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Control of Trypanodestructive Antibody Responses and Parasitemia in Mice Infected with *Trypanosoma (Duttonella) vivax*†

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After infection with a cloned population of *Trypanosoma vivax*, C57BL/6 mice controlled parasitemia during the exponential growth phase and survived, with intermittent parasitemia, for several weeks. In contrast, most mice of the C3H/He strain did not control the first wave of parasitemia and died within 9 to 13 days after infection. Control of parasitemia in C57BL/6 mice was mediated by the production of a variant surface glycoprotein-specific trypanodestructive antibody response which was accompanied by production of antibodies against antigens shared between procyclic and bloodstream *T. vivax* as well as antibodies against trinitrophenyl (TNP) and sheep erythrocytes. The infected C3H/He mice did not produce trypanodestructive antibodies or antibodies against procyclic antigens or TNP but did produce antibodies against sheep erythrocytes. Although infected C57BL/6 mice produced levels of serum immunoglobulin M four times higher than infected C3H/He mice, their parasite-induced B-cell DNA synthetic responses were similar, and both sets of mice developed similar numbers of spleen cells with cytoplasmic immunoglobulin M, a proportion of which could react with TNP. In vitro biosynthetic labeling studies accompanied by immunoglobulin precipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated that the immunoglobulin-containing cells of infected C3H/He mice synthesized and secreted less immunoglobulin than similar cells from infected C57BL/6 mice. We concluded that some parasite-induced antibody-forming cells in C3H/He mice, perhaps including parasite-specific and certainly including TNP-specific cells, had an impaired capacity to make and release immunoglobulin. Within 24 h after Berenil-mediated elimination of *T. vivax* from infected C3H/He mice, a population of cyclophosphamide-sensitive spleen cells produced large amounts of parasite-specific and TNP-specific antibody. We concluded that the defect in terminal B-cell function leading to suppressed parasite-specific and TNP-specific antibody responses was induced either by living trypanosomes or short-lived factors from degenerating trypanosomes or by short-lived parasite-induced host responses.

The severity of infections initiated with *Trypanosoma brucei* (5, 7, 18, 21, 37) and *Trypanosoma congolense* parasites in mice (27, 28, 43) correlates with the level, duration, and frequency of parasitemic waves (4, 26). Control of the level of parasitemia is predominantly mediated by nonimmunological reactions which regulate the rate of parasite multiplication (4, 26, 29), whereas control of the duration of parasitemic waves is mediated by parasite-specific antibody responses which lead to parasite destruction (4, 26, 27). Although the molecular basis for parasite population expansion rates has been only partially resolved (4), it appears that the efficiency of parasite-specific antibody responses is regulated by the degree of trypanosome-induced immunodepression (33), which differs between hosts (5, 21, 37) and may involve the interaction of trypanosomes with macrophages (13, 34).

The isolation of rodent-infective *Trypanosoma vivax* parasites (11, 20) which retain the characteristics of the species (S. M. Mahan, Ph.D. thesis, University of Birmingham, United Kingdom, 1984) and establish infections with differing degrees of severity in different inbred strains of mice (10) has provided material to examine host responses which influence the severity of infections with *T. vivax*. In this paper we show that unlike *T. brucei* and *T. congolense*, both the level and duration of *T. vivax* parasitemic waves in mice are predominantly controlled by the efficiency of the host antibody response, which in turn is controlled by a trypanosome-induced inhibition of antibody secretion from parasite-specific and some other plasma cells.

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MATERIALS AND METHODS

Mice and serum. Three-month-old female C3H/He and C57BL/6 mice, bred and maintained at the International Laboratory for Research on Animal Diseases, were used in all studies. Blood, collected from the retro-orbital plexus of mice under ether anesthesia, was left at room temperature (21 to 23°C) for 1 h, then at 4°C for 16 h before centrifugation and collection of serum.

Trypanosomes. The cloned *T. vivax* 1223b parasites (Mahan, Ph.D. thesis) were prepared in BALB/c mice from the stock stabilate ZARIA Y486 (20) and stored in liquid nitrogen. The thawed stabilate was taken up in a phosphate-buffered saline glucose solution at pH 8.0 (19) and grown in irradiated (800 rads) C3H/He mice before use. Methods for Percoll purification of *T. vivax* parasites from blood have been described (12), and purified trypanosomes were irradiated (60,000 rads) by a standard method (38). Trypanosomes were injected intravenously (i.v.), and parasitemia was monitored by staining with Ziehl-Neelsen carbolfuchsin and counting in a hemacytometer (29).

Analyses of serum antibodies. Serum antibodies specific for *T. vivax* 1223b were measured in the trypanolysis test of Van Meirvenne et al. (41). Parasites were examined with a phase-

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contrast microscope at 250× magnification, and the serum dilution which yielded 50% parasite lysis was recorded as the endpoint. No agglutinating parasite-specific antibodies were detected. Serum antibodies specific for the hapten trinitrophenyl (TNP) were measured in a solid-phase radioimmunoassay (RIA) (40).

Quantitation of serum immunoglobulin M (IgM). IgM levels in serum were measured by the single radial immunodiffusion test of Mancini et al. (24) with sheep anti-mouse IgM (Serotec Ltd. Bicester, Oxon, United Kingdom) incorporated into the gel.

