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# Cellular Mechanisms in the Immune Response to Malaria in *Plasmodium vinckei*-Infected Mice

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**Infection of mice with the malaria parasite *Plasmodium vinckei vinckei* is 100% lethal. However, after two infections followed by drug cure, BALB/c mice develop a solid immunity which is antibody independent but mediated by $CD4^+$ T cells. To elucidate the mechanisms of this immunity, spleen cells from immune mice were challenged in vitro with lysates of *P. vinckei*-infected or uninfected erythrocytes. The parasite antigen induced proliferation of T cells from immune mice but not from nonimmune mice. When gamma interferon production by cells from immune mice was assayed at the single-cell level, 1 to 3 cells per 1,000 cells were found to release this cytokine when exposed to antigen. In contrast, the numbers of interleukin 4 (IL-4)-producing cells from both immune and control mice were ≤4 per $10^6$ cells, regardless of antigen exposure. Investigation in a bioassay showed that *P. vinckei* antigen induced the release of IL-4 from spleen cells of immune mice but not from those of control mice. Nevertheless, that IL-4 is of minor significance in this system is also suggested by the absence of elevation of immunoglobulin E levels in blood samples from these mice, in contrast to what is seen with *P. chabaudi* infection, in which IL-4-producing Th2 cells are of major importance for immunity during later phases of infection. Taken together, the present results indicate that immunity to *P. vinckei* is a Th1 response, with gamma interferon being an important protective factor. Whether or not the Th1 response, through overproduction of tumor necrosis factor alpha, is also responsible for pathology and death in this infection remains to be clarified.**

Malaria continues to be one of the major infectious diseases, with approximately 300 million cases and 2 to 3 million deaths each year. In the life-cycle stages at which the malaria parasite causes disease, the parasites are in asexual erythrocytic forms. Although immunity to these plasmodial stages may be acquired, this is a slow process which requires repeated infections for the immunity to become efficient. Improved knowledge of the factors regulating immunity to the malaria parasite is important for the rational design of malaria vaccines.

Plasmodial species causing malaria in rodents may provide a basis for the understanding of malaria immunity in humans. Several studies have shown that $CD4^+$ T cells, with or without participation of B cells, play a crucial role in the development of immune protection of mice against blood-stage infection (for references and discussion, see reference 46). For the murine malaria parasite *Plasmodium chabaudi chabaudi* AS, available evidence suggests that $CD4^+$ T cells of the Th1 type, producing the marker lymphokines interleukin 2 (IL-2) and gamma interferon (IFN-γ), are of importance during the acute phase of an infection, while control of this parasite during later phases also involves $CD4^+$ T cells of the Th2 type, providing B-cell help by producing IL-4 and other lymphokines. Thus, *P. chabaudi* represents a malaria model in which both cell-mediated immunity and humoral immunity act sequentially to clear an infection (23, 24, 41). However, the relative roles of the different arms of the immune system vary greatly in the different models, dependent on the parasite species, the genetic constitution of the host, and the host's immune status (50, 52).

To further elucidate the cellular mechanisms regulating the immune response to the erythrocytic phase of malaria, we studied a mouse model in which antibodies play a minor role in controlling infection. Thus, we investigated the T-cell-dependent regulation of blood-stage immunity to the lethal parasite *P. vinckei vinckei* in BALB/c mice. Reinfection immunity to *P. vinckei* may be induced in normal as well as B-cell-deficient mice, and as evident from both in vivo depletion and adoptive-transfer experiments, it is mediated by $CD4^+$ T cells but is independent of $CD8^+$ T cells (22). Our results suggest that the cells executing the protective response in these mice are of the Th1 type, while IL-4-producing Th2 cells appear to have no roles or only minor roles in this context.

## MATERIALS AND METHODS

**Mice.** Female BALB/c mice, 6 to 8 weeks old, were obtained from the National Institutes of Health (NIH) breeding facility (Bethesda, Md.), where they were kept under standard conditions (22).

