INFECTION AND IMMUNITY, Dec. 1988, p. 3081–3088
0019-9567/88/123081-08$02.00/0

Vol. 56, No. 12

Roles of CD4- and CD8-Bearing T Lymphocytes in the Immune Response to the Erythrocytic Stages of *Plasmodium chabaudi*

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Received 20 May 1988/Accepted 3 September 1988

The possible role of CD4- and CD8-bearing T lymphocytes in parasite clearance in vivo was investigated, using *Plasmodium chabaudi* in C57BL/6 mice as a model. Monoclonal antibodies specific for the CD4 and CD8 molecules were administered in vivo to deplete selectively the appropriate subset of T cells. The efficacy of depletion was ascertained by flow cytometry and functional studies. These mice were then infected with *P. chabaudi*, and the course of infection was followed. The control groups had maximum parasitemias of approximately 30% 10 days after infection, and the infection was cleared within 27 days. Mice without $CD4^+$ cells had significantly higher parasitemias which they were unable to reduce below 20% for the duration of the experiment. Mice without CD8 cells had slightly higher parasitemias which were cleared after 34 days. Because of the possibility that $CD8^+$ cells alone could not be activated in the absence of growth factors, exogenous interleukin-2 was administered to the mice depleted of CD4 cells. This did not significantly affect parasitemias, and the mice were still unable to clear their infections. The data suggest that $CD4^+$ T cells play a crucial role in the protective immune response to the erythrocytic stages of *P. chabaudi*.

Antibody-mediated effector mechanisms have been considered to be a major component of the protective immune response against the erythrocytic stages of malaria parasites in humans and in experimental models (14, 15). In animals, there are indications that there may be a cellular or antibody-independent effector pathway. Early adoptive transfer experiments in a model of *Plasmodium yoelii* in the mouse and of *P. berghei* in the rat demonstrated that T cells alone could transfer immunity (4, 5, 19, 23). This can be explained by the fact that T helper cells are contained within the transferred population and these cells provide help for the production of antibody; however, partial protection can be achieved by transfer of Lyt-2-bearing T cells (36). In addition, Grun and Weidanz (21) have shown that an acute infection with *P. chabaudi adami* can be cleared in the absence of B cells, suggesting that antibody-independent mechanisms might be involved. Recently, it has been clearly demonstrated, using mice depleted of T cells in vivo and in adoptive transfer experiments, that the protective immune response to the sporozoite stage of *P. berghei* in the mouse in absolutely dependent on the presence of CD8-bearing T cells (38, 41). Moreover, gamma interferon (IFN-γ) appears to be a necessary component of that immune response (17, 38).

Major efforts are now being directed towards delineating the necessary T-cell response to malaria and the relevant antigens which stimulate it. It is therefore important to determine the protective effector mechanisms involved and the subset of T cells which mediates these effects in order to design a vaccine which incorporates epitopes which are appropriately presented to the T cells. In the experiments described here, we have examined the roles of CD4- and CD8-bearing T cells in the development of a protective immune response to a primary infection with the erythrocytic stages of *P. chabaudi chabaudi* in the C57BL/6 mouse. Mice have been depleted in vivo of the two subsets of T cells, using specific monoclonal antibodies (13). The use of these monoclonal antibodies in vivo allows manipulation of the immune response without the concomitant disturbance of the experimental system by whole-body irradiation (13, 31). Further, the T-cell subpopulations can be manipulated at any time during the response, allowing determination of the T-cell role in immunoregulation.

In the experiments described here, the effect of these selective depletions on the ability of mice to control a primary erythrocytic infection of *P. chabaudi* has been studied. The data suggest that the major effector mechanism relies on the presence of CD4-bearing T cells.

MATERIALS AND METHODS

Mice and parasites. C57BL/6 female mice (aged 6 to 8 weeks) obtained from our breeding facility were used throughout this study. *P. chabaudi chabaudi AS* (*P. chabaudi*) was obtained from K. N. Brown, National Institute of Medical Research, London, U.K. For experiments, a sample of frozen stock of the erythrocytic stages of the parasite was injected into mice. Upon development of a patent infection, blood was taken and the experimental mice were injected intraperitoneally with an erythrocyte suspension containing 5×10^3 trophozoite stages of the parasite. The parasitemia was monitored by analysis of Giemsa-stained thin blood films.

Antibodies. Rat monoclonal antibodies specific for CD4 and CD8 determinants (YTS 191.1 and YTS 169.4.2, respectively) were a gift of S. Cobbold and H. Waldmann, Cambridge University, Cambridge, U.K. (13). A control rat monoclonal antibody (BA1.2b) of the same isotype (immunoglobulin G subclass 2b [IgG2b]) specific for the O antigen of lipopolysaccharide was a gift of G. Pluschke, Basel Institute for Immunology, Basel, Switzerland. These antibodies were purified from tissue culture supernatants by affinity chromatography on protein A-Sepharose (Pharmacia, Uppsala, Sweden). The immunoglobulin concentration was determined by spectrophotometric measurement at 280 nm. The antibodies were concentrated by vacuum dialysis and sterilized by filtration. Before use in vivo, their specificity and cytotoxic potential in the presence of complement were ascertained by antibody-mediated complement lysis assays and flow cytometric (FC) analysis.