Preparation of spleen cells. Mice were killed by ether anesthesia, and spleens were excised, placed into RPMI 1640 medium (Flow Laboratories, Irvine, Ayrshire, Scotland) containing 10 μg of DNase per ml, 2 mM L-glutamine, and 10% fetal bovine serum (FBS; Flow Laboratories) (referred to as complete medium), and gently disrupted by using tissue teasers. Released cells were filtered through nylon screens (pore size, 30 μm; Swiss Silk Bolting Cloth Mg. Co. Ltd., Zurich, Switzerland) and washed twice in complete medium.

[^{125}I]Iododeoxyuridine labeling of spleen cells. Cultures of 2 × 10^5 nucleated spleen cells in 100 μl of complete medium were set up in triplicate in 96-well microtiter plates (Costar, Cambridge, Mass.). To each well, 0.5 μCi of [^{125}I]iododeoxyuridine (IM 355, Amersham, Buckinghamshire, United Kingdom) in 50 μl of complete medium was added, and cultures were incubated for 4 h at 37°C in a 5% CO_2 in air atmosphere. The cells were harvested onto filter papers by using a Titertek Cell Harvester (Skatron, Lierbyen, Norway) and dried, and the radioactivity was determined in a gamma scintillation spectrometer. The filter-bound radioactivity was associated with DNA, because when wet filters were incubated for 2 h at 37°C in RPMI 1640 medium containing 20 μg of DNase per ml or 20 μg of trypsin per ml, only the former treatment released the bound activity from the filter. The mean number of counts per minute (cpm) was prepared from results of the triplicate cultures, and the stimulation index was calculated as: (mean cpm of [^{125}I]iododeoxyuridine in spleen cells from infected mice)/(mean cpm in the same number of spleen cells from normal mice).

Fluorescence-activated cell sorter analysis. Spleen cells (2 × 10^6) were stained with an optimal concentration of affinity-purified fluorescein-conjugated rabbit anti-mouse immunoglobulin serum (Nordic, Tilberg, The Netherlands) or fluorescein-conjugated monoclonal anti-Thy-1.2 (ion-exchange chromatographically purified; Becton Dickinson, Mountain View, Calif.). Staining was done in complete medium containing 0.01% sodium azide for 40 min at 4°C. Stained cells were washed twice and analyzed on a fluorescence-activated cell sorter (FACS II, Becton Dickinson) for fluorescence intensity and forward angle (1 to 13°) light scatter. The FACS II was adjusted with scatter gates set to exclude dead cells from the analysis. This was achieved by first running cells stained with fluorescein diacetate (32). The cells were then processed at the rate of 1,000 to 1,500/s with the photomultiplier tube at 500 V. Fluorescence intensity and light scatter profiles were acquired on a linear scale by using an X-Y recorder (7040A/7041A; Hewlett Packard, San Diego, Calif.). The proportions of B and T cells in the cell preparations were calculated from fluorescence intensity histograms and were expressed as a percentage of the total cell numbers analyzed.

Analysis of cytoplasmic immunoglobulin. Isolated spleen cells were adjusted to 10^8 nucleated cells per ml of FBS, and thin films were prepared by the cover slip technique (35), air dried for 5 min at room temperature (21 to 23°C), and fixed for 10 min at −20°C in an equal volume of absolute ethanol and acetone. The cells were air dried and stained for direct immunofluorescence with an optimal concentration of fluorescein-conjugated rabbit anti-mouse immunoglobulin (Nordic) or fluorescein-conjugated monoclonal rat anti-mouse IgM (a gift from P. Lalor, Stanford University, Calif.) or for indirect immunofluorescence either with an optimal concentration of biotin-conjugated monoclonal anti-IgG2a or IgG1 (anti-Ig1a or -Ig1b; anti-Ig4a or -Ig4b) followed by fluorescein-conjugated avidin (gifts from P. Lalor) or with rabbit anti-mouse κ chain (a gift from R. Asofsky, National Institutes of Health, Bethesda, Md.; absorbed on purified IgM from the myeloma MOPC 104E) followed by fluorescein-conjugated anti-rabbit immunoglobulin (Vector Labs, Burlingame, Calif.). Stained films were examined by using a UV microscope; cells with bright generalized fluorescence were counted. For each cell preparation, two films were stained, and 500 cells were examined on each film.

Analysis of antibody activity of cytoplasmic immunoglobulin. Spleen cells were washed three times, adjusted to 10^8/ml in complete medium, and subjected to two cycles of freezing in liquid N_2 and thawing at 37°C. The samples were centrifuged at 10,000 × *g* for 10 min, and supernatants were collected and stored at −80°C. Supernatant antibodies against the hapten TNP were measured in a solid-phase RIA (40).

Analysis of antibody responses against *T. vivax* procyclic antigens. Uncoated procyclic trypanosomes were prepared by cultivation in vitro at 28°C and were provided by Robert Nelson at the International Laboratory for Research on Animal Diseases. The organisms were suspended in FBS at 10^8/ml, and thin films were prepared and fixed as described for the analysis of spleen cell cytoplasmic immunoglobulin. The trypanosomes were stained for indirect immunofluorescence. The first-step antibody was the sera prepared from blood of uninfected and *T. vivax* 1223b-infected C3H/He or C57BL/6 mice, and the second step antibody was an optimal concentration of fluorescein-conjugated rabbit anti-mouse immunoglobulin antisera (Nordic). The stained films were then examined with a UV microscope.

Analysis of sheep erythrocyte (SRBC)-specific plaque-forming cells (PFCs). Spleen cells from infected and uninfected mice were used in the analysis to detect IgM PFC against SRBC. The PFC analyses were conducted in Cunningham chambers by a standard method (14). The cells from infected mice were used unwashed since they were extremely fragile.

