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# T-Cell-Mediated Immune Response in Murine Malaria: Differential Effects of Antigen-Specific Lyt T-Cell Subsets in Recovery from *Plasmodium yoelii* Infection in Normal and T-Cell-Deficient Mice

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**For analysis of the role of immune T cells in protective immunity against murine malaria, *Plasmodium yoelii*-immune Lyt T-cell subsets were functionally characterized in vitro and in vivo. Selected Lyt2$^-$ and Lyt2$^+$ T cells from *P. yoelii*-immune C57BL/10 mice differed in their capability to proliferate in response to *P. yoelii* antigen in vitro. Only the Lyt2$^-$ T-cell population produced T-cell growth factor upon restimulation, and none of the selected T-cell subsets produced detectable amounts of macrophage activating factor. Lyt2$^-$ but not Lyt2$^+$ lymphocytes were capable of transferring protection to normal C57BL/10 mice. When transferred into T-cell-deficient C57BL/6-*nu/nu* mice, adoptive resistance to *P. yoelii* by Lyt2$^-$ lymphocytes was only demonstrable after prior reconstitution of recipients with normal T cells. These results suggest an interaction between *P. yoelii*-immune Lyt2$^-$ T cells and normal T lymphocytes via T-cell growth factor in the development of protective immunity to malaria.**

Protective immunity against malaria is highly complex and only poorly understood (1, 7, 11). In the murine malaria model, activated macrophages have been found to play a crucial role in the elimination of plasmodia (1), and the production of reactive oxygen metabolites may be an important killing mechanism (20, 21). Indeed, we have shown recently that the nonlethal parasite *Plasmodium yoelii* markedly stimulates oxygen metabolism in splenic macrophages from infected mice (4). Activation of macrophages for increased antimicrobial effects is generally thought to be effected by specific T lymphocytes (19), and the success in transferring protective immunity with specific T cells indicates that this may be true for malaria as well (13). To further elucidate the role of T lymphocytes in malarial infection, we attempted to identify the protective T-cell subpopulations generated after infection of mice with *P. yoelii* and to functionally characterize them in vitro and in vivo. We show that selected Lyt2$^-$ and Lyt2$^+$ T cells differed in their capability to respond to *P. yoelii* antigen by proliferation and production of T-cell growth factor (TCGF) and that only Lyt2$^-$ T lymphocytes could transfer protection upon normal animals. Furthermore, when transferred to T-cell-deficient *nu/nu* mice, adoptive resistance to *P. yoelii* by Lyt2$^-$ lymphocytes was only demonstrable after prior reconstitution of recipients with normal T cells. The results suggest an interaction between *P. yoelii*-immune Lyt2$^-$ T cells and resident T lymphocytes in the development of protective immunity to malaria.

## MATERIALS AND METHODS

**Mice.** C57BL/10 mice were bred in our own specific-pathogen-free colony, and C57BL/6-*nu/nu* mice were obtained from GJ Bomholtgard Ltd. (Ry, Denmark). Female mice were used at 8 to 12 weeks of age.

**Parasites and parasite antigen.** Parasites of the nonlethal strain *P. yoelii* 17XNL were kindly provided by J. H. L. Playfair, Middlesex Hospital Medical School, London, England, and kept virulent by continuous mouse passage. Mice were infected with $10^4$ parasitized erythrocytes (PRBCs), and parasitemia was determined microscopically in Giemsa-stained tail blood smears. PRBCs obtained from infected whole blood at 20% parasitemia were isolated by separation on a cellulose powder column (3) and subsequent centrifugation on a Percoll density gradient (25). PRBCs were then lysed by freeze-thawing (three times) and stored at −20°C at a concentration of $2 \times 10^9$ PRBCs per ml in phosphate-buffered saline. Erythrocytes from normal uninfected mice (NRBCs) treated in the same way were used as a control for testing antigen specificity.

***P. yoelii*-immune spleen cells.** C57BL/10 mice were immunized with self-limiting *P. yoelii* by intraperitoneal injection of $10^4$ PRBCs at days 0 and 30. On day 45, infected mice were immune to rechallenge with $10^4$ PRBCs, and parasites were not detectable in the blood. At this time, spleens were removed, and cell suspensions were treated with $NH_4Cl$ to lyse erythrocytes. T cells were enriched by passage of spleen cells over nylon wool columns by the method of Julius et al. (14).

**Selection of Lyt T-cell subpopulations and enrichment of B lymphocytes.** Nylon wool-enriched T cells ($3 \times 10^7$/ml) were treated with anti-Lyt1.2 antiserum (final dilution, 1:10) or anti-Lyt2.2 antiserum (final dilution, 1:10) at room temperature for 30 min and subsequently with selected rabbit complement (final dilution, 1:11) at 37°C for 30 min. Viable cells were determined by trypan blue exclusion. B cells were enriched by treatment of $3 \times 10^7$ unselected spleen cells per ml with anti-Thy1.2 antiserum and selected rabbit complement (15, 24).

**Accessory cells.** Nucleated spleen cells ($2 \times 10^5$) from immune mice were cultured in round-bottomed 96-well multidish trays (Nunc, Wiesbaden, Germany) for 2 h. Nonadherent cells were removed by washing, and the adherent fraction was irradiated with 2,200 rads.