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Depletion of CD4- and CD8-bearing T lymphocytes in vivo. Groups of experimental mice (10 mice per group) were injected with purified antibodies in phosphate-buffered saline (PBS) intraperitoneally. Groups of mice were injected with 500 μg of purified antibody 5 days before infection with 5×10^3 *P. chabaudi* organisms followed by 250 μg 4 days and 1 day before infection (total of three pretreatments). The efficiency of the depletion at the outset of the experiment was determined in individual mice sacrificed from each group. Once a week following infection, experimental mice were given 250 (for 4 weeks) or 100 (for the remainder of the experiment) μg of antibody intraperitoneally until the termination of the experiment (83 days). The experimental groups were as follows: mice treated with (i) anti-CD4 antibodies, (ii) anti-CD8 antibodies, (iii) both antibodies, (iv) control antibodies, or (v) PBS. From each group of 10 mice, 8 mice were infected with malaria; 2 were retained to determine their viability in the absence of infection. The mice were maintained under sterile conditions for the duration of the experiment. At intervals, individual mice were sacrificed to determine whether the depletion of the relevant subset was maintained. This was determined functionally and by FC (see below).

Experimental groups which were treated with recombinant interleukin-2 (IL-2) in addition to anti-CD4 antibodies were injected intraperitoneally every 48 h following infection for a total of 10 times with 2,500 U of recombinant IL-2 (a gift of Fritz Melchers, Basel Institute for Immunology) in each injection. An extra control group of mice given only IL-2 was included.

FC analyses. FC analyses of lymph node and splenic cells of infected mice from each group were carried out essentially as described previously (28). Briefly, for single-parameter analyses, lymph node or splenic lymphocytes were incubated with fluorescein-labeled anti-Lyt-2, anti-Thy-1.2 (Becton Dickinson GmbH, Heidelberg, Federal Republic of Germany), or fluorescein-labeled goat anti-mouse immunoglobulin (a gift of B. J. Fowlkes, National Institutes of Health, Bethesda, Md.) to detect B lymphocytes. $CD4^+$ cells were detected by using phycoerythrin-labeled anti-L3T4 antibodies (Becton Dickinson). In two-color analyses, when appropriate, biotin-labeled anti-Lyt-2, anti-Thy-1.2, and anti-L3T4 were used together with fluorescein-labeled avidin or phycoerythrin-avidin (Becton Dickinson). To rule out the possibility that cells isolated from mice treated with anti-CD4 or anti-CD8 antibodies were seen as negative on the fluorescence-activated cell sorter (FACS) due to blocking of the CD4 or CD8 molecules by the rat monoclonal antibodies, a fluorescein-labeled mouse anti-rat K reagent (Becton Dickinson) as direct stain was also used. Labeled cells were analyzed on a FACSCAN and with the FACSCAN software programs (Becton Dickinson).

Functional studies. (i) Cytotoxic cells. The presence of alloreactive (anti-$H\text{-}2^d$) and concanavalin A-induced lectin-dependent cytotoxic T-cell precursors was determined by limiting-dilution analyses of splenic or lymph node cells as described previously (27, 29). In the allogeneic mixed-lymphocyte reaction, spleen cells from BALB/c ($H\text{-}2^d$) were used as stimulators. Supernatants from rat spleen cells activated by concanavalin A were used as a source of growth factors. In both assays, ^{51}Cr-labeled P815 mastocytoma cells ($H\text{-}2^d$) were used as target cells in a 4-h ^{51}Cr-release assay. The frequency of responding cells was determined from the zero-order term of the Poisson distribution as described before (39).

(ii) Helper T cells. (a) The presence of T helper cells in splenic lymphocytes was determined by their ability to help B cells in culture make a primary antibody response to sheep erythrocytes (a T-cell-dependent antigen). Unfractionated splenic cells (3×10^5) from all experimental groups of mice were cultured with washed sheep erythrocytes in Iscove medium (GIBCO/BRL, Karlsruhe, Federal Republic of Germany) containing 10% fetal calf serum, 1 mM L-glutamine, 0.5 mM sodium pyruvate, and 5×10^{-5} M β-mercaptoethanol for 5 days in 7% CO_2 as described previously (35). After 5 days, the number of plaque-forming cells making sheep cell-specific antibodies in the cultures was measured by a slide modification of the Jerne plaque assay (1).