Biosynthetic labeling of spleen cells with L-[^{35}S]methionine, immunoprecipitation of immunoglobulin, and analysis on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Nucleated spleen cells (10^8; pooled from five mice) were cultured in vitro in 1 ml of methionine-free RPMI 1640 medium, which was supplemented with 2% dialysed FBS and 0.1 mCi L-[^{35}S]methionine (Amersham International, about 1,000 Ci/mmol). The cells were incubated at 37°C in a 5% CO_2 air atmosphere in 24-well tissue culture plates (Costar). Three hours later the cell supernatant was collected after spinning down the cells for 5 to 10 s in an Eppendorf centrifuge (Netheler + Hinz GmbH, Hamburg) at 10,000 × *g*. To the pellet, 1 ml of lysis buffer (50 ml of PBS, 50 ml of water, 0.5% sodium deoxycholate, 0.5 ml of Nonidet P-40) was added, and the cells were vortexed vigorously. The cell lysate was collected after spinning down the cellular debris for 2 min in the Eppendorf centrifuge.

Immunoprecipitation of the immunoglobulin in the cell

supernatant and cell lysate was achieved by the addition of an excess of rabbit anti-mouse IgM/G, (1 mg/ml; Nordic) antisera. The mixture was left overnight at room temperature, and the next morning 100 μl of preswollen protein A-Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) was added to each sample. The immunoprecipitated immunoglobulin was allowed to bind to the gel for 1 h at room temperature. The immunoglobulin-protein A-Sepharose 4B was washed four times (each wash was for 2 min) in washing buffer, pH 7.6 (0.05 M Tris, 0.4 M NaCl, 0.5% sodium deoxycholate, 0.5 ml of Nonidet P-40) by using the Eppendorf centrifuge. After the last wash, the buffer was carefully removed, and to each sample a volume of 100 μl of reducing gel sample buffer (62 mM Tris, 2% sodium dodecyl sulfate, 7.5% glycerol, and 5% 2-mercaptoethanol) was added to elute the bound immunoglobulin from the gel. The samples were spun down to obtain the immunoglobulin in the sample buffer, and the gel was discarded.

Each sample was boiled for 10 min on a heating block or in a water bath before loading (20 μl) onto a 10% polyacrylamide slab gel for separation by electrophoresis (23). The gels were washed, stained with Coomassie blue, saturated with salicylic acid for fluorography, and dried. Autoradiography of the dried gel was performed with Fuji X-ray film (Fuji Photo Film Co., Ltd.) which had been preflashed.

Chemotherapy. Mice were injected with the trypanocidal drug diminazene aceturate (Berenil; Hoechst AG, Frankfurt, Federal Republic of Germany). The drug was administered intraperitoneally in water at a concentration of 40 mg/kg of body weight (17). Control mice were injected with saline. Cyclophosphamide was dissolved in distilled water and injected at a dose of 150 mg/kg of body weight.

Splenectomy and sham splenectomy. Mice were anesthetized with ether and swabbed with 70% ethanol. An incision 0.5 to 1 cm long was made on the skin parallel to the last rib, and a similar incision was made through the underlying abdominal wall. The spleen was exteriorized, blood vessels were ligated by using 5/0 Mersilene (Ethicon Ltd., Edinburgh, Scotland), and the spleen was excised. The same procedure was followed for sham splenectomy, but the spleen vessels were not ligated and the spleen was not excised. The abdominal wall and skin were closed by inserting sutures with 5/0 Mersilene. The wound was sprayed with Pieti-Chloram antibiotic spray (Veto-quinol; S. A.-Magny-Vernois, Lure, France).

Presentation of results. Parasitemias were recorded as $\log_{10}$ parasites per milliliter of blood ± population standard deviation (SD). Antibody responses measured in the RIA were presented as $\log_{10}$ reciprocal titer ± SD. Trypanolytic antibody responses were presented as the reciprocal end titer ± SD. Cells containing cytoplasmic immunoglobulin were presented as the arithmetic mean ± SD. Unless otherwise stated, 10 mice were used in each experimental group.

RESULTS

C57BL/6 and C3H/He mice differed in susceptibility to infection with *T. vivax* 1223b parasites. An infectivity titration with between 5 and 10^4 *T. vivax* 1223b parasites gave identical results in C57BL/6 and C3H/He mice. The parasites were equally infective for both strains of mice, and the prepatent periods (time to detection of 10^5 parasites per ml of blood) were also the same. A few C3H/He mice infected with 10^3 or fewer *T. vivax* 1223b parasites controlled the first parasitemic wave; however, most C3H/He mice (>90%) infected with 10^3 or more parasites did not control the first parasitemic wave and died between 9 and 13 days after infection. In contrast, all C57BL/6 mice controlled the first parasitemic wave, irrespective of the infecting dose of parasites, and survived for 43 ± 19 days.

TABLE 1. Parasitemia, serum antibody responses, and changes in IgM levels in C57BL/6 and C3H/He mice infected i.v. with 10^3 *T. vivax* 1223b parasites

Mouse strain and day after infection[a]	$\log_{10}$ parasites/ml of blood (mean ± SD)	Trypanolytic antibody[b] reciprocal titer ± SD	IgM concn (mg/ml) in serum[c]
C57BL/6			
NI	NI	0	0.88
4	7.14 ± 0.23	0	NT
5	7.63 ± 1.23	NT	1.39 (1.5)
6	4.20 ± 0.40	240 ± 101	NT
7	4.10 ± 0.3	NT	8.27 (9.4)
8	5.53 ± 1.05	320 ± 0	NT
9	6.59 ± 1.44	NT	10.68 (12)
C3H/He			
NI	NI	0	1.21
4	7.48 ± 0.23	0	NT
5	7.89 ± 0.23	NT	2.28 (1.9)
6	8.00 ± 0.33	0	NT
7	8.78 ± 0.29	NT	2.06 (1.7)
8	8.91 ± 0.32	0	NT
9	8.78 ± 0.82	NT	4.02 ± 1.12 (2.4–3.3)

[a] NI, Not infected.