**Proliferation assay.** Cultures were set up in RPMI 1640 medium (GIBCO, Pacely, England) supplemented with L-glutamine (2 mM), kanamycin (100 μg/ml), tylosin (10 μg/ml), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic

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acid) buffer (25 mM), 2-mercaptoethanol ($2 \times 10^{-5}$ M), and 10% selected fetal calf serum (complete medium; Seromed, Munich, Germany) in an atmosphere of 5% $CO_2$ at 37°C. Immune T cells ($2 \times 10^5$) or equivalent numbers of selected immune cells were cultured with adherent cells from $2 \times 10^5$ unfractionated spleen cells from immune animals or without additional accessory cells in the presence of $10^6$ lysed PRBCs, $10^6$ lysed NRBCs, or 1 μg of concanavalin A (ConA), respectively. Cells were incubated in a total volume of 0.2 ml in round-bottomed 96-well multidish trays (Nunc) for 3 days. A 1.25-μCi amount of [$^3$H]thymidine (specific activity, 2 Ci/mmol; Radiochemical centre, Amersham, England) was added to the cultures for the last 24 h. Cells were harvested, and samples were counted in a liquid scintillation counter.

**Production of lymphokines in vitro.** Cell cultures were set up as described for the proliferation assay. After 24 h of incubation, supernatants were harvested and tested for their lymphokine activity (macrophage activating factor [MAF] and TCGF).

**Test for TCGF activity.** Cells ($3 \times 10^3$/0.2 ml) of a TCGF-dependent cytotoxic T lymphocyte line were cultured in complete medium in the presence or absence of supernatant (50%) in round-bottomed 96-well multidish trays (Nunc). After 24 h, cultures were pulsed with 1.25 μCi of [$^3$H]thymidine for an additional 24 h. Afterwards, cells were harvested, and samples were counted in a liquid scintillation counter.

**Test for MAF activity in the chemiluminescence assay.** Thioglycolate-induced peritoneal exudate cells ($2 \times 10^5$) were cultured in 0.5 ml of Dulbecco minimal essential medium supplemented with 200 mM asparagine and 10% fetal calf serum in chemiluminescence vials (Abimed, Düsseldorf, Germany). After 24 h, fresh medium containing 50% supernatant was added. After another 24 h, 0.5 ml of medium containing 10 μl of luminol (2 mg/ml; Sigma, Munich, Germany), and 10 min afterwards, 10 μl of zymosan (50 mg/ml; Becton-Dickinson, Orangeburg, N.Y.) was added. Chemiluminescence responses were then determined as described previously (4, 8, 9).

**Test for MAF activity in the cytotoxicity assay.** Supernatants were added to thioglycolate-induced peritoneal macrophages ($2 \times 10^5$/0.2 ml of complete medium) in flat-bottomed 96-well multidish trays (Nunc) at a final concentration of 50%. After 24 h, $2 \times 10^4$ $^{51}$Cr-labeled P815 cells were added, and $^{51}$Cr release was determined after another 24 h as described previously (17). Percent lysis was calculated as (test counts per minute)/(maximum counts per minute released) × 100. Maximum release was induced by osmotic rupture of $^{51}$Cr-labeled P815 cells.

**Adoptive transfer experiments.** Three modes of cell transfers were performed. (i) C57BL/10 mice were injected intravenously with $10^7$ to $5 \times 10^7$ unselected or selected immune syngeneic lymphocytes. After 24 h, animals were infected with $10^4$ PRBCs intraperitoneally, and the course of parasitemia was monitored by differential cell counts on Giemsa-stained tail blood smears. (ii) C57BL/6-*nu/nu* mice received unselected or selected immune lymphocytes from congenic C57BL/10 donors. (iii) C57BL/6-*nu/nu* mice were first injected intravenously with either $10^8$ unselected spleen cells or $3 \times 10^7$ enriched T or B lymphocytes from congenic C57BL/10 mice. After 24 h, recipients received $10^7$ to $5 \times 10^7$ immune spleen cells, unselected immune T cells, or immune Lyt2$^-$ T cells. After another 24 h, animals were infected with $10^4$ PRBCs intraperitoneally, and the course of parasitemia was scored as described above.

## RESULTS

**Proliferation of and lymphokine production by *P. yoelii*-immune T lymphocytes in vitro.** Proliferative responses of *P. yoelii*-immune spleen lymphocytes were tested in cultures containing either lysed NRBCs or PRBCs. The antigen-specific proliferative response of unselected spleen cells was dependent on T lymphocytes since pretreatment with anti-Thy1.2 plus complement completely abrogated the response (Table 1). In addition, T-cell-depleted responder cells did not show antigen-specific proliferation in the presence of either ConA supernatant or irradiated immune spleen cells, indicating that B lymphocytes from presensitized mice are not stimulated by *P. yoelii* to proliferate under these conditions. Activation of both selected Lyt2$^-$ lymphocytes and the mixture of Lyt2$^+$ and Lyt2$^-$ populations resulted in proliferative responses similar to those of unselected T cells (Table 2). Selected Lyt2$^+$ lymphocytes did not respond under these conditions and showed antigen-specific proliferation only in the presence of additional accessory cells. When tested for lymphokine production in response to *P. yoelii* antigen, Lyt2$^-$ but not Lyt2$^+$ cells were able to secrete TCGF to an extent comparable to that of the unselected population (Table 3). Furthermore, MAF activity was not detected in either population upon stimulation with PRBCs. These results indicate that both the Lyt2$^-$ and Lyt2$^+$ lymphocyte population from immune mice contain *P. yoelii*-reactive T cells which, however, differ in their induction