(b) The frequency of T helper cells able to respond to malaria parasites was measured as described elsewhere (J. Langhorne, S. Slade, and S. Gillard, manuscript in preparation) and modified from a previously described method (32). To determine the frequency of malaria-specific T helper cells, *P. chabaudi*-immune B cells (as indicator cells for antibody production) were provided in excess in each well such that, if a single precursor T cell able to help is present, then these B cells will proliferate, differentiate into plasma cells, and produce antibody. Thus, a culture well positive for malaria-specific antibody indicates that there was one or more precursors of specific T helper cells present. Graded cell doses (24 replicates of each cell dose) of T cells were cocultured with 20,000 splenic B cells (determined by previous experiments to contain sufficient B cells specific for malaria antigen such that, when appropriately stimulated, antibody production will result) obtained from mice 2 months after a single infection with *P. chabaudi*. B cells and macrophages were purified by removal of T cells by treatment with monoclonal anti-Thy-1.2 and anti-Ly-1 antibodies and complement (monoclonal antibodies J1j [6] and C3PO [30]) and removal of dead cells by centrifugation over Ficoll-Hypaque solutions (Lympholyte, Cedar Lane, Ontario, Canada). After 1 week of culture, the IgM antibody response to *P. chabaudi* was measured in the culture supernatants by an enzyme-linked immunosorbent assay (ELISA), using a solubilized extract of the erythrocytic stages of the parasite as antigen (25).

Malaria-specific serum antibody measurements. Malaria-specific antibodies in the serum of depleted and intact mice were measured by an isotypic-specific ELISA, as described before (26). Alkaline phosphatase-labeled goat anti-mouse immunoglobulin isotype-specific antisera were obtained from Southern Biotechnology, Birmingham, Ala.

Statistics. Significant differences between parasitemias were ascertained by using a Student's *t* test on $\log_{10}$-transformed data. For clarity, the standard errors are not shown in the figures. Significant differences are indicated in the text. The limiting-dilution assays were analyzed by maximum likelihood, as described by Taswell (39).

RESULTS

Mice treated with monoclonal anti-CD4 and -CD8 antibodies in vivo are depleted of the appropriate subset of T cells. Individual mice were sacrificed from each experimental group at the outset of infection (day 0) and 7, 21, 64, and 83 days (termination of experiment) thereafter. Throughout this time period, anti-CD4 or anti-CD8 antibodies were administered weekly by intraperitoneal injection of 100 to 250 μg of purified antibody per mouse as described in Materials and Methods. FC analyses performed on lymph node cells 7 days after infection are shown in Fig. 1. It can clearly be seen that there is substantial depletion of the appropriate subset of T