[b] Antibodies reacted with *T. vivax* 1223b but not *T. vivax* 10E25 parasites, both of which were derived from stock stabilate ZARIA Y486. NT Not tested.

[c] Sera from groups of 10 mice were pooled, except in the case of strain C3H/He, day 9, in which sera were tested individually from mice which survived to this time point. Fold increase above level in noninfected sera is given in parentheses. Little or no change was observed in serum IgG levels in either mouse strain.

The capacity to control parasitemia correlated with the efficiency of the parasite-specific antibody response. After i.v. inoculation with 10^3 *T. vivax* 1223b parasites, C57BL/6 and C3H/He mice had identical levels of peripheral blood parasitemia for 5 days. Thereafter the parasitemic wave was controlled by C57BL/6 mice, and parasite variant-surface glycoprotein (VSG) specific antibody responses were detected in the serum. In contrast, the first parasitemic wave was not controlled, nor were parasite VSG-specific antibody responses detected in the serum from the C3H/He mice (Table 1). Despite the fact that the C3H/He mice did not mount parasite-specific antibody responses, a threefold increase in serum IgM levels was recorded by day 9 postinfection (Table 1). The IgM levels in the sera of infected C57BL/6 mice were raised to 12-fold by the same time after infection, and in these mice there was a more rapid increase in serum IgM levels (Table 1). Sera from the two strains of mice were analyzed by immunofluorescence assay for antibodies against antigens shared between bloodstream and procyclic *T. vivax* organisms and by solid-phase RIA for antibodies against the bystander antigen TNP. In addition, SRBC-PFC assays were conducted with spleen cells from infected C57BL/6 and C3H/He mice. It was found that the C57BL/6 mice mounted antibody responses against shared bloodstream and procyclic *T. vivax* antigens, SRBC, and TNP. The C3H/He mice mounted responses against SRBC, and only one of five C3H/He mice mounted antibody responses against shared bloodstream and procyclic *T. vivax* antigens on day 9 postinfection. No C3H/He mice produced antibodies against TNP (Table 2). The PFC response against

TABLE 2. *T. vivax* 1223b-induced antibody responses in C57BL/6 and C3H/He mice

Mouse strain and day after infection[a]	No. of SRBC-specific IgM PFC ± SD/5 × 10^5 spleen cells[b]	Log_{10} reciprocal titer of IgM anti-TNP[c] ± SD	Anti-*T. vivax* procyclic antigen[d] reciprocal titer ± SD
C57BL/6			
NI	1 ± 0	0	0
4	4 ± 3	0	NT
5	NT	NT	NT
6	3 ± 2	1.8 ± 0.2	NT
7	NT	NT	6.2 ± 3.4
8	9 ± 3	3.8 ± 0.4	NT
9	6 ± 3	NT	20 ± 0.0
C3H/He			
NI	2 ± 1		0
4	14 ± 5	0	NT
5	NT	NT	NT
6	12 ± 2	0	NT
7	NT	NT	0
8	11 ± 6	0	NT
9	11 ± 5	NT	1 ± 2.0

[a] NI, Not infected.
[b] Spleen cells were collected from five mice at each time point. NT, Not tested.
[c] Sera were collected from ten mice at each time point. Values were corrected for preinfection antibody levels, which were 0.9 ± 0.2 for C57BL/6 and 1.3 ± 0.1 for C3H/He mice.
[d] Sera were collected from five mice at each time point and tested by indirect fluorescence assay individually. Mice were infected with 10^3 *T. vivax* 1223b parasites i.v.

SRBC, although slight, was greater in the infected C3H/He than in the C57BL/6 mice, and the sizes of the plaques were similar in the two strains of mice.

C3H/He and C57BL/6 mice mounted antibody responses against irradiated *T. vivax* 1223b parasites. To examine whether C57BL/6 and C3H/He mice had intrinsically different capacities to respond to *T. vivax* 1223b antigens, the mice were inoculated with lethally irradiated parasites. After i.v. inoculation with 10^8 irradiated *T. vivax* 1223b parasites, C3H/He mice produced similar levels of parasite-specific and somewhat lower levels of TNP-specific antibodies in comparison with C57BL/6 mice (Table 3).

T and B cells from C3H/He and C57BL/6 mice infected with *T. vivax* 1223b parasites underwent activation. Spleen cells collected from infected mice were incubated with [^{125}I]iododeoxyuridine, and the amount of radioisotope incorporated was compared with that of spleen cells from uninfected animals. DNA-synthetic responses were of greater magnitude in C3H/He than in C57BL/6 mice infected with *T. vivax* 1223b parasites (Table 4). In C57BL/6 mice, the uptake of the label increased with the parasitemia and decreased after parasitemic wave remission.