TABLE 1. Effect of anti-Thy1.2 antiserum plus complement on the proliferative response of *P. yoelii*-immune spleen cells

| Treatment of immune spleen cells | Addition | Proliferative response ([$^3$H]thymidine uptake [cpm]) after activation with[a]: | | | |
|---|---|---|---|---|---|
| | | No activation | ConA | NRBCs | PRBCs |
| None | None | 1,705 | 24,770 | 1,835 | 7,268 |
| Complement | None | 236 | 29,713 | 96 | 1,349 |
| Anti-Thy1.2 + complement | None | 262 | 112 | 88 | 74 |
| | ConA supernatant | 596 | 744 | 270 | 179 |
| | Irradiated immune spleen cells | 571 | 1,906 | 460 | 275 |

[a] Immune spleen cells were treated with anti-Thy1.2 antiserum plus complement and adjusted to a volume corresponding to $2 \times 10^5$ complement-treated spleen cells, and equivalent numbers were cultured with antigen ($10^6$ lysed NRBCs, PRBCs, or 1 μg of ConA) in a total volume of 0.2 ml in the presence or absence of 10% ConA-spleen cell supernatant or $2 \times 10^5$ irradiated immune spleen cells for 4 days. 24 h before harvest, cells were pulsed with 1 μCi of [$^3$H]thymidine. Means of triplicates are given; standard deviation, ≤20%.

TABLE 2. Effect of posttreatment with either anti-Lyt1.2 or anti-Lyt2.2 antiserum plus complement on the proliferative response of *P. yoelii*-immune T cells

| Treatment of nylon-enriched immune T cells[a] | T-cell subset | Accessory cells[b] | Proliferative response ([$^3$H]thymidine uptake [cpm]) after activation with[a]: | |
|---|---|---|---|---|
| | | | NRBCs | PRBCs |
| Complement | Unselected T | − | 354 | 2,503 |
| | | + | 1,526 | 6,104 |
| Anti-Lyt2.2 + complement | Lyt2$^-$ | − | 452 | 4,103 |
| | | + | 565 | 5,379 |
| Anti-Lyt1.2 + complement | Lyt2$^+$ | − | 310 | 476 |
| | | + | 405 | 2,551 |
| Mixture (1:1) of anti-Lyt1.2 + complement and anti-Lyt2.2 + complement | Lyt2$^-$ + Lyt2$^+$ | − | 293 | 2,733 |
| | | + | 796 | 5,281 |

[a] Immune nylon-purified T cells were treated with anti-Lyt1.2 or anti-Lyt2.2 antiserum plus complement. Cells were adjusted to a volume corresponding to $2 \times 10^5$ complement-treated T cells, and equivalent numbers of T cells were cultured with antigen ($10^6$ lysed NRBCs or PRBCs) in a total volume of 0.2 ml in the presence or absence of accessory cells.

[b] Immune spleen cells ($2 \times 10^5$) were cultured for 2 h, and the adherent cell fraction was irradiated (2,200 rads) and used as accessory cells. Means of triplicates are given; standard deviation, ≤20%.

requirements (accessory cells) and their ability to produce soluble mediators.

**Transfer of immune T-lymphocyte subsets to syngeneic C57BL/10 mice results in protection against *P. yoelii* infection.** C57BL/10 mice received intravenous injections of unselected or selected immune lymphocyte populations and were infected with an intraperitoneal inoculum of $10^4$ PRBCs 24 h after cell transfer. Parasitemia was determined at various times thereafter. Control animals inoculated with *P. yoelii* in the absence of immune lymphocytes controlled the infection within 20 to 25 days and exhibited maximum parasitemia of 20% (Fig. 1). Recipient mice which had received $10^7$ unselected immune spleen cells, the same number of enriched immune T cells, selected Lyt2$^-$ lymphocytes, or a mixture of Lyt2$^-$ and Lyt2$^+$ cells were capable of controlling the infection within 10 to 13 days. They showed parasitemia which at maximum was less than 2.5% of the control. On the other hand, the transfer of selected immune Lyt2$^+$ T cells had no effect on either the course of infection or on the magnitude of parasitemia in recipient mice. Furthermore, the immune Lyt2$^+$ T cells did not influence the outcome of protection mediated by the Lyt2$^-$ population. These results show that protection against *P. yoelii* infection can be transferred upon normal mice by Lyt2$^-$ but not by Lyt2$^+$ T lymphocytes from immune donors.