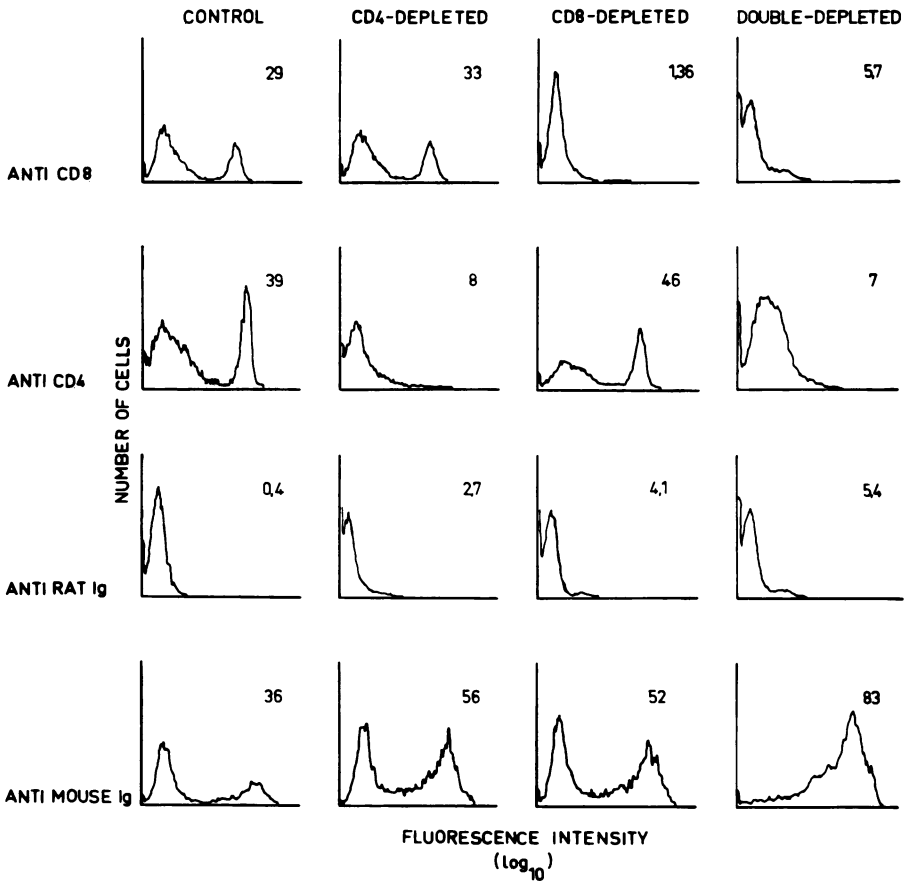

FIG. 1. FC analysis of lymph nodes of mice treated with anti-CD4 antibodies, anti-CD8 antibodies, both antibodies, or PBS taken 7 days after infection with *P. chabaudi*. The four vertical panels represent the different antibody treatments of mice. The four horizontal panels represent fluorescent staining with (top to bottom) anti-CD8, anti-CD4, mouse anti-rat K, and goat anti-mouse immunoglobulin. The numbers on each graph represent the percentage of positive cells determined from comparison with unstained cells or cells stained only with a second-step antibody.

cells. For example, in lymph nodes of mice treated with anti-CD8 antibodies, there are only 1.36% of the cells positive for CD8 (top panel, Fig. 1) compared with 29% in untreated or 33% in CD4-treated mice. The depletions of $CD4^+$ cells resulted in about 5 to 8% of $CD4^+$ cells (second panel, Fig. 1) remaining; this is in agreement with previous investigations (13, 31, 38, 41). However, as described below, these mice are functionally depleted.

The lack of staining with the anti-CD4 or anti-CD8 antibodies (different monoclonal antibodies from those used to deplete the mice) was not due to masking of the molecules by bound antibodies since staining with labeled mouse antibodies specific for rat kappa chains did not reveal more positive cells. In addition, the number of Thy-1-positive cells was also correspondingly reduced and there was no significant appearance of $CD4^-$ $CD8^-$ T cells (data not shown). The amount of depletion of the CD4- or CD8-bearing T cells remained constant in lymph nodes and spleens in these groups for the 83-day duration of the experiment and for 30 days in the case of the double-depleted groups. In those mice depleted of $CD4^+$ cells, there was a clear increase in the proportion and total number of B cells (>50 compared with 35%). Lymph nodes were comparable in size or smaller than those of intact mice, and the mononuclear cell content of the spleen, although increased in size due to hematopoeisis, was never greater than twice that of normal spleens (data not shown).

Functional studies supported the FC analyses and demonstrated clearly that those mice depleted of CD8 T cells were unable to make a cytotoxic response to $H\text{-}2^d$ alloantigens (Fig. 2B) despite the addition of excess help in the form of antigen-presenting cells and IL-2 (frequency, <1 in 6,000; this was the highest concentration of cells added, and there were 2 positive wells out of 24). In addition, the frequency of lectin-dependent cytotoxic precursors was substantially reduced (1 in 28 in controls compared with <1 in 400 in CD8-depleted mice; Fig. 2A). In both cases the response of the CD4-depleted mice was comparable to that of the control groups. The T helper cell response, as determined by a limiting-dilution assay to measure malaria-specific T helper cells, was insignificant in the CD4-depleted mice (frequency is <1 in 60,000), whereas they were unaltered or enhanced in the CD8-depleted mice compared with control mice (frequency is 1 in 9,337 and 1 in 8,046, respectively). Similarly, the primary in vitro response to sheep erythrocytes was substantially depressed in CD4-depleted mice (<200 plaque-forming cells per 10^6 cells compared with 2,200 plaque-forming cells per 10^6 cells in CD8-depleted mice), demonstrating that T helper cells were insufficient in the cultures to activate a sheep erythrocyte-specific B-cell response. Double-depleted animals could make neither cytotoxic responses to alloantigens nor helper cell responses which resulted in antibody production by B cells (Fig. 2B and D).