To ascertain that the uptake of the label was by activated B or T cells or both cell types, the spleen cells from the infected C3H/He and C57BL/6 mice were stained with either fluorescein-conjugated anti-mouse immunoglobulin for detecting B cells or anti-mouse Thy-1.2 reagents for T cells and then analyzed on the FACS II for changes in fluorescence intensities and capacity to scatter incident light. These parameters correlate with cell activation (15, 42). In both strains of mice the levels of surface immunoglobulin or Thy-1.2 on spleen cells decreased and the light-scattering capacity of the immunoglobulin-positive or Thy-1.2$^+$ cells increased after infection with *T. vivax*; i.e., both B and T cells were induced to blast during infection of C3H/He and C57BL/6 mice. No differences were noted in the kinetics with which these events occurred in both strains of mice.

Experiments were conducted to examine the stage of activation of the B-cell populations in *T. vivax*-infected C57BL/6 and C3H/He mice. Spleen cells from both C3H/He and C57BL/6 mice which had been infected with 10^3 *T. vivax* 1223b parasites were analyzed by immunofluorescence for the proportion of cells which contained cytoplasmic immunoglobulin and, after cell lysis, for the presence of supernatant immunoglobulin which could bind to TNP or to the surface coat of the *T. vivax* parasites. A similar dramatic increase in splenic cytoplasmic immunoglobulin-positive (cIg$^+$) cells was detected in both C57BL/6 and C3H/He mice (Table 4). The cells predominantly contained IgM heavy chains and κ light chains (data not shown). Lysates prepared from these cells contained antibodies specific for TNP. The concentration of parasite surface coat-specific antibodies in the spleen cell lysates was too low to be detected by the

TABLE 3. C57BL/6 and C3H/He mice mount parasite-specific and TNP-specific antibody responses after inoculation with 10^8 irradiated[a] *T. vivax* 1223b

Mouse strain and day after immunization	Trypanolytic antibody[b] reciprocal titer ± SD	Log_{10} reciprocal titer of IgM anti-TNP ± SD
C57BL/6		
0	0	1.7 ± 0.2
4	9 ± 6	3.8 ± 0.1
6	10 ± 0	3.7 ± 0.7
8	30 ± 10	2.8 ± 0.1
C3H/He		
0	0	2.3 ± 0.1
4	12 ± 4	3.2 ± 0.1
6	16 ± 5	2.8 ± 0.1
8	22 ± 10	2.8 ± 0.1

[a] Parasites were subjected to 60,000 rads and were viable but noninfective when injected.
[b] Antibodies reacted with *T. vivax* 1223b but not *T. vivax* 10E25 parasites, both of which were derived from stock stabilate ZARIA Y486.

immune lysis test. As yet, *T. vivax* VSG has not been purified and hence more sensitive RIAs or enzyme-linked immunosorbent assays could not be performed to look for VSG-specific antibodies in the lysates. It was also not possible to conduct an RIA or an enzyme-linked immunosorbent assay with whole *T. vivax* bloodstream parasites because the *T. vivax* organisms were very fragile and disrupted when washed to remove contaminating immunoglobulin after isolation from mouse blood on Percoll gradients.

Spleen cells of C57BL/6 mice synthesized and secreted more immunoglobulin than cells of C3H/He mice during *T. vivax* 1223b infections. The lack of production of serologically detectable parasite VSG- or TNP-specific antibody responses in the *T. vivax*-infected C3H/He mice was not due to a general failure of induction of B-cell DNA synthetic responses, or due to a failure in development of cIg^+ (including some demonstrably TNP-specific) cells in the spleen of these mice. These data indicated that the immunological defect in the infected C3H/He mice might be related to a control of immunoglobulin secretion from some parasite-induced antibody-containing cells. To examine this possibility, immunoglobulin biosynthesis and secretion by the spleen cells of infected C57BL/6 and C3H/He mice were compared. Nucleated spleen cells (10^8; pooled from five mice per group) were cultured in vitro in the presence of L-[^{35}S]methionine for 3 h, and immunoglobulin was precipitated from cell pellets and supernatants and examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

During the critical stage of the infection in C3H/He mice, i.e., day 7 to 9 after infection, less immunoglobulin (μ, γ, and light chains) was synthesized and secreted by the cells of infected C3H/He mice than by the cells of the infected C57BL/6 mice (Fig. 1). The cells of infected C3H/He mice, however, clearly had raised levels of immunoglobulin synthesis and secretion compared with cells of normal C3H/He mice.

TABLE 4. Lymphocyte activation and B-cell development in C57BL/6 and C3H/He mice infected with *T. vivax* 1223b parasites

Mouse strain and day after infection	SI of pooled cells[a]	% cIg^+ spleen cells[b]	Log_2 reciprocal titer of cytoplasmic IgM anti-TNP[c] ± SD
C57BL/6			
0	1.0	1.0	0
3	3.6	2.2	NT
4	NT	1.6	NT
5	7.3	2.2	NT
6	NT	4.8	2.6 ± 0.6
7	5.0	13.4	2.9 ± 0.3
8	NT	9.6	NT
9	7.8	14.0	NT
C3H/He			
0	1.0	1.0	0
3	6.0	0.6	NT
4	NT	2.8	NT
5	8.7	3.2	NT
6	NT	5.2	2.1 ± 0.6
7	20.0	10.0	2.7 ± 0.4
8	NT	12.0	NT
9	20.6	11.0	NT

[a] NT, Not tested; SI, stimulation index.