**Parasites and parasite antigen.** The rodent malaria parasites *P. vinckei vinckei* ATCC 30091 (American Type Culture Collection, Rockville, Md.) were maintained in mice by weekly intraperitoneal injections of $10^6$ infected (parasitized) erythrocytes ($E_i$) or stable pathogen-free cryopreservates preserved in liquid nitrogen. *P. chabaudi chabaudi* AS parasites (obtained from D. Walliker, University of Edinburgh, Edinburgh, Scotland) were maintained in 4- to 8-week-old BALB/c mice by serial injections of $10^6$ to $10^7$ $E_i$ per mouse. Soluble plasmodial antigen was prepared by freeze-thawing washed $E_i$ at a concentration of $10^9$ erythrocytes per ml and a parasitemia of ≥90%, with up to six repetitions of the freeze-thaw cycle. *Schistosoma mansoni* (NMRI strain) parasites from the Biomedical Research Institute (Rockville, Md.) were maintained in C57Bl/6 mice, and soluble *Schistosoma* egg antigen was prepared as described previously (3).

**Immunizations.** BALB/c mice were infected with *P. vinckei* by intraperitoneal injection with $10^6$ $E_i$. Following infection, smears of blood samples from the mice were performed until the levels of parasitemia were ~10% (about 7 days), and then the mice were cured by treatment for 3 days with 500 μg of chloroquine-HCl. In most instances, the mice were reinfected after about 2 weeks and were again treated with drugs as described above. Mice immunized by two infections were efficiently protected and did not develop parasitemias when rechallenged for a third time (22, 51).

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Hyperimmune sera against *P. chabaudi* were from BALB/c mice injected three times with parasitized blood (level of parasitemia, 50 to 70%) as described elsewhere (18). Anti-*S. mansoni* sera (generously provided by E. Pearce, NIH) were from female C57Bl/6 mice experimentally infected with cercariae by percutaneous infection via the tail.

**Preparation and fractionation of spleen cells.** Single-cell suspensions were prepared from the spleens of naive or immune mice (22). In some experiments, erythrocytes were removed by osmotic lysis followed by washing and resuspension in complete tissue culture medium. In other experiments, the splenic cells were also depleted of adherent cells and B cells by fractionation over columns of glass beads coated with rabbit anti-mouse immunoglobulin (Ig) (Beckman Instruments Inc., Fullerton, Calif.), according to the manufacturer's instructions. Mononuclear cells prepared in this way will hereafter be called T cells in this work.

**Spleen cell cultures.** Unfractionated or fractionated spleen cells ($2 \times 10^6$ mononuclear cells per ml) were cultured in round-bottomed Falcon tubes in complete tissue culture medium (Iscove's medium with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, pH 7.2, and supplemented with $10^{-5}$ M mercaptoethanol, 2 mM L-glutamine, 100 μl of gentamicin per ml, and 10% fetal bovine serum). In experiments with splenocytes depleted of adherent cells and B cells, 5% irradiated (3,000 R) normal spleen cells were added as filler cells. Cultures with parasite antigen ($10^7$ $E_i$ per ml), control erythrocyte lysates, or mitogen (concanavalin A [ConA], 2 μg/ml) or in medium alone were set up in parallel.

**Proliferation.** After 1 to 6 days of culture, 0.1-ml samples of the cell suspensions were transferred in triplicate to 96-well tissue culture plates and pulsed with 1 μCi of [$^3$H]thymidine per well (6.7 Ci/mmol; ICN, Irvine, Calif.). The cells were harvested 16 h later by means of an automated cell harvester (Skatron, Sterling, Va.), and cellular incorporation of tritium was measured in a liquid scintillation counter (LKB Wallac, Turku, Finland). The results are expressed as the stimulation index, defined as mean counts per minute in test cultures/counts per minute in antigen-free control cultures.

**ELISPOT assay.** For detection of IFN-γ- or IL-4-producing cells, we used the ELISPOT assay (10, 31). After being cultured in tubes with or without antigen for 2 to 5 days, the cells were washed and 0.1-ml aliquots of the cell suspensions ($2 \times 10^5$ mononuclear cells) were transferred to the wells of nitrocellulose-bottomed 96-well Millititer hemagglutination plates (Millipore, Bedford, Mass.) which had been precoated with 10 μg of affinity-purified anti-IFN-γ rat monoclonal antibody R4-6A2 (9) or rat anti-IL-4 monoclonal antibody 11B11 (30) per ml. After 20 h of incubation in a $CO_2$ incubator under tissue culture conditions, the plates were washed and treated sequentially either with rabbit anti-mouse IFN-γ (31) followed by biotinylated goat anti-rabbit IgG (Vector Labs Inc., Burlingame, Calif.) or with a goat antibody specific for mouse IL-4 (kindly provided by R. Asofsky, NIH) followed by biotinylated rabbit anti-goat IgG (Sigma, St. Louis, Mo.). After the addition of streptavidin alkaline phosphatase (Sigma), the spots were developed with freshly prepared enzyme substrate solution (15 mg of 5-bromo-4-chloro-3-indolylphosphate toluidinium salt and 30 mg of *p*-nitroblue tetrazolium chloride, separately dissolved in 1 ml of dimethyl formamide and added to 100 ml of 0.1 M carbonate buffer [pH 9.8]–1 mM $MgCl_2$, filtered to remove particulate material). After a few minutes, the plates were thoroughly rinsed under running tap water. After drying overnight, the spots were counted under low magnification with a Nikon dissection zoom microscope, equipped with a grid in the ocular.

