}$ mice were injected with hamster Ig. All mice were infected i.v. with 1×10^5 *P. chabaudi*-parasitized erythrocytes. Parasitemia was subsequently monitored as described above. Whereas treatment with the depleting mAb had little, if any, effect on the course of *P. chabaudi* malaria in $\delta^{o/o}$ mice (Fig. 2*A*), it prevented the suppression of parasitemia in $J_H^{o/o}$ mice (Fig. 2*B*), as reported previously (18).

Flow cytometric analysis of spleen cells obtained from P. chabaudi*-infected $\delta^{o/o}$ mice*

To determine whether $\delta^{o/o}$ mice harbored aberrant $CD3^+$ T cells (e.g., bearing TCR$\gamma\beta$ (28)) that would not be depleted by treatment with mAb GL-3, nor detected by flow cytometric analysis of spleen cells stained with mAb GL-3, we analyzed single cell suspensions from spleens of uninfected and *P. chabaudi*-infected $\delta^{o/o}$ and δ-chain-intact control mice. Spleens were harvested from infected mice 3 wks postinoculation i.p. with 1×10^6 *P. chabaudi*, and cells suspensions were stained with mAb specific for Vγ1.1, Vγ2, Vγ3, and Vγ5. Approximately 90% of the $CD3^+$ TCR$\gamma\delta$ splenocytes from infected or uninfected δ-chain-intact mice belonged to the V$\gamma1.1^+$ and V$\gamma2^+$ subsets (data not shown.) None of the cells were stained with anti-Vγ3 or anti-Vγ5. Moreover, Vγ-expressing $CD3^+$ cells were not detected in splenocyte preparations from $\delta^{o/o}$ mice, regardless of their infection status.

The course of P. chabaudi *parasitemia in $J_H^{o/o}$, $\delta^{o/o}$ mice*

We previously reported that acute *P. chabaudi* infections failed to clear in $J_H^{o/o}$ treated with anti-δ-chain mAb (18). To confirm this observation, we produced $J_H^{o/o}$, $\delta^{o/o}$ as described above. $J_H^{o/o}$, $\delta^{o/o}$ and $J_H^{o/o}$, $\delta^{o/+}$ control mice were infected i.p. with 1×10^6 *P. chabaudi*-parasitized erythrocytes. Whereas control mice deficient in B cells suppressed their acute infections as described previously, double KO mice deficient in both B cells and γδ T cells were unable to do so and instead developed progressive infection with relatively high levels of parasitemia (Fig. 3).

Passive immunization against P. chabaudi *infection with immune sera*

To determine whether the sera of infection-immunized mice was capable of protecting $J_H^{o/o}$, $\delta^{o/o}$ mice against challenge infection with 1×10^5 *P. chabaudi*-parasitized erythrocytes injected i.v. on day 0, $J_H^{o/o}$ mice were injected i.p. as described above with 0.45 ml of immune sera obtained from infection-immunized $\delta^{o/o}$ mice on days −1, 0, and +1. Control double KO mice were injected identically with pooled serum from uninfected $\delta^{o/o}$ mice or saline and challenged identically. The results (Fig. 4) indicate that the onset of parasitemia in the mice given immune sera was not detected until the 11th day following the inoculation of parasites. In contrast, all the control mice injected with nonimmune sera were parasitemic by day 5 following the initiation of infection. A comparison of the two groups of mice revealed significant differences in mean parasitemia ($p < 0.05$) on days 11, 13, 15, and 17. One of three mice treated with immune sera did not exhibit parasitemia during the 21-day observation period.