[b] Spleen cells were pooled from five mice for preparation of smears. For analysis of cIg^+ cells, 500 cells were counted on each slide. The proportion of immunoglobulin-positive and Thy-1.2$^+$ spleen cells remained relatively unchanged during the 9 days of infection (Mahan, Ph.D. thesis): for C3H/He, day 0, 60% immunoglobulin-positive and 25% Thy-1.2$^+$ cells, and day 9, 55% immunoglobulin-positive and 30% Thy-1.2$^+$ cells; for C57BL/6, day 0, 50% immunoglobulin-positive and 31% Thy-1.2$^+$ cells, and day 9, 65% immunoglobulin-positive and 29% Thy-1.2$^+$ cells.

[c] Assayed in solid-phase RIAs with the clarified supernatant of spleen cells from five mice per group which were subjected to two cycles of freezing and thawing.

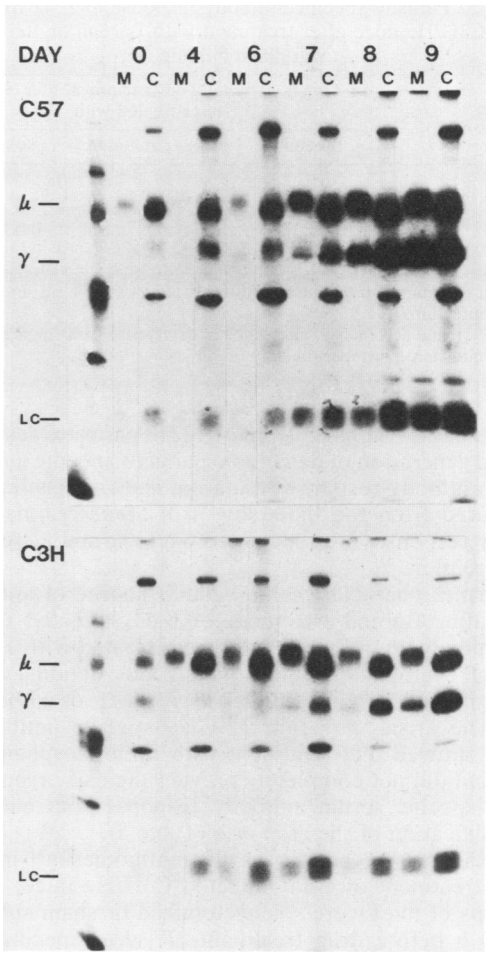

FIG. 1. Biosynthesis and secretion of immunoglobulin from spleen cells of *T. vivax* 1223b-infected C57BL/6 and C3H/He mice. Spleen cells were prepared from uninfected C57BL/6 and C3H/He mice and from mice (in groups of five) which had been infected i.v. 4, 6, 7, 8, or 9 days earlier with 10^3 *T. vivax* 1223b parasites. The cells were cultured for 3 h at 37°C (10^8 cells per ml of medium) in methionine-free RPMI 1640 medium supplemented with 2% dialyzed FBS and 0.1 mCi L-[^{35}S]methionine. Immunoglobulin was precipitated from cells and cell supernatants and solubilized in the presence of 5% 2-mercaptoethanol, and electrophoresis was performed on 10% sodium dodecyl sulfate-polyacrylamide gels. The left track is of ^{14}C-labeled standards: myosin (200 kDa), phospholipase B (95 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), and methylated lysozyme (14.3 kDa). Day of infection is indicated at the top of the figure. M, Supernatant (medium) immunoglobulin; C, cell-associated immunoglobulin; μ, IgM heavy chain; γ, IgG heavy chain; LC, light chain. The gel was saturated with salicylic acid before drying, and autoradiography was performed on preflashed Fuji X-ray film.

Splenic antibody responses after Berenil or Berenil and cyclophosphamide treatment of C3H/He mice infected with *T. vivax* 1223b parasites. Administration of the trypanocidal drug Berenil to C3H/He mice which had been infected 5, 6,

TABLE 5. Parasite-specific antibody response in *T. vivax* 1223b-infected C3H/He mice after treatment with Berenil or Berenil and cyclophosphamide

Time posttreatment (h)	Trypanolytic antibody reciprocal titer ± SD in mice treated with:	
	Berenil[a]	Berenil and cyclophosphamide[b]
10	0	0
16	35 ± 26	3.6 ± 3.9
48	252 ± 205	0.4 ± 0.5
72	520 ± 240	0

[a] Berenil was administered intraperitoneally at a dose of 1 mg per mouse on day 6 postinfection.
[b] Berenil (l mg per mouse) and cyclophosphamide (150 mg/kg of body weight) administered intraperitoneally on day 6 postinfection.

or 7 days earlier with 10^3 *T. vivax* 1223b parasites resulted in the rapid generation of detectable parasite-specific and TNP-specific antibody responses (data not shown). The parasites disappeared from the blood by 10 h after treatment, and antibody responses were detected 6 h later and increased in titer thereafter.

To further characterize the cellular source of antibodies arising after Berenil treatment of 6-day-infected C3H/He mice, animals (in groups of five) were treated with a combination of cyclophosphamide, which kills dividing cells of host origin, and Berenil, which kills *T. vivax*, or with Berenil alone. The arisal of serum *T. vivax*-specific antibody responses showed that treatment with cyclophosphamide inhibited but did not completely prevent the early recovery of parasite-specific serum antibody responses but eliminated the amplification of the response (Table 5).

To examine the source of the antibodies arising after Berenil treatment of 6-day-infected C3H/He mice, animals (in groups of five) were splenectomized or sham splenectomized 2 h before drug treatment. *T. vivax*-specific serum antibodies were assayed 16, 24, and 48 h later. Spleen cells were essential for the generation of the rapid parasite-specific antibody response in Berenil-treated C3H/He mice (Table 6).