**IL-4 bioassay.** IL-4 activities in supernatants of spleen cell cultures were determined with the IL-4-sensitive cell line CT.4S (19) (generously provided by W. Paul, NIH). Five thousand cells were plated in flat-bottomed 96-well microplates together with 50 μl of supernatant in a total volume of 0.2 ml. After 48 h, proliferation was assayed by measuring [$^3$H]thymidine incorporation and the amount of cytokine in supernatants was quantitated by comparison with the proliferation induced by known amounts of recombinant IL-4 (Genzyme Co., Cambridge, Mass.). The specificity of the assay was ascertained by inhibition in parallel cultures with the anti-IL-4 monoclonal antibody 11B11 (30) or the rat anti-mouse IL-2 monoclonal antibody S4B6 (4).

**Ig enzyme-linked immunosorbent assay (ELISA).** For determinations of total IgE in serum samples from *P. vinckei*-, *P. chabaudi*-, and *S. mansoni*-infected mice, microtiter plates were coated with a monoclonal rat anti-mouse IgE antibody (PharMingen, San Diego, Calif.), and for assays of parasite-specific antibodies (IgG), the plates were coated with lysates of *P. vinckei*- or *P. chabaudi*-infected erythrocytes (50 μl per well). The assay has been described in detail elsewhere (18, 32). In brief, the plates were incubated for 1 h at 37°C with test serum (diluted 1:100). In the IgE assay, a biotinylated rat anti-mouse IgE antibody (Biosource International, Camarillo, Calif.) was added for another hour of incubation at 37°C; this was followed by washing and incubation (1 h, 37°C) with alkaline phosphatase-conjugated streptavidin (Mabtech, Stockholm, Sweden). Finally, substrate was added and the $A_{405}$ was measured. For detection of IgG anti-malarial antibodies, alkaline phosphatase-conjugated rabbit anti-mouse IgG antibody (Sigma) was added to the parasite lysate-coated plates processed as described above.

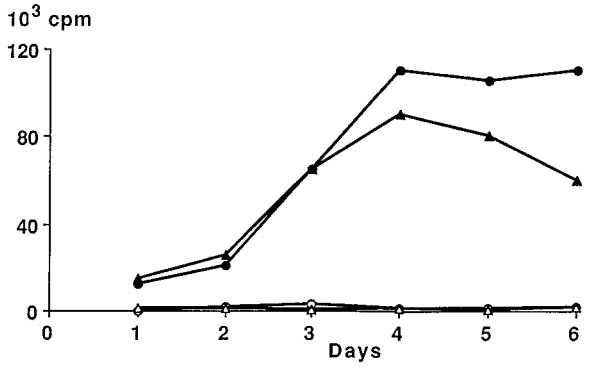


FIG. 1. Spleen cells ($2 \times 10^6$ mononuclear cells per ml) harvested from two *P. vinckei*-immune mice (I-1 and I-2) 3 weeks after a second infection were incubated with lysates of *P. vinckei*-infected (P) or uninfected (C) mouse erythrocytes and pulsed with [$^3$H]thymidine. Solid circles, I-1 with P; solid triangles, I-2 with P; empty circles, I-1 with C; empty triangles, I-2 with C. Abscissa, days of incubation; ordinate, $^3$H incorporation (see text for details; all values are means of triplicate determinations, with the standard deviation being 19% of the mean).