Course of infection of *P. chabaudi* in mice depleted of their

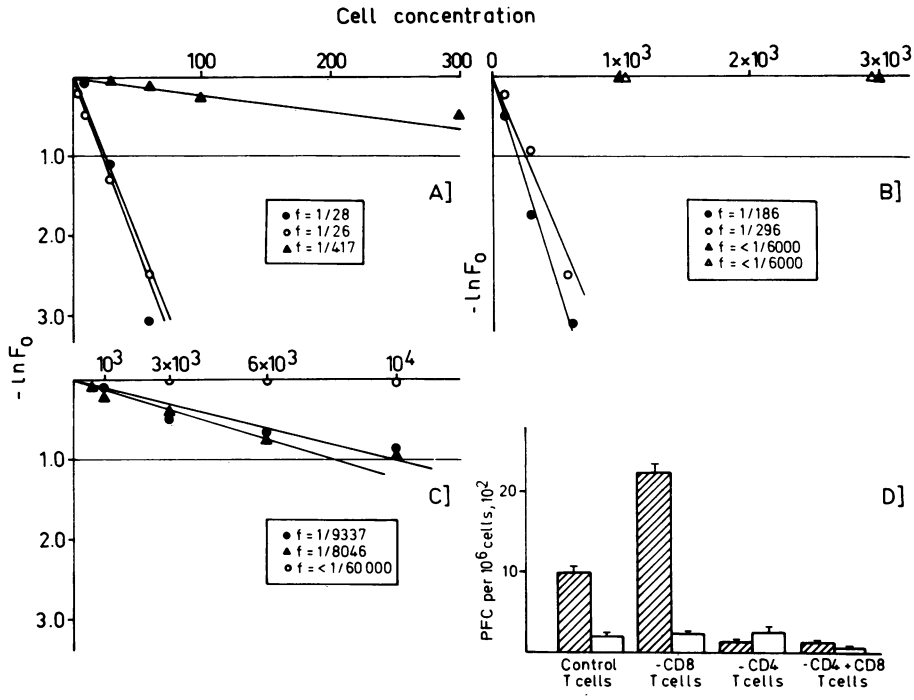

FIG. 2. Functional analysis of mice depleted of $CD4^+$ or $CD8^+$ T cells. (A) Limiting-dilution analysis of concanavalin A-activated phytohemagglutinin-dependent cytotoxic cells among spleen cells taken from control mice (●), CD4-depleted mice (○), or CD8-depleted mice (▲). The frequencies were calculated as described in Materials and Methods. The horizontal line at an Ln F_0 value of 1.0 indicates 37% nonresponding cultures. (B) Limiting-dilution analysis of *H-2*^{d}-specific cytotoxic precursors among lymph node cells taken from depleted and control mice. Symbols are as for panel A, plus CD4- plus CD8-depleted mice (△). (C) Limiting-dilution analysis of the T helper cell response to malaria antigens. Symbols are as in for panel A. (D) Primary in vitro plaque-forming cell (PFC) response to sheep erythrocytes of splenic cells from depleted and control mice cultured at 3×10^5 splenic cells per culture for 5 days: cells cultured in the presence (hatched bars) or absence (open bars) of sheep erythrocytes. Vertical lines are the standard deviations of triplicate cultures.

CD4 or CD8 subset of T cells. Five days after the first injection of the monoclonal anti-T-cell antibodies, mice were infected with 5×10^3 trophozoite forms of *P. chabaudi*. The course of infection in the different experimental groups is shown in Fig. 3. It can be seen that the courses of infection in the PBS-treated group and control antibody-treated group were essentially the same (Fig. 3A, B, and C). That is, parasites could be observed in the peripheral blood 5 to 6 days after injection, and the peak of infection (26 to 29% parasitemia) occurred after 10 days. This parasitemia was reduced to <0.5% after 18 days, and parasites remained at a low level (<0.2% parasitemia) for the duration of the experiment. The prepatent period of 5 to 6 days was the same in CD4-depleted mice as in the control groups (Fig. 3B). However, in contrast, mice depleted of CD4 cells developed a significantly higher peak parasitemia (mean, 57%; $P < 0.001$). This parasitemia was reduced to about 20%, at which it remained with small variations for 80 days. The erythrocyte count after the clearance of the peak parasitemia remained below normal levels ($<4 \times 10^9$ erythrocytes per ml) for at least 2 weeks in these mice. Thus, the actual total number of parasites is substantially less than that recorded at the peak of infection. It thus appears that the acute infection can be reduced to some extent in CD4-depleted mice but the parasitemia cannot be cleared. In CD8-depleted mice (Fig. 3A), the prepatent period was the same as in the other groups, but again, the peak parasitemia was significantly higher than that observed in the two control groups (40%; $P < 0.05$), although lower than the CD4-depleted group. This parasitemia was reduced to <0.5% after about 23 days, about 5 days after the control groups. After this time the parasite counts were indistinguishable from those of the controls. A fifth experimental group in which mice were depleted of both subsets of T cells was also monitored (Fig. 3C). The course of infection of this group resembled that of the CD4-depleted mice in that the peak parasitemia was higher (50%) than controls, and mice were unable to clear their infections. These data suggest that the first reduction of parasitemia between 10 and 12 days after infection can be achieved in the relative absence of T cells. These experiments also suggest that $CD8^+$ T cells have only a marginal effect on parasite development, since in their absence parasitemias are only slightly higher and clearance takes longer than in control animals. Those mice which have only $CD8^+$ T cells (CD4 depleted) are unable to clear their infection. This may have been a consequence of the inability to activate $CD8^+$ T cells in vivo in the absence of growth factors provided by $CD4^+$ helper cells. Therefore, the CD4 depletion experiments were repeated, with the concomitant injection of exogenous recombinant IL-2 as a source of growth factors (Fig. 3D). IL-2 was injected (2,500 U per injection) intraperitoneally on alternate days during the acute phase of the infection (from days 3 to 22) to a total of 25,000 U. The lower peak parasitemias shown in Fig. 3D compared with Fig. 3A to C reflect the different times at which blood smears were made. It can be seen that in the presence of IL-2 the parasitemias of the CD4-depleted mice developed slightly slower and were somewhat lower than those of depleted mice given no IL-2. However, similar differences were observed in intact animals. Depleted animals were still unable to clear their infections.