A comparison of the ability of protein G-fractionated immune sera to protect $J_H^{o/o}$, $\delta^{o/o}$ mice against P. chabaudi *challenge*

Having observed that the sera of infection-immunized mice delayed the onset of patent parasitemia in $J_H^{o/o}$, $\delta^{o/o}$ recipients, we fractionated immune sera from $\delta^{o/o}$ mice by affinity chromatography on a protein G column into Ig-rich and Ig-poor fractions. Double KO mice were injected i.p. with 0.5 ml of either fraction on days, −1, 0, and +1, relative to the time of challenge infection, with 1×10^5 *P. chabaudi*-parasitized erythrocytes. The onset of parasitemia in mice injected with Ig-rich fractions was delayed in comparison to control mice receiving Ig-poor fractions (Fig. 5). Whereas parasitemia became patent in one of three test mice on day 9 of infection, parasitemia was patent in the three control mice on day 5 of infection. A comparison of parasitemia in test vs control mice indicated significant ($p < 0.05$) mean differences between groups on days 9 and 11 following the initiation of infection.

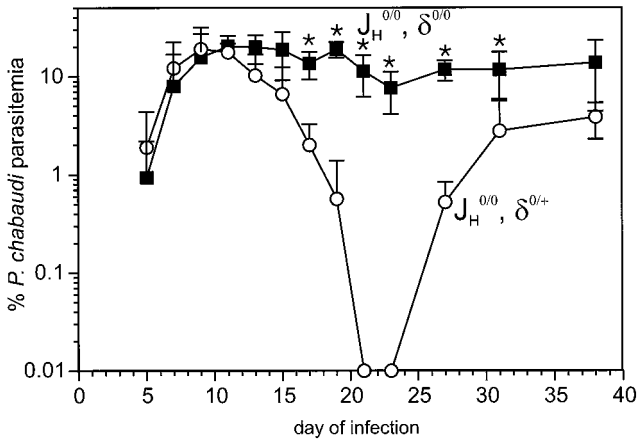

FIGURE 3. Time course of *P. chabaudi* parasitemia (mean ± SD) in double KO ($J_H^{o/o}$, $\delta^{o/o}$) and control ($J_H^{o/o}$, $\delta^{o/+}$) mice (four mice per group) on the days shown. *, Statistical differences ($p < 0.05$) in parasitemia between groups of double KO and control mice.

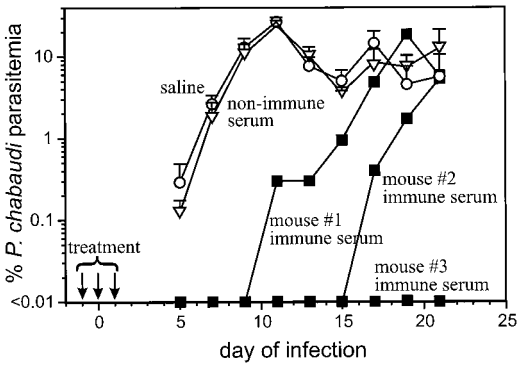

FIGURE 4. Time course of *P. chabaudi* infection in $J_H^{o/o}$, $\delta^{o/o}$ mice passively immunized with serum obtained from infection-immunized $\delta^{o/o}$ mice, serum from noninfected $\delta^{o/o}$ mice, or saline (four mice per group). The results are expressed as the parasitemia for individual mice injected with immune serum and the mean (±SD) for the groups of control mice.

Discussion

The role of γδ T cells in immunity to malaria has been difficult to ascertain. We originally proposed a protective function for these cells based on our observation that the number of γδ T cells was elevated in peripheral blood during acute *P. falciparum* malaria and remained elevated for at least 4 wk during convalescence (29). We also observed that cloned human γδ T cells were cytotoxic for *P. falciparum* in vitro (17). However, different conclusions regarding the significance of γδ T cells were derived from the analysis of experimental malaria in γδ T cell-deficient mice, including mice depleted of γδ T cells with mAb and $\delta^{o/o}$ mice (19, 30). The results of these studies indicate that mice deficient in γδ T cells, but otherwise possessing an intact immune system, display exacerbated levels of parasitemia but suppress acute *P. chabaudi* blood-stage infections in approximately the same time frame as γδ T cell-intact control mice or after a short delay. The results of the present study in which $\delta^{o/o}$ mice were infected with *P. chabaudi* confirm and extend the findings of the above published reports in which $\delta^{o/o}$ mice were infected with the more virulent chabaudi subspecies of *P. chabaudi* (19) or utilized mice depleted of γδ T cells by treatment with mAb (30). Whereas we observed that $V\gamma1.1^+$ and $V\gamma2^+$ subsets comprised ~90% of the splenic γδ T cells in intact mice, we failed (data not shown) to demonstrate the presence of γ-chain-expressing splenocytes in $\delta^{o/o}$ mice. Thus, the ability of $\delta^{o/o}$ mice to resolve *P. chabaudi* malaria could not be attributed to the presence of an aberrant γ-chain-expressing T cell population in these mice.