## RESULTS

**Induction of proliferation.** Female BALB/c mice were rendered immune to *P. vinckei vinckei* by two infections followed by drug cure. For mice immunized in this way, parasite challenge results in the formation of crisis forms before immunity reaches its full potential. This occurs within 1 to 2 weeks and remains at an optimal level, with no circulating parasites, for at least 60 days (22). When spleen cells from such immune mice were incubated with lysates of $E_i$, they responded with proliferation, as measured by [$^3$H]thymidine incorporation. As shown in Fig. 1, this response was optimal from 4 to 6 days after the beginning of incubation. Proliferation was parasite specific, since lysates of noninfected erythrocytes had no effect. Table 1 shows the results of three experiments with cells from five immune and three control mice. As the spleen cells used in these experiments were depleted of B cells by passage over anti-Ig columns, the responding cells were considered to be T cells.

**Induction of IFN-γ.** In order to assess the number and function of the malaria antigen-responsive lymphocytes, the

TABLE 1. Induction of DNA synthesis in spleen cells[a]

| Expt no. | Mouse | Stimulation index[b] | | cpm |
|---|---|---|---|---|
| | | P | C | |
| 1 | I-1 | 38.0 | 1.3 | 400 |
| | N-1 | 1.2 | 1.0 | 120 |
| 2 | I-2 | 22.0 | 1.4 | 78 |
| | N-2 | 1.1 | 1.3 | 84 |
| 3 | I-3 | 34.0 | 1.2 | 2,500 |
| | I-4 | 91.0 | 1.4 | 941 |
| | I-5 | 147.0 | 3.0 | 540 |
| | N-3 | 1.5 | <1.0 | 280 |

[a] Spleen cells taken from five immune (I) mice 3 weeks after a second infection and drug cure and spleen cells from three normal (N) mice were treated with *P. vinckei*-infected (P) or normal (C) erythrocyte lysates. The cells were depleted of adherent cells and B cells as described in the text and were pulsed with [$^3$H]thymidine after 4 days in culture. The results are given as stimulation indices. All values are means of triplicate determinations, with a standard deviation being 19% of the mean.

[b] cpm in test samples divided by cpm in untreated control samples.

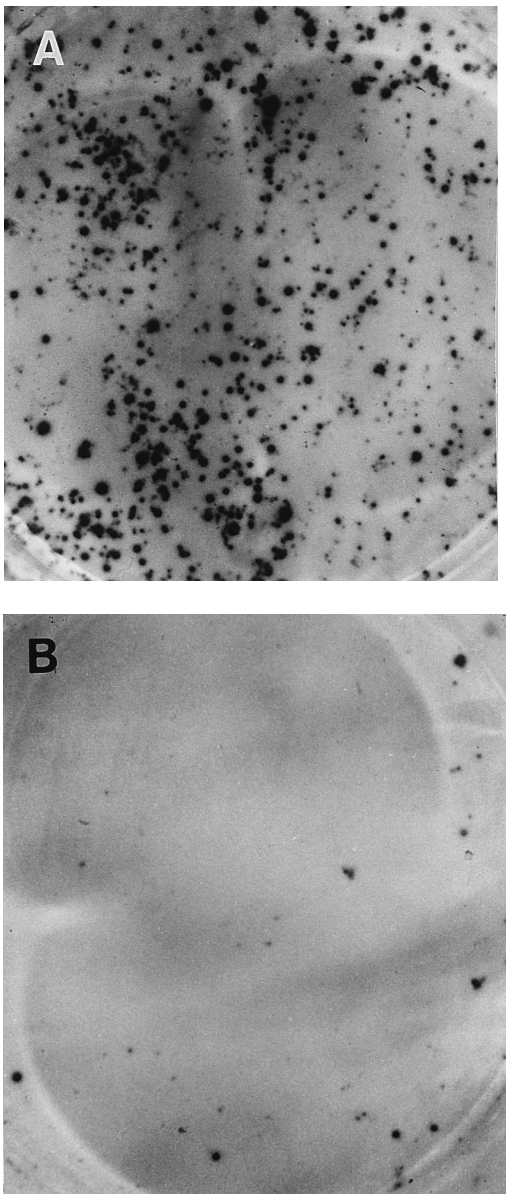


FIG. 2. Spleen cells ($2 \times 10^6$ mononuclear cells per ml) from an immune mouse were incubated with lysates of *P. vinckei*-infected erythrocytes ($10^7$ $E_i$ per ml) (A) or in medium alone (B) in tubes for 2 days, and thereafter they were incubated in anti-IFN-γ-coated wells ($2 \times 10^5$ cells per well) in ELISPOT microplates. For a description of the development of spots and other details, see Materials and Methods.