**Failure to transfer protection upon C57BL/6-*nu/nu* mice with immune T-lymphocyte subsets from normal mice.** To find out whether the protection seen after transfer of immune Lyt2$^-$ T cells into normal animals was directly mediated by this T-cell subpopulation or depended on the presence of additional T cells from the recipient, immune lymphocytes were transferred intravenously into T-cell-deficient *nu/nu* mice 24 h before intraperitoneal inoculation of *P. yoelii*. In contrast to normal animals, T-cell-deficient *nu/nu* mice did

TABLE 3. Production of lymphokines by *P. yoelii*-immune T-cell subsets

| Cells | Treatment | Primary culture[a] | | Secondary culture | | |
|---|---|---|---|---|---|---|
| | | | | MAF[b] | | TCGF[c] |
| | | Cell subset | Stimulus | Chemiluminescence (cpm, $10^3$) | Specific lysis of P815 (%) | Proliferative response of CTLL ([$^3$H]thymidine uptake [cpm]) |
| Spleen | None | Spleen | None | 348 | 1.2 | 280 |
| | None | Spleen | NRBCs | 211 | 1.3 | 521 |
| | None | Spleen | PRBCs | 438 | 1.1 | 9,174 |
| | None | Spleen | ConA | 1,938 | 24.2 | 15,956 |
| Spleen | Nylon + complement | Unselected T | PRBCs | 498 | 1.9 | 8,775 |
| Unselected T | Anti-Lyt2.2 + complement | Lyt2$^-$ | PRBCs | 392 | 1.1 | 9,138 |
| Unselected T | Anti-Lyt1.2 + complement | Lyt2$^+$ | PRBCs | 362 | 1.8 | 1,028 |
| Lyt2$^-$ + Lyt2$^+$ | Mixture (1:1) of anti-Lyt1.2 + complement and anti-Lyt2.2 + complement | Lyt2$^-$ + Lyt2$^+$ | PRBCs | 421 | 2.3 | 7,639 |

[a] A total of $2 \times 10^5$ immune spleen cells, T cells, or equivalent numbers of Lyt T-cell subsets were cultured in a total volume of 0.2 ml in the presence of antigen ($10^6$ lysed NRBCs or PRBCs with 1 μg of ConA) and accessory cells (irradiated adherent cell fraction of $2 \times 10^5$ immune spleen cells) for 24 h. Supernatants were harvested and used in the secondary culture.

[b] A total of $2 \times 10^5$ thioglycolate-induced peritoneal macrophages per 0.2 ml were cultured for 24 h in the presence of 50% culture supernatants from primary cultures. Afterwards, zymosan-induced chemiluminescence or cytotoxicity against P815 cells was determined as described in the text.

[c] A total of $3 \times 10^3$ cells of a TCGF-dependent T cell line (CTLL) were cultured for 48 h in the presence of 50% supernatants from primary cultures. Proliferation during the last 24 h of culture was detected by [$^3$H]thymidine uptake as described in the text. Means of triplicates are given; standard deviation, ≤20%.

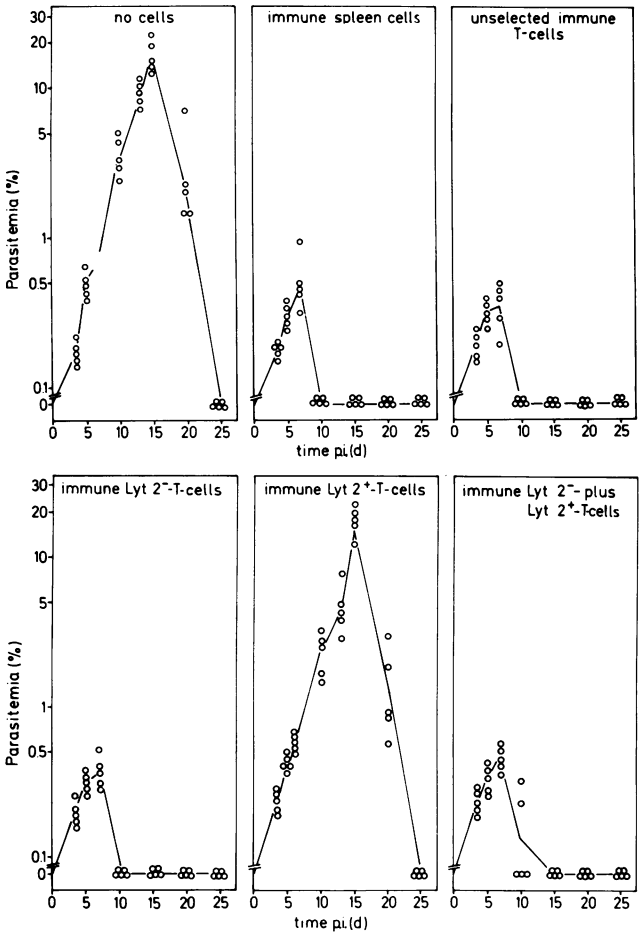


FIG. 1. Role of Lyt T-cell subsets in mediating protection against *P. yoelii* in C57BL/10 mice. Mice received $10^7$ immune syngeneic spleen cells, nylon-enriched T cells, or selected Lyt T lymphocytes. After 24 h, the animals were infected intraperitoneally with $10^4$ PRBCs, and parasitemia was determined in Giemsa-stained tail blood smears (each data point represents one individual mouse).

not recover from *P. yoelii* infection and died within 3 to 5 weeks (Fig. 2). In addition, none of the immune lymphocyte populations, i.e., unselected spleen cells, enriched T cells, or selected Lyt2$^-$ T cells, could transfer protection against lethal *P. yoelii* infection in T-cell-deficient mice. Since even higher numbers of unselected immune spleen cells ($5 \times 10^7$) had no effect on the course of parasitemia, these results suggest at least three alternative possibilities: (i) the transferred immune lymphocyte population was either inactivated or suppressed in *nu/nu* mice; (ii) the transferred cells expressed different homing patterns in the different mouse strains, or (iii) the immune cells required additional cells for elicitation of their effector function which were absent in T-cell-deficient mice.