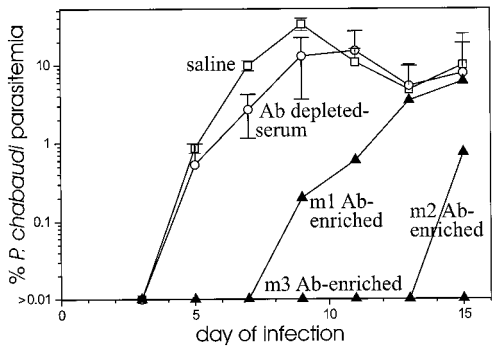

FIGURE 5. Time course of *P. chabaudi* parasitemia in groups of $J_H^{o/o}$, $\delta^{o/o}$ mice passively immunized with fractionated Ab-enriched or Ab-depleted serum or saline (three mice per group). The results are expressed as the parasitemia for individual mice injected with immune serum and the mean (±SD) for the control groups of mice

As reported previously (18), very different results were observed when γδ T cells were depleted from B cell-deficient mice, which were then challenged with *P. chabaudi*. These doubly deficient mice failed to suppress their acute malaria; instead, they developed unremitting parasitemia. Our observations have been confirmed by Seixas and Langhorne (31), who recently reported that γδ T cells contribute to the control of chronic *P. chabaudi chabaudi* malaria in B cell-deficient mice lacking γδ T cells. B cell-deficient mice, whether anti-μ-treated or gene KO, suppress acute *P. chabaudi* malaria by CMI (23, 32); the observation that they failed to do so when depleted of γδ T cells suggests that γδ T cells are essential for the expression of CMI against the parasites. An alternate explanation for these results is that the depleting mAb was toxic for or removed an essential cell type in addition to γδ T cells. To test this possibility, both $J_H^{o/o}$ and $\delta^{o/o}$ mice were treated with mAb GL-3, the γδ T cell-depleting Ab, and then challenged with *P. chabaudi*. Whereas the treated $J_H^{o/o}$ mice failed to resolve their acute infections, $\delta^{o/o}$ mice treated with mAb suppressed parasitemia in the same time frame as control $\delta^{o/o}$ mice treated with hamster Ig. These results indicate that the mAb treatment regimen functions solely by depleting γδ T cells from the host. The observation that $J_H^{o/o}$, $\delta^{o/o}$ double KO mice are unable to suppress acute *P. chabaudi* malaria provides additional support that γδ T cells are crucial for CMI against *P. chabaudi*. The question remains whether murine γδ T cells are directly cytotoxic for the parasites or may also function by secreting cytokines, which in turn, activate effector mechanisms. As indicated above, we observed that clonal human γδ T cells kill *P. falciparum* in vitro (17). Human γδ T cells have been reported to be the major source of IFN-γ when peripheral blood cells are stimulated in vitro with *falciparum* Ags (12), and TNF-α production appears to be depressed in $\delta^{o/o}$ mice (33). Although $CD4^+$ T cells and macrophages are present in the spleens of mice deficient in B cells and γδ T cells (data not shown), they fail to constitute a major parasite-killing system on their own.