DISCUSSION

Mice of the C57BL/6 strain controlled parasitemia more efficiently and survived for a longer time than mice of the C3H/He strain after infection with *T. vivax* 1223b parasites. The superior capacity of infected C57BL/6 mice to control parasitemia was not related to a capacity to inhibit infection or to control the initial phases of parasite population expansion. The parasites were equally infective for both strains of mice, had similar prepatent periods in both strains of mice, and parasitemia increased at a similar rate postpatency in both strains of mice. Similar results have been obtained by De Gee et al. (10) with *T. vivax* parasites of the same strain and C57BL/6 and BALB/c mice as recipients.

The capacities of infected C57BL/6 mice to control parasitemia was correlated with their ability to mount a serologically detectable parasite surface antigen-specific antibody response, an anti-*T. vivax* internal antigen-specific antibody response, a bystander TNP-specific antibody response, and a bystander splenic PFC response against SRBC. This was accompanied by a 12-fold increase in total serum IgM levels. We do not know the specificity of all of the *T. vivax*-induced antibody responses which led to this 12-fold increase in total serum IgM levels. The presence of anti-TNP antibodies, which do not cross-react with trypanosomes (38; S. J. Black, unpublished data), together with the large proportion of activated B cells in the C57BL/6 mice, suggests that some of the response might result from trypanosome-induced polyclonal B-cell activation (39). It should be noted, however, that most if not all mice kept under standard laboratory conditions have a detectable titer of low-affinity serum antibodies against TNP. It is therefore possible that environmental antigens cross-reacting with TNP were present in the *T. vivax*-infected mice and that the parasite acted as an adjuvant for these antigens. Similarly, although the presence of trypanosome-induced heterophile antibodies against SRBC and various other erythrocyte membrane antigens in trypanosome-infected animals has also been taken as evidence for trypanosome-induced polyclonal B-cell activation (39), we found that some if not all of these antibodies cross-react with trypanosomes including *T. vivax*, *T. congolense*, *T. brucei brucei*, *T. brucei rhodesiense* and *Trypanosoma evansi* (C. N. Sendashonga and S. J. Black, unpublished data). The nature of the cross-reacting antigens is not yet known, but monoclonal antibodies which react with erythrocytes and trypanosomes have been raised by fusing spleen cells from trypanosome-infected BALB/c mice with myeloma cells. It is therefore premature to speculate on how much, if any, of the antibody response induced by *T. vivax* or other African trypanosomes is a result of a trypanosome-derived B-cell polyclonal activator.

In contrast to *T. vivax*-infected C57BL/6 mice, neither parasite-specific nor bystander anti-TNP responses were detected in the serum of infected C3H/He mice, although these mice mounted similar SRBC-PFC responses to infected C57BL/6 mice. Furthermore, total serum IgM levels, although raised relative to normal C3H/He mice, were fourfold lower in the infected C3H/He mice than in the infected C57BL/6 mice.

The results described above are similar to those obtained in susceptible and resistant inbred strains of mice infected with *T. brucei* parasites (5, 21). Black et al. (5) showed that C3H/He mice infected with a pleomorphic strain of *T. brucei brucei* parasites either produce a weak (5) or do not produce any serologically detectable parasite surface coat-specific antibody response and do not eliminate the first parasitemic wave. Levine and Mansfield (21) also showed that C3H/He mice infected with *T. brucei rhodesiense* parasites do not mount a detectable antibody response against the parasite surface coat and do not eliminate the first parasitemic wave. The animals do, however, mount a weak but detectable serum antibody response against trypanosome common antigens. This latter result was taken to show a selective bias in suppression of antibody responses in the *T. brucei rhodesiense*-infected C3H/He mice, although it could conceivably be interpreted to result from a selective in vivo absorption of

TABLE 6. Influence of splenectomy[a] on recovery of antibody responses after Berenil treatment of 6-day *T. vivax* 1223b-infected C3H/He mice

Time posttreatment[b] (h)	Trypanolytic antibody reciprocal titer ± SD[c]	
	Sham splenectomized mice	Splenectomized mice
0	0	0
16	32 ± 10	0
24	288 ± 206	2 ± 2
48	320 ± 0	14 ± 13

[a] Conducted 2 h before drug administration.
[b] Berenil, administered at a dose of 1 mg per mouse.
[c] Titers for untreated mice were 0 at all times posttreatment.

surface coat-specific antibody which was of too low a titer to cause parasite elimination. In the case of the *T. vivax* studies reported above, we were unable to detect parasite surface antigen-specific antibodies in the serum of infected C3H/He mice by a trypanolysis test. We were also unable in most mice to detect antibodies against *T. vivax* internal antigens shared between bloodstream and procyclic organisms. We were, however, able to detect a slight splenic IgM PFC response against SRBC. As discussed above, we consider that this response may well be induced by a trypanosome common antigen and hence consider that the infected C3H/He mice may be induced to mount some degree of an antibody response against the infecting trypanosome.

After *T. congolense* infection of susceptible and resistant inbred strains of mice, parasite-specific responses and TNP-specific bystander responses were always detected in serum of resistant mice, and these mice also had elevated serum IgM levels (27). Some susceptible mice infected with *T. congolense* were also able to control first-wave parasitemia and mounted parasite-specific and other antibody responses. However, the majority behaved like susceptible C3H/He mice infected with *T. vivax* or *T. brucei* parasites, and neither produced detectable parasite-specific antibody responses nor controlled first-wave parasitemia (27).