capacity of the lymphocytes to release cytokines following antigen stimulation was analyzed by the ELISPOT assay. Antigen-induced IFN-γ release by single cells from immune animals could be seen already after 1 day of in vitro incubation. The number of IFN-γ-producing cells reached a maximum after 2 to 4 days of stimulation and declined thereafter. A representative experiment is shown in Fig. 2. Occasionally however, larger numbers of IFN-γ-producing cells were seen in mice (approximately 1 mouse of 20), even without antigen stimulation, and the presence of these cells was obviously unrelated to plasmodial infection.

The results for IFN-γ release by the immune spleen cells from five mice are shown in Table 2. In all cases, the number

TABLE 2. Numbers of antigen-treated spleen cells releasing IFN-γ[a]

| Expt no. | Cell treatment[b] | No. of cells[c]/well | No. of spots[d]/well | No. of spots[d]/$10^3$ cells[c] |
|---|---|---|---|---|
| 1 | U | 200,000 | 118 | 0.6 |
| 2 | U | 200,000 | 100 | 0.5 |
| 3 | $B^-$ | 250,000 | 184 | 0.7 |
| | | 120,000 | 95 | 0.8 |
| 4 | $B^-$ | 200,000 | 480 | 2.4 |
| | | 100,000 | 316 | 3.2 |
| 5 | $B^-$ | 200,000 | 228 | 1.1 |
| | | 100,000 | 184 | 1.8 |

[a] Spleen cells from mice immune to *P. vinckei* after two infections and drug cure were taken ~2 weeks after rechallenge with parasites and incubated with lysates of $E_i$ for 4 days before addition to the ELISPOT plates.
[b] U, unfractionated; $B^-$, T cell enriched by depletion of adherent cells and B cells on anti-Ig columns.
[c] Mononuclear cells.
[d] Spots in ELISPOT assay, representing numbers of IFN-γ-releasing cells (means of duplicate determinations, with a standard deviation being 2.1% of the mean). The number of spots in antigen-free controls was ≤5 per well (see text for details).

of spots per well in antigen-free as well as in normal mouse controls was ≤5. After 4 days of incubation with antigen, approximately 1 to 3 cells per 1,000 T cells (mononuclear cells depleted of adherent cells and B cells) from immune mice were found to release IFN-γ. The pattern of antigen response in vitro shown in Table 2 persists for at least 60 days after infection but probably persists as long as a mouse maintains its immunity (usually up to 6 months).

**Induction of IL-4.** Helper cells of the Th2 type are important for the development of antimalarial immunity in other rodent malaria models (23, 41, 43) as well as in the human *P. falciparum* system (47). Therefore, we also investigated the release of IL-4, a Th2-type cytokine (27), from spleen cells (of *P. vinckei*-immune mice) incubated in vitro with antigen. In the ELISPOT assay, the number of IL-4-producing cells harvested 1 to 3 weeks after parasite challenge varied from 0 to 4 per 1 million T cells. However, this number was independent of antigen exposure in vitro and was the same as that found for control mice. Spleen cells taken from immune mice 2 to 4 months after challenge gave the same results. Fig. 3A shows an example of a result from an ELISPOT experiment in which spleen cells from one immune BALB/c mouse were incubated in tubes for 2 days and thereafter overnight in the ELISPOT microplate. Longer incubations of up to 5 days gave similar results. As a positive control for the IL-4 ELISPOT procedure, we also included spleen cells taken from mice 11 weeks after *S. mansoni* infection. When treated with soluble *S. mansoni* egg antigen, such cells produce significant amounts of IL-4 (17, 31) (Fig. 3B).

In parallel with the ELISPOT, we also used a bioassay (19) to analyze the supernatants of activated spleen cells for the presence of IL-4. Table 3 shows the results of a typical experiment in which either total spleen cells or erythrocyte-depleted spleen cells from four immune mice and one control mouse were incubated with *P. vinckei* antigen or the mitogen ConA (as a positive control) or in medium only. Four months prior to the assay, the mice had been infected twice and drug cured. They were reinfected from 4 to 14 days before the assay or not reinfected at all. As seen from Table 3, the addition of *P. vinckei* antigen to the cells from immune mice induced IL-4 release at a level which was elevated over that seen with the controls. Under such conditions, IL-4 release was not noticeably affected by parasite challenge shortly before the assay.