The first possibility was tested by transferring $3 \times 10^7$ unselected *nu/nu* spleen cells to congenic C57BL/10 mice. After 24 h, these mice were given $10^7$ unselected immune T cells, and after another 24 h, they were infected with $10^4$ PRBCs. C57BL/6-*nu/nu* spleen cells had no demonstrable effect on the final outcome of adoptive protection in C57BL/10 mice (data not shown). The second possibility was tested by comparing the homing pattern of $10^7$ intravenously injected $^{51}$Cr-labeled immune spleen cells in mice of both congenic strains by scoring radioactivity 24 and 48 h after transfer in the organs of the animals. No differences between the strains could be detected (data not shown).

**Influence of normal splenocytes on adoptive protection in *nu/nu* mice.** Since *nu/nu* mice are deficient in mature T cells (16) it was possible that resident T lymphocytes were required for elicitation of optimal protection activities by the transferred immune T cells. Therefore, *nu/nu* mice were reconstituted intravenously with $10^8$ unselected normal spleen cells from congenic C57BL/10 mice. After 24 h, these mice received selected lymphocyte subpopulations from immune animals, and after an additional 24 h, they were infected with $10^4$ PRBCs. As expected, transfer of spleen cells from normal animals did not result in protection against *P. yoelii* infection in T-cell-deficient recipient mice (Fig. 3). However, when *nu/nu* mice received *P. yoelii*-immune spleen cells, enriched T cells, or selected Lyt2$^-$ lymphocytes in addition to normal spleen cells, protection against *P. yoelii* infection developed in some but not all animals within each

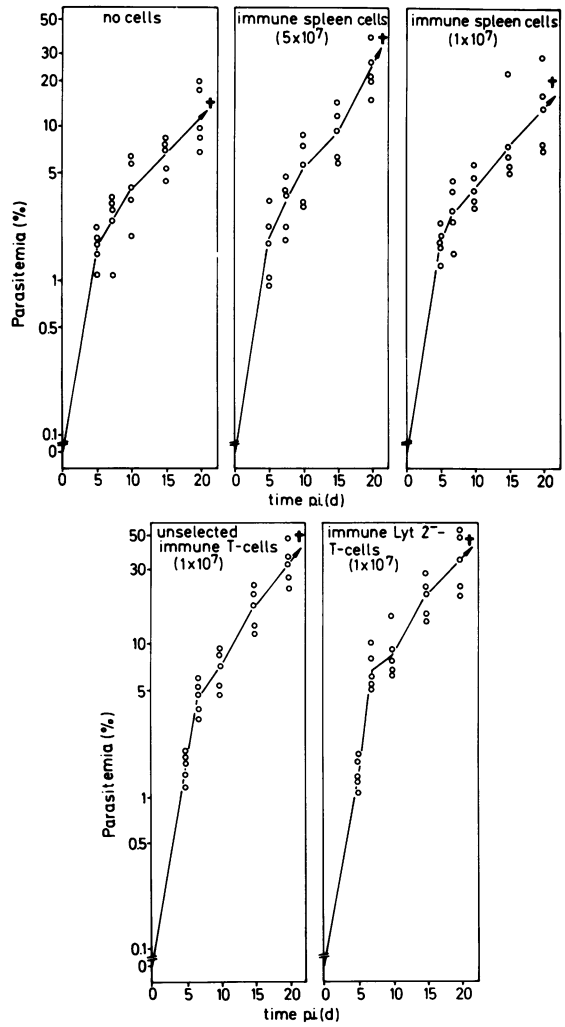


FIG. 2. Role of Lyt T-cell subsets in protection against *P. yoelii* in C57BL/6-*nu/nu* mice. C57BL/6-*nu/nu* mice received the indicated numbers of immune cells from congenic C57BL/10 donors. After 24 h, the animals were infected intraperitoneally with $10^4$ PRBCs, and parasitemia was determined in Giemsa-stained tail blood smears (each data point represents one individual mouse).