Mice depleted of $CD4^+$ T cells or of both subsets of T cells

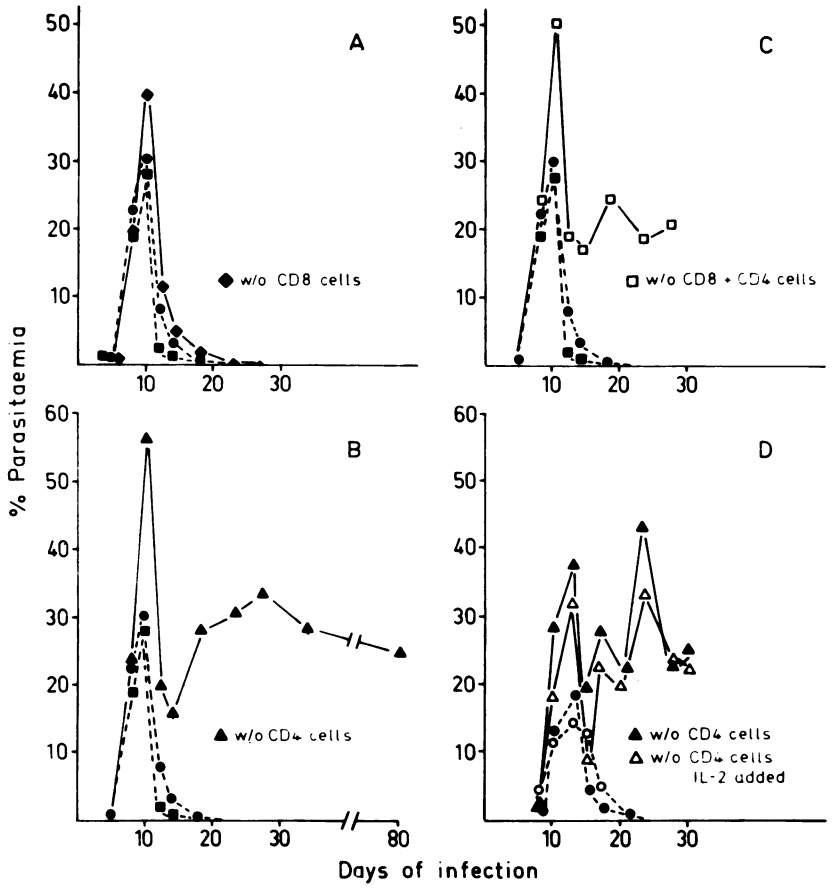

FIG. 3. Course of infection of *P. chabaudi chabaudi* in C57BL/6 female mice treated in vivo with antibodies. (A to C) Controls treated with PBS (●) or an irrelevant IgG2b antibody (■); (A) mice treated with anti-CD8 antibodies (◆); (B) mice treated with anti-CD4 antibodies (▲); (C) mice treated with both anti-CD8 and -CD4 antibodies (□). (D) Second course of treatment with anti-CD4 antibodies (▲) and anti-CD4 antibodies plus IL-2 (△), PBS (●), and PBS plus IL-2 (○). The values are the geometric means of the parasitemias of six to eight mice. The standard deviations (not shown) are on the order of 10 to 15% of the means.

had no detectable levels of malaria-specific IgG antibodies of any isotype at any time tested throughout the experiments (days 23 and 44 of infection are shown in Fig. 4). However, a transient low titer of IgM antibodies was detectable earlier in infection (day 23). Subsequent tests of sera at 44 (Fig. 4), 63, and 80 days demonstrated that despite a persistent parasitemia no further malaria-specific IgM could be detected. It is possible that the small amounts of circulating IgM are adsorbed by the abundant parasite material present in the blood. Mice depleted of $CD8^+$ T cells had levels of IgG and IgM antibodies comparable to or slightly higher than those of the control groups at all times tested throughout the infection.

DISCUSSION

The experiments described here demonstrate the importance of CD4-bearing T cells in the development of protective immunity to the erythrocytic stages of *P. chabaudi*. The absence of $CD4^+$ T cells had no impact on the rising parasitemia of infected mice but did affect the subsequent peak of parasitemia and clearance of parasites. Parasitemias of the CD4-depleted mice were significantly higher than those of control animals, but were reduced within 2 to 3 days to approximately 20%. However, CD4-depleted mice were unable to reduce this parasitemia further.