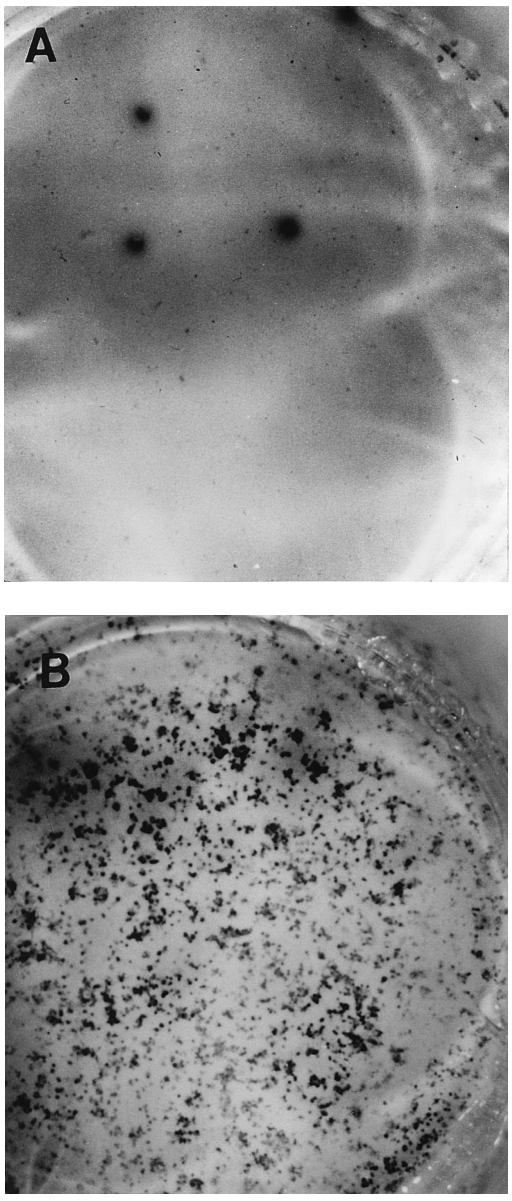


FIG. 3. Spleen cells ($4 \times 10^6$ mononuclear cells per ml) from an immune mouse were incubated with lysates of *P. vinckei*-infected erythrocytes ($10^7$ $E_i$ per ml) for 2 days and thereafter were incubated in anti-IL-4-coated wells in ELISPOT microplates (A). As a positive IL-4 ELISPOT control, panel B shows IL-4 release from spleen cells of *S. mansoni*-infected mice. (Incubation for panel B was performed as in panel A but with *S. mansoni* egg antigen [see Materials and Methods for details]).

Removal of erythrocytes by osmotic lysis did not significantly affect cytokine release. In two additional experiments, immune mice infected and drug cured either 3 weeks or 3 months before the assay gave similar results. When spleen cells from *S. mansoni*-infected mice were stimulated with ConA or antigen, IL-4 release was similarly increased while IFN-γ release was reduced (21). Others have also reported that *S. mansoni* infection in mice is associated with down regulation of IL-2 and IFN-γ secretions after stimulation but is accompanied by a very significant increase in IL-4 secretion (17, 31). As supported by the results of the ELISPOT assay, the IL-4 release seen here probably reflects increased IL-4 production at the single-cell level rather than a recruitment of more IL-4-producing cells as occurs in the *S. mansoni* system. Thus, the comparison of Fig. 3A and B indicates that the amount of IL-4 produced per spleen cell was very significantly greater in *P. vinckei* than that seen in *S. mansoni*. The basis for this difference remains to be elucidated.

IL-4 is an important factor switching Ig isotype production from IgM or IgG to IgE (11). In this context, IgE is found in elevated concentrations in the blood of helminth-infected mice as well as in mice infected with *P. chabaudi* (18). Similar findings have been made for the human *P. falciparum* system (32). In contrast, no IgE level elevation was found in the plasma of BALB/c mice immune to *P. vinckei* after three infections and drug cure (Table 4). In agreement with this, these mice had no IgE anti-plasmodial antibodies (not shown), which is in contrast to results obtained with mice responding to *P. chabaudi* (18). Nevertheless, that *P. vinckei* also induced strong antibody responses is shown by the occurrence of significant amounts of IgG antibodies binding to the lysates of infected erythrocytes (Table 4).