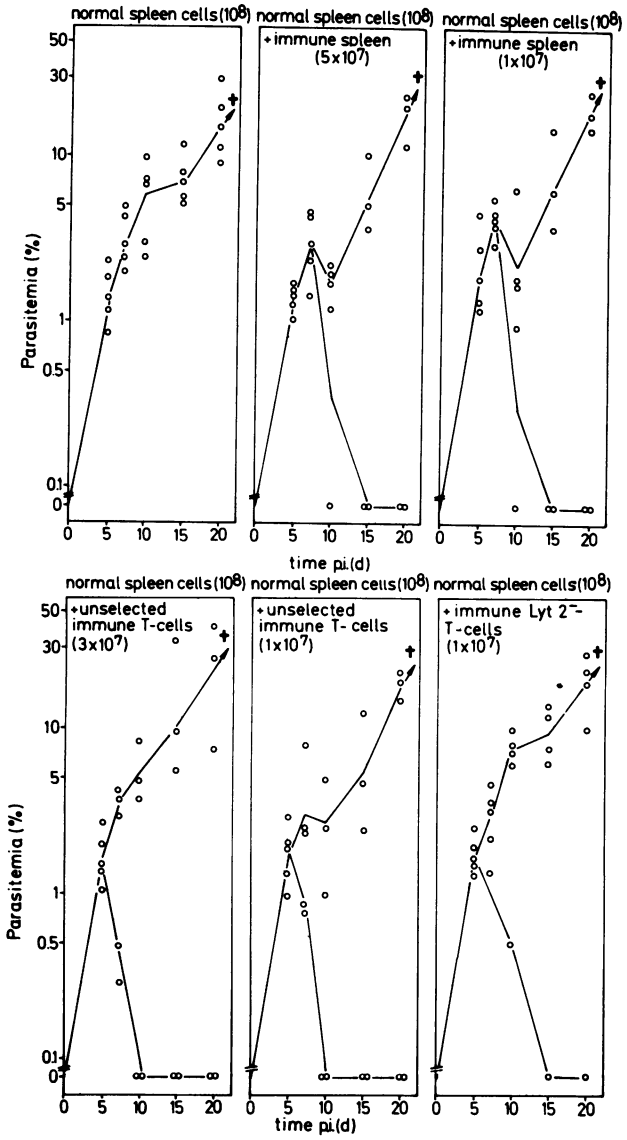


FIG. 3. Role of normal spleen cells in cooperation with immune T cells in protection against *P. yoelii* in C57BL/6-*nu/nu* mice. C57BL/6-*nu/nu* mice received $10^8$ normal spleen cells and 24 h later the indicated numbers of congenic immune cells. After another 24 h, mice were infected intraperitoneally with $10^4$ PRBCs, and parasitemia was determined in Giemsa-stained tail blood smears (each data point represents one individual mouse).

experimental group. In additional experiments of the same design, it was consistently found that a certain proportion (40%) of recipient mice was protected.

To determine which lymphocyte subset within the normal spleen cell population was required for the protective effect, *nu/nu* mice were reconstituted with normal unselected T cells or T-cell-deprived lymphocyte populations before transfer of immune lymphocytes. Unselected T cells but not T-cell-deprived normal spleen cells were able to facilitate adoptive protection against *P. yoelii* by immune T cells in *nu/nu* mice (Fig. 4). These results suggest that *P. yoelii*-immune T cells after transfer upon recipient mice have to interact with a resident T-cell population to effectively protect the host from malarial infection.

## DISCUSSION

The importance of T lymphocytes in the induction and effector phases of immunity to malaria is well established (11). This is implied by the findings that T-cell-deficient mice fail to resist *P. yoelii* infections (6) and that T cells from immune mice, when transferred to nonimmune recipients, confer protection against malaria (11). Although a role for helper T cells in the humoral immune response to plasmodia has been demonstrated (5), evidence for additional activities of T cells in the regulation of parasite multiplication exists (12, 23). However, the exact mechanisms by which plasmodia-immune T cells induce protection are far from being understood.

To further analyze the mechanisms involved in the generation of aquired resistance to malaria, we investigated the capacity of selected Lyt T-cell subsets from *P. yoelii*-immune mice to produce lymphokines in vitro and to adoptively mediate protection in normal C57BL/10 and congenic thymusless *nu/nu* mice. It was found that the Lyt2$^-$ T-cell-subset constituted the major component involved in adoptive resistance in normal mice (Fig. 1). In contrast, when comparable numbers of unselected or selected immune spleen cells were injected intravenously into *nu/nu* mice, these cells failed to induce protection (Fig. 2). Three alternatives were considered as possible explanations for the failure to transfer protection upon *nu/nu* mice: (i) the transferred cells exerted different homing patterns in the two mouse strains; (ii) *nu/nu* mice possess resident cells with nonspecific suppressive activity; or (iii) the transferred immune cell population required additional cells for protection which were absent in *nu/nu* mice.

In transfer experiments with $^{51}$Cr-labeled cells, we found that the homing pattern of *P. yoelii*-immune T cells was comparable in both mouse strains (data not shown). This finding strongly argues against an aberrant migration pattern of transferred T cells in *nu/nu* mice. Recently, it has been shown that a population of *nu/nu* spleen cells is capable of suppressing cytotoxic T-cell responses in vitro (18). Therefore, it was tested whether such cells also had a suppressive effect on adoptively transferred immunity. Because administration of even high numbers of *nu/nu* spleen cells to congenic normal mice did not influence the outcome of protection transferred by immune T cells (data not shown), inhibitory influences on *P. yoelii*-immune T cells in *nu/nu* mice seem unlikely.