Phenotypical analysis of these depleted mice showed that, although there was substantial depletion of $CD4^+$ T cells, it was not absolute. This is in agreement with reports by other investigators (13, 18, 31). It could therefore be argued that the partial control of parasitemia observed was due to the few remaining T cells. However, our studies show that functional helper cells for malaria-specific antibody production or for the response to sheep erythrocytes are lacking in these mice when tested in vitro. Further, no IgG antibodies specific for malaria antigens could be detected at any time tested throughout the experiment in the CD4-depleted mice, suggesting that T helper cell function was lacking.

Our data are in agreement with previous adoptive transfer experiments carried out with immune T cells taken from mice infected with *P. yoelii* or *P. chabaudi* (4, 8, 23) which demonstrated that protective immunity is best transferred by immune Lyt-2^- ($CD8^-$) L3T4^+ ($CD4^+$) T cells. These experiments also support the results found with T-cell clones and T-cell lines showing that a *P. chabaudi*-specific $CD4^+$ T-cell clone can transfer resistance (2, 3). Therefore, the $CD4^+$ T cells are important not only in the generation of a protective immune response in unprimed mice but also in ability to transfer resistance to infection, suggesting that these cells are not just providing help for $CD8^+$ T cells which are then the effector cell responsible for clearing the infec-

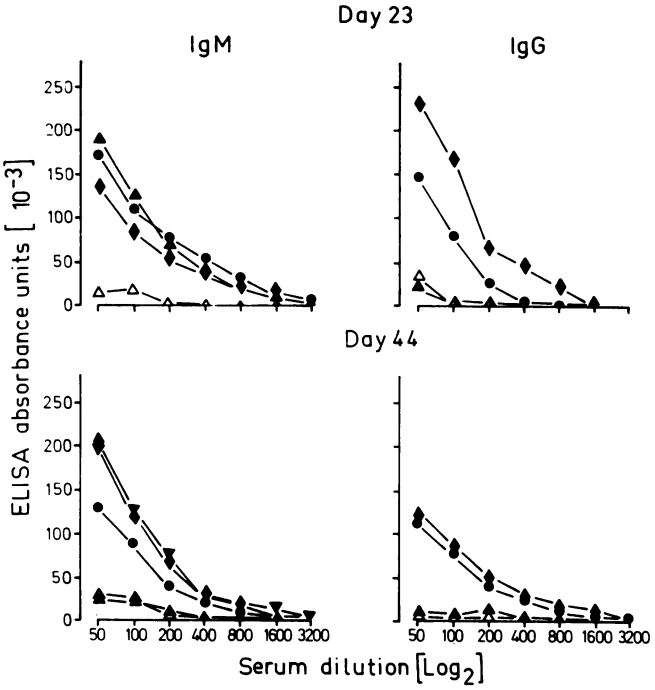

FIG. 4. Titration of sera from antibody-treated mice taken 23 and 44 days after infection with *P. chabaudi* in a malaria-specific ELISA: PBS treated (●); anti-CD4 treated (▲); anti-CD8 treated, (◆) control antibody treated (▼); serum from normal uninfected mice (△). The ELISA absorbance values (optical density at 405 nm) shown are those of pooled sera from three or more mice and are given with the background subtracted (alkaline phosphatase-conjugated anti-mouse IgM or anti-mouse IgG plus antigen in the absence of sera).

tion. Interestingly, even in those mice deprived of both subsets of T cells, the parasitemias were controlled at about 20% for the duration of the experiment (30 days). These mice were demonstrated to be substantially depleted of T cells. It is possible that the few remaining CD4- or CD8-bearing cells are responsible or that $CD4^-$ $CD8^-$ T cells have some immune function. However, since these mice were also depleted of Thy-1^+ cells in all lymphoid organs examined and were functionally severely impaired in their immune responsiveness, this is unlikely. The courses of infection in the double-depleted mice and the CD4-depleted mice were similar to those observed for *P. chabaudi adami* in nude mice (2, 3, 21) and *P. chabaudi chabaudi AS* in adult thymectomized, irradiated mice (33, 34) in that the parasites are not cleared but the majority of mice remain alive for at least 30 to 65 days. The low transient malaria-specific IgM antibodies may play a role in this. To rule this out, these experiments would have to be repeated in mice without circulating immunoglobulins. The control of infection may also be partly due to the preference of this parasite for mature erythrocytes. After the initial high peak of parasitemia, there is a persistent reticulocytosis in the peripheral blood which may limit the extent of the parasitemia. Also, the products of activated macrophages which are not depleted by the experiment may be directly parasiticidal (10, 12, 16).