Previously, we reported (23, 34) that B cell-deficient mice suppress the acute parasitemia of *P. chabaudi* malaria, but then develop chronic low-grade malaria with parasitemia ≤1%. As shown in Fig. 3, double KO mice do not suppress the parasitemia of acute malaria. Instead, parasitemia reaches a peak and then remains at constant levels in these mice. In contrast, the parasitemia of acute malaria is suppressed in B cell-deficient mice having γδ T cells. The parasitemia then ascends to a level between 1 and 10% with the passage of time during chronic malaria. Similar findings have been reported by Seixas and Langhorne (31). We do not know how parasitemia is stabilized in these mice with chronic malaria. We know that when B cell-deficient mice with chronic malaria are depleted of γδ or $CD4^+$ T cells with mAb, their parasitemia is markedly exacerbated, indicating that both cell types are crucial for CMI (our unpublished data).

Langhorne et al. (19) reported that $\delta^{o/o}$ mice produce Abs in response to *P. chabaudi* infection. Production of both IgG3 and IgG1 isotypes of Ab is greater in $\delta^{o/o}$ mice compared with controls, with IgM and IgG3 Abs being made in approximately equal amounts. The quantities of IgG2a Abs in the sera of $\delta^{o/o}$ mice either equaled or exceeded those found in control mice. In collaboration with Dr. James Burns (Meharry Medical College, Nashville, TN), we assessed the Ab response of $\delta^{o/o}$ mice to *P. chabaudi* infection by Western blot analysis and observed that these mice produce an array of Ab reactivities similar to those seen in C57BL/6 mice infected with *P. chabaudi* (data not shown). The availability of double KO mice with mutated J_H and δ-chain genes

provided a model with which to determine whether sera from infection-immunized $\delta^{o/o}$ mice could passively transfer protection. The results indicate that the onset of patent parasitemia was significantly delayed in the recipient mice. Further, the protective activity of affinity-purified immune sera was associated with the Ig-rich fraction retained on the protein G column vs the Ig-poor pass-through fraction. Together, these findings indicate that $\delta^{o/o}$ mice produce protective Abs when infected with *P. chabaudi*.

Earlier, we had reported that the nonlethal murine malarial parasites could be compartmentalized into two major groups, depending upon the outcome of their infections in B cell-deficient mice (34, 35). Whereas acute infections with *P. chabaudi* and *P. vinckei* resolved in these hosts, acute *P. yoelii* infections failed to do so and eventually terminated in death. T cell-deficient mice were unable to resolve infections caused by those parasites (23). We thus concluded that acute infection(s) caused by *P. chabaudi* and *P. vinckei* are suppressed by CMI, whereas those caused by *P. yoelii* are cured by AMI. *P. chabaudi* produced chronic malaria in B cell-deficient mice; a finding that led us to conclude that the subsequent sterilization of this infection requires B cells and presumably Abs (23, 34, 35). A similar conclusion was recently reached by those who observed that $\mu MT^{o/o}$ mice that lack B cells did not sterilize their *P. chabaudi* infections (36). The present findings may not seem surprising on first sight; they are, however, quite different from those reported previously. In the present study, mice suppressed acute *P. chabaudi* infections by CMI or AMI, depending upon the restrictive immunologic environment. Mice lacking B cells used $\gamma\delta$ T cell-dependent CMI to suppress infection, whereas B cell-sufficient mice lacking $\gamma\delta$ T cells produced Abs and appeared to suppress their *P. chabaudi* infections by means of AMI. Our recent findings (37) with different cytokine KO mice indicate that both CMI and AMI against *P. chabaudi* are dependent on the presence of type 1 but not type 2 cytokines, as proposed previously (38). Although the parasitemia curves in both model infections are too similar to suggest that either CMI or AMI alone suppresses acute *P. chabaudi* malaria in an immunologically intact mouse, it is possible that one response dominates, while the other develops to a protective level. In falciparum malaria, the $\gamma\delta$ T cell response is seen in acutely infected humans (28, 39); individuals, either parasitemic or aparasitemic, living in areas of endemic malaria transmissions have undetectable or low levels of $\gamma\delta$ T cells in their blood (4, 5). Similarly, the expansion of the splenic $\gamma\delta$ T cell subset observed when mice are infected the first time with *P. chabaudi* fails to occur when infection-immunized mice are rechallenged (our unpublished observations). It is thus possible that the $CD4^+$ T cell-dependent $\gamma\delta$ T cell response to acute infection modulates the expansion of the parasite population until a more efficient protective Ab response occurs. What determines which mechanism(s) are activated in the intact host to suppress infection and whether these are activated in some ordered fashion are presently unknown, but the choice might depend on achieving the greatest functional efficiency with the greatest economy of energy. By deliberately removing components of what appears to be a successful immune response, we force the host to select other immune mechanisms capable of suppressing parasitemia. Similar blocks, or the activation of selected immune responses, may occur in nature due to prior or concurrent infection or intoxication by environmental chemicals.