From these results, we concluded that resident T cells of the recipients were required for expression of adoptively transferred antimalarial immunity and that the failure to confer protection upon *nu/nu* mice was due to their defective T-cell compartment. Indeed, when *nu/nu* mice were reconstituted with normal T cells, but not B cells, before adoptive immunization, protective effects were observed in 40% of the recipients of immune cells (Figs. 3 and 4). Therefore, it appears likely that for effective protection to occur, the transferred immune T-cell population required cooperation with resident T cells which are absent in *nu/nu* mice. These findings are at variance with the results of Jayawardena et al. (13), who could transfer protection to thymectomized, irradiated mice which had been reconstituted with T-cell-depleted bone marrow cells before adoptive immunization. This discrepancy might be interpreted to mean that *nu/nu* mice lack a cell population relevant to the development of protection, which is present not only in normal but also in thymectomized, irradiated, B-cell-reconstituted mice. From our findings, we therefore conclude that cooperation of the

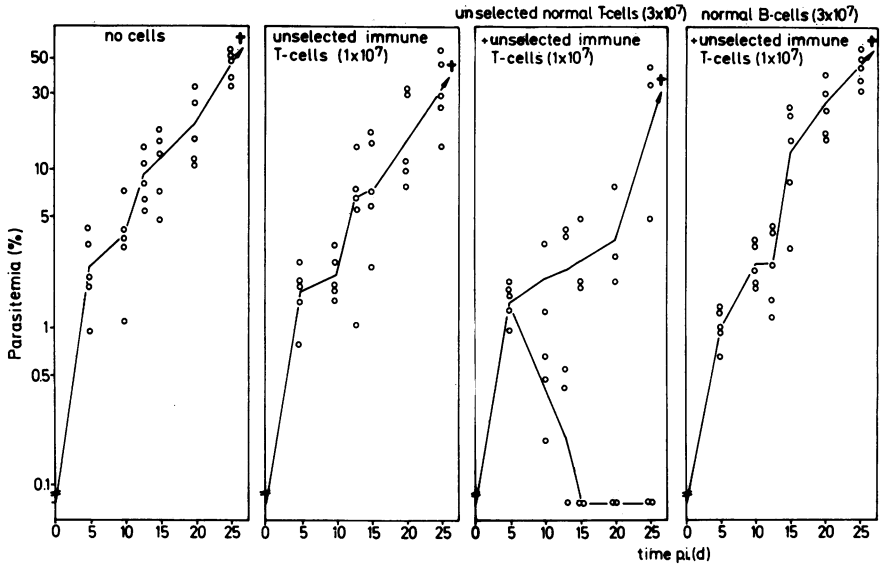


FIG. 4. Role of normal T cells in cooperation with immune T cells in protection against *P. yoelii* in congenic C57BL/6-*nu/nu* mice. C57BL/6-*nu/nu* mice received $3 \times 10^7$ normal T or B cells or no cells and 24 h later $10^7$ immune T cells. Another 24 h later, the animals were infected intraperitoneally with $10^4$ PRBCs, and parasitemia was determined in Giemsa-stained tail blood smears (each data point represents one individual mouse).

transferred immune T cells with additional resident T cells was required for mediation of optimal protection. Because cooperation between different leukocyte populations is known to be mediated by lymphokines (19), we tested the capacity of *P. yoelii*-immune Lyt T-cell subpopulations to produce lymphokines. It was found that immune mice possessed antigen-reactive $Lyt2^-$ and $Lyt2^+$ cells. However, the two T-cell subpopulations expressed differential requirements for antigen-presenting cells (Table 2). The $Lyt2^+$ T-cell subpopulation showed antigen-specific proliferation only in the presence of additional accessory cells, indicating that nylon treatment removed a quantitative or qualitative portion of antigen-presenting cells essential for the proliferation of $Lyt2^+$, but not of $Lyt2^-$, T lymphocytes. Under optimal proliferation conditions, *P. yoelii*-immune $Lyt2^-$ lymphocytes secreted high amounts of TCGF after restimulation. On the other hand, none of the selected T-cell populations secreted detectable levels of MAF upon restimulation with specific antigen. This is in accordance with our recent data (4) which show that splenic macrophages of *P. yoelii*-infected mice produce high quantities of parasite-toxic $O_2$ species preferentially during the induction phase of infection (peak values at day 3 postinfection). Also, Huang et al. (10) demonstrated production of significant levels of gamma interferon, and Okenhouse and Lustig-Shear (22) demonstrated secretion of MAF only during the first days of infection. Although immune $Lyt2^+$ T cells proliferated after restimulation in vitro, they had no influence on the course of infection. Therefore, their role in antimalarial immunity remains unclear.

From recent data (4, 10, 22) and our findings, two phases of antimalarial immunity can be distinguished: (i) the acute phase, in which activated macrophages producing high amounts of microbicidal $O_2$ metabolites (1, 4) as well as $Lyt2^-$ T cells helping to develop a humoral response by B cells are involved (5, 13); and (ii) later stages of infection or after reinfection of immune animals, in which an additional T-cell-dependent mechanism seems to become essential for the maintenance of secondary immunity. The latter assumption is supported by the finding (12) that CBA/N mice possessing functionally defective B cells are highly resistant to *P. yoelii* reinfection and by our experiments, which indicate that secondary immunity, at least in part, results from the induction of additional T-cell-dependent mechanisms by *P. yoelii*-immune $Lyt2^-$ T lymphocytes via TCGF secretion.