## DISCUSSION

The balance between T-cell subsets producing different lymphokines upon activation is important for the development of immunity to the blood stages of the malaria parasite. In this study, we found that for mice immune to the lethal parasite *P. vinckei vinckei* after two infections followed by drug cure, a majority of the responding splenic lymphocytes produced IFN-γ but not IL-4 when stimulated with parasite antigen in vitro. As we have shown previously by means of both in vivo depletion and adoptive-transfer experiments, immunity to *P. vinckei* is strictly dependent on $CD4^+$ but not $CD8^+$ T cells (22); the results of this study suggest that *P. vinckei* immunity in this model is a Th1-dependent process in which IFN-γ-mediated activation of effector cells probably has a central role (20, 33). Whether other potentially IFN-γ-producing and *P. vinckei*-reactive T cells such as γδ T cells also are involved is presently unknown.

In the murine malaria *P. chabaudi* cell transfer experiments, T-cell subset depletion and studies of malaria-specific cell lines have all shown that both Th1- and Th2-type $CD4^+$ responses are of importance for the development of immunity (26, 44). With *P. yoelii*, for which antibodies are essential for immunity (34, 53), infection of mice with a nonlethal strain induces stronger IFN-γ responses of their splenocytes than that with a closely related lethal variant (39). In agreement with this, *P. yoelii*-resistant CBA/J mice produce more IFN-γ than do susceptible BALB/c mice. Similar results were obtained with lethal or nonlethal strains of *P. chabaudi* (38). IFN-γ protects both mice and monkeys against infection with sporozoites from *P. berghei*, *P. cynomolgi* or *P. vivax* (12, 25), and administration of IFN-γ also prolongs the survival of *P. vinckei*-infected mice (2).

Acute primary infection of resistant C57Bl/6 mice with *P. chabaudi chabaudi* AS results in an early activation of Th1 cells (23, 24, 41). Later when the infection is being finally cleared, this is switched to a Th2 response. On the other hand, infection of susceptible A/J mice with these parasites leads to a severe and lethal course of disease, characterized by a strong Th2 response early in infection (41). The latter results also underline the importance of correct timing of the different phases of response for the final outcome of the infection, in terms of protection or illness. Evidently, the situation in the *P. chabaudi* model is distinct from that described herein for *P. vinckei*, for which there appears to be no switch of the Th1 response to a

TABLE 3. Determination of IL-4 levels in supernatants of spleen cell cultures[a]

| Mouse[b] | IL-4 level (U/ml) in supernatant (mean ± SD)[c] | | | | | |
|---|---|---|---|---|---|---|
| | Total spleen cells | | | Erythrocyte-depleted spleen cells[d] | | |
| | ConA | P | Medium | ConA | P | Medium |
| N | 6.2 ± 0.1 | <1 | <1 | 4.8 ± 0.1 | <1 | <1 |
| I-1 | 20.8 ± 2.3 | 10.8 ± 0.7 | <1 | 14.4 ± 0.7 | 8.0 ± 0.1 | <1 |
| I-2 | 25.0 ± 2.4 | 6.0 ± 0.4 | <1 | 20.8 ± 0.1 | 5.2 ± 0.4 | <1 |
| I-3 | 24.2 ± 0.4 | 8.2 ± 0.1 | <1 | 22.4 ± 1.0 | 9.0 ± 0.3 | <1 |
| I-4 | ND[e] | 15.2 | 1.2 | ND | ND | ND |

[a] Bioassay with the IL-4-dependent cell line CT.4S. Most values given are means ± standard deviations from determinations in duplicate wells.
[b] One normal mouse (N) and four mice immunized (I) by two infections and drug cure 4 months prior to the assay were used for determinations. The latter were reinfected 14 (I-1), 10 (I-2), or 4 (I-3) days before the assay or not at all (I-4).
[c] Mononuclear cells ($4 \times 10^6$/ml) were incubated for 2 days with mitogen (ConA), lysates of $E_i$ (P), or medium only.
[d] Erythrocytes depleted by osmotic lysis.
[e] ND, not done.