Finally, the implications of other than expected immune mechanisms being activated during the course of infection may confound the interpretation of data and our understanding of immunological events. A mechanism of immunity identified as functional in one model may be replaced by a different mechanism in another. On the other hand, it is possible that the study of such models may reveal previously unrecognized mechanisms of immunity that might be exploited as targets for immunoprophylaxis or immunotherapy.

Acknowledgments

We thank our colleagues Dr. Dean Manning for editorial assistance and Dr. Dennis Heisey for performing statistical analysis.

References

1. van der Heyde, H. C., W. - L. Chang, and W. P. Weidanz. 1997. Specific immunity to malaria and the pathogenesis of disease. In *Host Response to Intracellular Pathogens*. S. Kaufman, ed. R. G. Landes Co., Austin, p. 195.
2. Chang, W. - L., H. C. van der Heyde, D. G. Maki, M. Malkovsky, and W. P. Weidanz. 1992. Subset heterogeneity among $\gamma\delta$ T cells found in peripheral blood during *Plasmodium falciparum* malaria. *Immunol. Lett. 32:273.*
3. Ho, M., P. Tongtawe, J. Kriangkum, T. Wimonwattrawatee, K. Pattanapanyatsat, L. Bryant, J. Shafig, P. Suntharsamai, S. Looareesuwan, H. K. Webster, and J. F. Elliot. 1994. Polyclonal expansion of peripheral $\gamma\delta$ T cells in human *Plasmodium falciparum* malaria. *Infect. Immun. 62:855.*
4. Goodier, M., M. Krause-Jauer, A. Sanni, A. Massougbodjii, B. C. Sadeler, G. H. Mitchell, M. Modell, K. Eichmann, and J. Langhorne. 1993. $\gamma\delta$ T cells in the peripheral blood of individuals from an area of holoendemic *Plasmodium falciparum* transmission. *Trans. R. Soc. Trop. Med. Hyg. 87:692.*
5. Hviid, L., J. A. Kurtzhalg, D. Dodoo, O. Rodrigues, A. Ronn, J. O. Commey, F. K. Nkrumah, and T. G. Theander. 1996. The γ/δ T cell response to *Plasmodium falciparum* in a population in which malaria is endemic. *Infect. Immun. 64:4359.*
6. Troye-Blomberg, M., W. P. Weidanz, and H. C. van der Heyde. The role of T cells in immunity to malaria and the pathogenesis of disease. In *Malaria: Molecular and Clinical Aspects*. M. Walgren and P. Perlmann, eds. Harwood Academic Publishers, Reading.
7. Elloso, M. M., H. C. van der Heyde, A. T. Troutt, D. D. Manning, and W. P. Weidanz. 1996. The human $\gamma\delta$ T cell subset proliferative response to malarial antigen in vivo depends on $CD4^+$ T cells or cytokines that signal through components of the IL-2R. *J. Immunol. 157:2096.*
8. van der Heyde, H. C., D. D. Manning, and W. P. Weidanz. 1993. Role of $CD4^+$ T cells in the expansion of the $CD4^-$, $CD8^-$ $\gamma\delta$ T cell subset in the spleens of mice during blood-stage malaria. *J. Immunol. 157:6311.*
9. Jones, S. M., M. R. Goodier, and J. Langhorne. 1996. The response to *Plasmodium falciparum* is dependent on activated $CD4^+$ T cells and the recognition of MHC class I molecules. *Immunology 89:405.*