## LITERATURE CITED

1. **Allison, A. C., and E. M. Eugui.** 1983. The role of cell mediated immune responses in resistance to malaria, with special reference to oxidant stress. Annu. Rev. Immunol. **1**:361–392.
2. **Berthold, F., H. Kubisiak, M. Ernst, and H. Fischer.** 1981. Six-channel luminescence analyzer for phagocytosis applications, p. 699–703. *In* M. A. DeLuca und M. D. McElroy (ed.), Bioluminescence and chemiluminescence. Academic Press, Inc., New York.
3. **Beutler, E., C. West, and K. G. Blume.** 1976. The removal of leucocytes and platelets from whole blood. J. Lab. Clin. Med. **88**:328–333.
4. **Brinkmann, V., S. H. E. Kaufmann, M. M. Simon, and H. Fischer.** 1984. Role of macrophages in malaria: $O_2$-metabolite production and phagocytosis by splenic macrophages during lethal *Plasmodium berghei* and self-limiting *Plasmodium yoelii* infection in mice. Infect. Immun. **44**:743–746.
5. **Brown, K. N.** 1971. Protective immunity to malaria provides a model for the survival of cells in an immunologically hostile environment. Nature (London) **230**:163–167.
6. **Clark, I. A., and A. C. Allison.** 1974. *Babesia microti* and *Plasmodium berghei yoelii* infections in nude mice. Nature (London) **252**:328–329.
7. **Cohen, S.** 1979. Immunity to malaria. Proc. R. Soc. Lond. B Biol. Sci. **203**:323–345.
8. **Ernst, M., H. Lang, H. Fischer, M. L. Lohmann Matthes, and H. J. Staudinger.** 1981. Chemiluminescence of cytotoxic macrophages, p. 609–616. *In* M. A. De Luca und M. D. McElroy (ed.), Bioluminescence and chemiluminescence. Academic Press, Inc., New York.
9. **Fischer, H., and H. Staudinger.** 1981. Chemilumineszenz, ein Indikator für Zellaktivierung. Klin. Wochenschr. **59**:199–201.
10. **Huang, K. Y., W. W. Schultz, and F. B. Gordon.** 1968. Inter-

feron induced by *Plasmodium berghei*. Science **162**:123–124.
11. **Jayawardena, A. N.** 1981. Immune responses in malaria, p. 85–136. *In* J. Mansfield (ed.), Advances in parasitic diseases: immunology, vol. 1. Marcel Dekker Inc., New York.
12. **Jayawardena, A. N., C. A. Janeway, and J. D. Kemp.** 1979. Experimental malaria in the CBA/N mouse. J. Immunol. **23**:2532–2539.
13. **Jayawardena, A. N., D. B. Murphy, C. A. Janeway, and R. K. Gershon.** 1982. T-cell mediated immunity in malaria. I. The Ly phenotype of T cells mediating resistence to *Plasmodium yoelii*. J. Immunol. **129**:377–381.
14. **Julius, H. H., E. Simpson, and L. A. Herzenberg.** 1973. A rapid method for the isolation of functional thymus derived murine lymphocytes. Eur. J. Immunol. **3**:645–649.
15. **Kaufmann, S. H. E., M. M. Simon, and H. Hahn.** 1982. Regulatory interactions between macrophages and T cell subsets in *Listeria monocytogenes* specific T cell activation. Infect. Immun. **38**:907–913.
16. **Kindred, B.** 1977. Nude mice in immunology. Prog. Allergy **26**:137–237.
17. **Lohmann-Matthes, M. L., H. Lang, D. M. Sun, E. Kniep, and B. Kickhöfen.** 1981. Macrophage activation to cytotoxicity by the macrophage cytotoxicity (activating) factor (MCF, MAF), p. 365–386. *In* E. Pick (ed.), Lymphokines, vol. 3. Academic Press, Inc., New York.
18. **Miller, R. G.** 1980. An immunological suppressor cell inactivating cytotoxic T lymphocyte precursor cells recognizing it. Nature (London) **287**:544–546.
19. **Möller, G. (ed.).** 1982. Interleukins and lymphocyte activation. Immunol. Rev. **63**:1–29.
20. **Nathan, C. F.** 1980. The release of hydrogen peroxide from mononuclear phagocytes and its role in extracellular cytolysis, p. 1143–1164. *In* R. van Furth (ed.), Mononuclear phagocytes. M. Nijhoff, The Hague.
21. **Nathan, C. F., and A. Nakagawara.** 1982. Role of reactive oxygen intermediates in macrophage killing of intracellular pathogens; a review, p. 27–291. *In* Self defense mechanisms: role of macrophages. University of Tokyo Press, Tokyo.
22. **Okenhouse, C. F., and H. Lustig-Shear.** 1984. Oxidative killing of the intraerythrocytic malaria parasite *Plasmodium yoelii* by activated macrophages. J. Immunol. **132**:424–431.
23. **Roberts, D. W., and W. P. Weidanz.** 1979. T cell immunity to malaria in the B cell deficient mouse. Am. J. Trop. Med. Hyg. **28**:1–3.
24. **Shen, F. W., E. A. Boyse, and H. Cantor.** 1975. Preparation and use of Ly antisera. Immunogenetics **2**:591–595.
25. **Tosta, C. E., M. Sedegah, D. C. Henderson, and N. Wedderburn.** 1980. *Plasmodium yoelii* and *Plasmodium berghei*: isolation of infected erythrocytes from blood by colloidal silica centrifugation. Exp. Parasitol. **50**:7–15.