Th2 response, even after repeated infections. The number of IL-4-producing splenocytes was always very low and did not differ for immune mice compared with naive animals. This was in contrast to what was seen with the *S. mansoni*-infected mice used as positive controls to prove the validity of the IL-4 ELISPOT assay. Nevertheless, when determined in a bioassay, the level of IL-4 release into culture supernatants from *P. vinckei*-immune splenocytes after activation with antigen was higher than that from naive cells. However, this IL-4 release was not enhanced by reinfection of immune mice and the nature of the cells involved is unknown. It appears likely that they are $CD4^+$ T cells, but the involvement of γδ T cells (48) or nonlymphoid splenocytes (55) is by no means excluded.

Immunity to *P. vinckei* has previously been shown to be antibody independent. Thus, *P. vinckei* infection has the same course in normal mice as it has in mice made functionally B cell deficient by infusion of anti-μ antibodies. *P. vinckei*-immune mice are also protected against reinfection with this parasite regardless of whether they are B cell deficient (16, 22). Moreover, although immune and immunologically intact mice contain parasite-specific antibodies, serum transfer experiments give only very limited protection. However, these results do not imply that antibodies and/or B cells may not also have a role in the clearance of *P. vinckei* infection, as they have roles in other rodent malaria models, such as *P. yoelii* or *P. berghei* (35, 53, 54). Thus, *P. vinckei*-infected mice treated with curative doses of chloroquine in combination with IFN-γ develop immunity characterized by an early induction of IgG2a antibodies and accompanied by a reduction of IgG1 antibodies (13). The transfer of antibody-treated $E_i$ suggests that these humoral immune responses may well contribute to solid anti-*P. vinckei* immunity. The IgG2a/IgG1 antibody ratio seen early in infection also appears to reflect the underlying Th1 predominance, as IFN-γ in mice is known to support IgG2a formation (28). In confirmation of this is the present finding of a lack of IgE level elevation in serum samples from *P. vinckei*-immune mice, a finding different from what we and others have reported recently for *P. chabaudi*-immune mice (18, 49).

Although there can be little doubt that *P. vinckei* immunity represents a cell-mediated type of protection, the underlying Th1 response also appears to be responsible for the pathogenesis of this infection. Thus, IFN-γ (8, 42) as well as certain parasite-derived antigens (1, 36) is known to induce the release of tumor necrosis factor alpha (TNF-α) from macrophages. Although TNF-α probably has a role in protection against the parasite (6, 40), there is now good evidence that an overproduction of TNF-α is intimately involved in illness and the pathology of infection (15). *P. vinckei* does not give rise to cerebral malaria, in which TNF-α is assumed to play a role (14). However, like severe *P. falciparum* infection in humans, *P. vinckei* infection in mice is characterized by hypoglycemia, liver injuries, and anemia, phenomena which are all induced by TNF-α (5, 7). Thus, the factors which would save the mouse by contributing to the acquisition of specific immunity are obviously causes of illness and possibly death when their rates of production go out of control. It may be assumed that in the *P. chabaudi* model an excessive Th1 response is inhibited by the subsequent Th2 response. This would imply that the cytokine balance between, for example, IL-4 and/or IL-10 on the one hand and IL-12 on the other (29, 30, 37, 45), crucial as it is for achieving efficient immune protection, is also critical for preventing pathology. The reason why *P. chabaudi* induces such a balanced response in these mice while *P. vinckei* does not is an important issue which remains to be resolved.

TABLE 4. Total IgE and anti-parasite IgG levels in mouse serum[a]

| Serum[b] | IgE[c] | Anti-parasite IgG[d] |
|---|---|---|
| Normal | 0.017 ± 0.001 | 0.021 ± 0.001 |
| *P. chabaudi* immune | 0.626 ± 0.021 | 0.244 ± 0.004 |
| *P. vinckei* immune | 0.053 ± 0.003 | 0.863 ± 0.021 |
| *S. mansoni* immune | 1.034 ± 0.043 | ND[e] |

[a] Amounts of Ig were determined by measuring the optical densities at 405 nm of the indicated Igs by ELISA (18).
[b] Pooled serum from five normal BALB/c mice or from five mice immune to the parasite indicated.
[c] Optical density corrected for background, 0.011 in IgE-ELISA (18). All sera were diluted 1:1,000.
[d] The optical density in IgG antibody-ELISA against lysates of *P. chabaudi*- and *P. vinckei*-infected erythrocytes (18) was measured. All sera were diluted 1:500.
[e] ND, not done.

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