10. van der Heyde, H. C., D. D. Manning, D. C. Roopenian, and W. P. Weidanz. 1993. Regulation of blood-stage malarial infections in $CD8^+$ cell-deficient β_2-$m^{o/o}$ mice. *J. Immunol. 151:3187.*
11. Behr, C., R. Poupot, M. A. Peyrat, Y. Poquet, P. Constant, P. Dubois, M. Bonneville, and J. J. Fournie. 1996. *Plasmodium falciparum* stimuli for human $\gamma\delta$ T cells are related to phosphorylated antigens of mycobacteria. *Infect. Immun. 64:2892.*
12. Goodier, M. R., C. Lundquist, M. L. Hammarstrom, M. Troye-Blomberg, and J. Langhorne. 1995. Cytokine profiles for human $V\gamma9^+$ T cells stimulated by *Plasmodium falciparum*. *Parasite Immunol. 17:413.*
13. Janeway, C. A., Jr. 1998. Frontiers of the immune system. *Nature 333:804.*
14. Perera, M. K., R. Carter, R. Goonewardene, and K. N. Mendis. 1994. Transient increase in circulating $\gamma\delta$ T cells during *Plasmodium vivax* paroxysms. *J. Exp. Med. 179:311.*
15. Roussilhon, C., M. Agrapart, I. Gugliemi, A. Bensussan, P. Brasseur, and J. J. Ballet. 1994. Human $TcR\gamma\delta^+$ lymphocyte response on primary exposure to *Plasmodium falciparum*. *Clin. Exp. Immunol. 95:91.*
16. Yañez, D. M., J. M. Batchelder, H. C. van der Heyde, D. D. Manning, and W. P. Weidanz. 1999. $\gamma\delta$ T cells function in the pathogenesis of cerebral malaria in mice infected with *Plasmodium berghei* ANKA. *Infect. Immun. 67:446.*
17. Elloso, M. M., H. C. van der Heyde, J. A. vande Waa, D. D. Manning, and W. P. Weidanz. 1994. Inhibition of *Plasmodium falciparum* in vitro by human $\gamma\delta$ T cells. *J. Immunol. 153:1187.*
18. van der Heyde, H. C., M. M. Elloso, W. - L. Chang, M. Kaplan, D. D. Manning, and W. P. Weidanz. 1995. $\gamma\delta$ T cell function in cell-mediated immunity to acute blood-stage *Plasmodium chabaudi adami* malaria. *J. Immunol. 154:3985.*
19. Langhorne, J., P. Mombaerts, and S. Tonegawa. 1995. $\alpha\beta$ and $\gamma\delta$ T cells in the immune response to the erythrocytic stages of malaria in mice. *Int. Immunol. 7:1005.*
20. Hohara, S., P. Mombaerts, J. Lafaille, J. Iacomini, A. Nelson, A. R. Clarke, M. - L. Hooper, A. Farr, and S. Tonegawa. 1993. T cell receptor mutant mice: independent generation of $\alpha\beta$ T cells and programmed rearrangement of $\gamma\delta$ TCR genes. *Cell 72:337.*
21. Chen, J., M. Trounstine, F. W. Alt, F. Young, C. Kurahara, J. F. Loring, and D. Huszar. 1993. Immunoglobulin gene rearrangement in B cell deficient mice generated by targeted deletion of the JH locus. *Int. Immunol. 5:647.*
22. Hoffmann, E. J., W. P. Weidanz, and C. A. Long. 1984. Susceptibility of C × B recombinant inbred mice to murine plasmodia. *Infect. Immun. 43:981.*
23. Grun, J. L., and W. P. Weidanz. 1981. Immunity to *Plasmodium chabaudi adami* in the B-cell deficient mouse. *Nature 290:143.*
24. van der Heyde, H. C., M. M. Elloso, D. C. Roopenian, D. D. Manning, and W. P. Weidanz. 1993. Expansion of the $CD4^-$, $CD8^-$, $\gamma\delta$ T cell subset in the spleens of mice during non-lethal blood-stage malaria. *Eur. J. Immunol. 23:1846.*