The $CD4^+$ T cell can be characterized functionally as a cell which can mediate delayed-type hypersensitivity and which can act as a helper cell for antibody production. Recent experiments (37) indicate that these functions may be carried out by separate subsets of CD4 cells (TH_1 and TH_2, respectively). It is proposed that the TH_1 cells which secrete IFN-γ, IL-2, and lymphotoxin among other factors are the mediators of delayed-type hypersensitivity whereas the TH_2 cells, which secrete IL-4 and IL-5, are efficient helper cells for B cells. The effector role of the CD4 cell in controlling a primary infection of *P. chabaudi* is not known. The experiments of Grun and Weidanz (21) demonstrate that mice lacking B cells are able to control infection, suggesting a role for T cells in clearance of parasites independent of their helper function for antibody production. Furthermore, the T-cell clone which has been shown to be protective against an infection with *P. chabaudi* is one which makes IFN-γ (3). It has been known for some time that degenerated forms or "crisis forms" of the intraerythrocytic stages of *Plasmodium* spp. occur in partially immune animals or mice treated with a variety of macrophage activators (7, 9, 10–12). This observation can be interpreted as an effect of antibody bound to merozoites before invasion of the erythrocyte or as an antibody-independent effect. In addition, it has been shown that a nonantibody, but undefined component of serum of immune patients, has an effect on intraerythrocytic parasites (24). It could be envisaged that $CD4^+$ T cells are activated by antigens presented in the context of class II major histocompatibility complex (MHC) molecules on macrophages or B cells within the spleen. A variety of mediators such as IFN-γ and TNF-β are then released which could act directly on the parasite or activate macrophages to produce other mediators such as TNF-α and reactive oxygen radicals, which are known to play important roles in the response to malaria infection (10, 16). An intact spleen is a necessary component for a protective immune response (20), and it may be that it provides a site for these interactions. The precise role of the $CD4^+$ cells in immunity to malaria remains to be defined, but our experiments indicate that a majority of $CD4^+$ cells elicited during the acute phase of infection with *P. chabaudi* are relatively poor helpers for antibody production, although they secrete IL-2 (Langhorne, et al. in preparation). The data presented here also demonstrate that in the absence of $CD4^+$ T cells no significant malaria-specific antibodies are made, with the exception of a transient low titer of *P. chabaudi*-specific IgM. This is in agreement with studies in the *P. yoelii* model system which indicated that T-cell-independent (type I or II) antigens were lacking in *P. yoelii* organisms (40).

Studies of *P. yoelii* in the mouse have suggested that Lyt-2^+ ($CD8^+$) cells can partially protect against challenge infection (36). This ability was correlated with an increase of expression of class I MHC molecules on infected erythrocytes and led to the postulation that $CD8^+$ may be directly cytotoxic for intraerythrocyte parasites (22, 23). Our studies show little indication in an erythrocytic infection with *P. chabaudi* that $CD8^+$ T cells have any significant role since, in their absence, parasitemias are only marginally higher and the infection is cleared. However, it is possible that under the conditions of these experiments the $CD8^+$ T cells could not be appropriately activated. Although is is not known whether $CD8^+$ T cells in vivo require an exogenous IL-2 source (from $CD4^+$ cells), recombinant IL-2 was given to CD4-depleted mice to determine whether under these conditions $CD8^+$ cells would be effective in clearing the parasitemias. Addition of 25,000 U of IL-2 did not result in the clearance of parasitemia. It is possible that the amount of IL-2 given was insufficient. However, mice which have been depleted of $CD4^+$ T cells at the peak of infection or shortly afterwards, when sufficient time would have elapsed for the

activation of $CD8^+$ effector cells by $CD4^+$ T cells, are still unable to clear their infections (Langhorne, unpublished observations). This, of course, does not mean that $CD8^+$ effector cells are not elicited in *P. chabaudi* infections, since it is possible that parasite antigens are processed by antigen-presenting cells and presented with MHC class I molecules. $CD8^+$ cells so elicited could be antiparasiticidal by the release of inflammatory mediators such as lymphotoxin and IFN-γ. However, these data taken together with adoptive transfer experiments in *P. chabaudi* infection (8) suggest that $CD8^+$ T cells do not play a major protective role. This is in direct contrast to the crucial role played by $CD8^+$ cells in the protective response against the exoerythrocytic stages of *P. berghei* (38, 41). Although it cannot be ruled out, the $CD8^+$ cell as a directly cytotoxic cell in *P. chabaudi* infection seem less likely than in *P. yoelii*, since *P. chabaudi* invades preferentially mature erythrocytes which present little or no class I MHC molecules.

In conclusion, the $CD4^+$ T cell is essential for a successful primary immune response to *P. chabaudi*. The search for necessary T-cell epitopes for a vaccine should include those structures which associate efficiently with class II MHC molecules.

ACKNOWLEDGMENTS

This study was supported by a grant from the Rockefeller Foundation.

We thank M. M. Simon, M. C. Lamers, and B. Stockinger for critical reading of the manuscript.

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