25. Pereira, P., D. Gerber, S. Y. Huang, and S. Tonegawa. 1995. Ontogenic development and tissue distribution of Vγ-1 expressing γ/δ T lymphocytes in normal mice. *J. Exp. Med. 182:1921.*
26. Dent, A. L., L. A. Matis, F. Hooshmand, S. M. Widacki, J. A. Bluestone, and S. M. Hedrick. 1990. Self-reactive γδ T cells are eliminated in the thymus. *Nature 343:714.*
27. Goodman, T., R. LeCarre, and L. Lefrancois. 1992. A T-cell receptor γδ-specific monoclonal antibody detects a Vγ5 region polymorphism. *Immunogenetics 35: 65.*
28. Stern, M. H., S. Lipkowitz, A. Aurias, C. Griscelli, G. Thomas, and I. R. Kirsch. 1989. Inversion of chromosome 7 in ataxia telangiectasia is generated by a rearrangement between T cell receptor, B and T cell receptor γ genes. *Blood 74: 2076.*
29. Ho, M., H. K. Webster, P. Tongtawe, K. Pattanapanyasat, and W. P. Weidanz. 1990. Increased γδ T cells in acute *Plasmodium falciparum* malaria. *Immunol. Lett. 25:139.*
30. Sayles, P. C., and L. Rakhmilevich. 1996. Exacerbation of *Plasmodium chabaudi* malaria in mice by depletion of TCR $\alpha\beta^+$ T cells, but not TCR $\gamma\delta^+$ T cells. *Immunology 87:29.*
31. Seixas, E. M. G., and J. Langhorne. 1999. γδ T cells contribute to control of chronic parasitemia in *Plasmodium chabaudi* infections in mice. *J. Immunol. 162:2837.*
32. van der Heyde, H. C., D. Huszar, C. Woodhouse, D. D. Manning, and W. P. Weidanz. 1994. The resolution of acute malaria in a definitive model of B cell deficiency, the JHD mouse. *J. Immunol. 152:4557.*
33. Nishimura, H., M. Emoto, K. Hiromatsu, S. Yamamoto, K. Matsuura, H. Gomi, T. Ikeda, S. Hohora, and Y. Yoshikai. 1995. The role of γδ T cells in priming macrophages to produce tumor necrosis factor-α. *Eur. J. Immunol. 25:1465.*
34. Weidanz, W. P., and J. L. Grun. 1981. Antibody-independent mechanisms in the development of acquired immunity to malaria. *Adv. Exp. Med. Biol. 162:409.*
35. Grun, J. L., and W. P. Weidanz. 1983. Antibody-independent immunity to reinfection malaria in B cell deficient mice. *Infect. Immun. 41:1197.*
36. von der Weid, T., N. Honavar, and J. Langhorne. 1996. Gene targeted mice lacking B cells are unable to eliminate a blood stage malaria infection. *J. Immunol. 156:2510.*
37. van der Heyde, H. C., B. Pepper, J. Batchelder, and W. P. Weidanz. 1997. The time course of selected malarial infections in cytokine-deficient mice. *Exp. Parasitol. 85:206.*
38. von der Weid, T., and J. Langhorne. 1993. The roles of cytokines produced in the immune response to the erythrocytic stages of mouse malarias. *Immunobiology 189:397.*
39. Roussilhon, C., M. Agrapart, J. J. Ballet, and A. Bensussan. 1990. T lymphocytes bearing the gamma delta T cell receptor in patients with acute *Plasmodium falciparum* malaria. *J. Infect. Dis. 162:283.*