It appears from the above that elimination of *T. vivax* parasitemia, like that of *T. brucei* and *T. congolense*, is likely to be antibody mediated. There are, however, important differences in the role played by antibody in controlling *T. brucei*, *T. congolense*, and *T. vivax* parasitemia. Antibody control of *T. brucei* parasitemia occurs after most organisms have differentiated to nondividing forms (1, 2, 5, 38) and hence when parasite population expansion in the blood is no longer exponential. A similar phenomenon has been observed with *T. congolense* (26, 43). In contrast, antibody control of parasitemia in C57BL/6 mice infected with *T. vivax* organisms occurred during the exponential phase of parasite population increase in the blood. The capacity of antibody responses to control exponentially growing *T. vivax* parasites but not *T. brucei* parasites might relate to the respective abilities of the parasites to stimulate antibody responses, the respective capacities of the parasites to eliminate antibody from their surface (3, 25, 36), and/or the absolute amounts of antibody needed to opsonize or lyse the two species of organisms.

Although C3H/He mice infected with *T. vivax* 1223b parasites did not produce detectable parasite surface antigen-specific antibody responses, the mice did mount these responses when stimulated with irradiated *T. vivax* 1223b parasites. This observation is again analogous to results obtained with *T. brucei* parasites (5, 6) and *T. congolense* parasites (26, 27, 43), where it was concluded (5, 6) that parasite surface antigen-specific and bystander TNP-specific responses were somehow suppressed in susceptible mice during the course of an ongoing infection but not when the mice were stimulated with irradiated organisms.

Whereas detectable parasite surface antigen-specific antibody responses were absent in infected C3H/He mice and overall immunoglobulin responses were low in comparison with infected C57BL/6 mice, parasite-induced splenic B- and T-cell DNA synthetic responses were detected in both strains of mice and were of greater magnitude in the infected C3H/He mice. Furthermore, similar numbers of IgM-containing cells were found to arise in the spleens of *T. vivax*-infected C3H/He and C57BL/6 mice. A proportion of these plasma cells contained functional antibody which was able to bind to the hapten TNP. It was unfortunately not possible to measure parasite-specific antibodies in lysates from either C57BL/6 or C3H/He spleen cells. In vitro biosynthetic labeling studies with L-[^{35}S]methionine followed by immunoprecipitation with anti-immunoglobulin demonstrated that parasite-induced antibody-containing spleen cells from infected C3H/He mice were less able to synthesize and secrete immunoglobulin than similar cells from C57BL/6 mice, perhaps accounting for the lower efficiency of the antibody responses in the *T. vivax*-infected C3H/He mice. Similar results were obtained with spleen cells from *T. brucei*-infected C3H/He mice (6). In the case of *T. vivax*-infected C3H/He mice, it is interesting to note that the few *T. vivax*-induced splenic SRBC-specific PFC which did arise appeared to be of similar size to SRBC-PFC in C57BL/6 mice, indicating that some immunoglobulin-containing cells in the infected C3H/He mice secreted normal amounts of antibody. Perhaps this can be taken as an indication that other immunoglobulin-containing cells in the infected C3H/He mice produced little or no antibody. If this is true, it follows that *T. vivax*-induced suppression of antibody secretion is clonotypic, is restricted to an as-yet-undefined B-cell subset, only occurs in certain splenic environmental niches, or includes some combination of these factors.

The mechanism leading to the apparent inhibition of secretion of parasite and TNP-specific antibodies from antibody-containing cells in C3H/He mice infected with *T. vivax* 1223b parasites is unknown. However, splenic plasma cells which produced large amounts of parasite-specific or TNP-specific antibodies arose from a cyclophosphamide-sensitive and hence proliferating (16) cell pool rapidly after parasite elimination from C3H/He mice with the trypanocidal drug Berenil. We do not know whether the antibody-secreting cells which arose after Berenil treatment originally were present in the cIg$^+$ nonsecreting population. Parasites were cleared from the blood by 10 h after treatment, and serum antibody responses were detected 6 h later. Treatment with Berenil has also shown to rapidly (within 48 h) reverse parasite-induced disruption of splenic red and white pulp areas in C3H/He mice infected with *T. vivax* (Mahan, Ph.D. thesis) or in mice infected with *T. congolense* (31) and to alleviate trypanosome-induced immunodepression in mice infected with *T. congolense* (31) or *T. brucei* (30). Therefore, it is likely that splenic disorganization, immunodepression, and inhibition of antibody secretion resulting from infection with *T. vivax*, *T. brucei*, or *T. congolense* are maintained by exposure of the host to living trypanosomes or short-lived components of degenerating organisms.

It was recently reported by Cox et al. (9) that rats infected with *T. rhodesiense* parasites have plasma factors which can inhibit antibody secretion by SRBC-specific plasma cells. These authors postulate that the active factors might be antigen-antibody complexes or complement-fixing soluble immune complexes which bind to plasma cells and act as a target for immunoconglutinin. While our studies do not exclude this mechanism of plasma cell blockade in C3H/He mice infected with *T. vivax* 1223b, we consider it unlikely because some normal immunoglobulin-secreting cells (e.g., SRBC-specific PFC) were detected in the spleens of *T. vivax*-infected C3H/He mice and recovery of parasite-specific antibody responses was rapid after chemotherapy. Other studies have shown that mice can be immunodepressed by the administration of trypanosome membrane or membrane components (8, 37) which induce, over a period of 24 to 48 h, suppressor macrophage activity (44). Once again, the incapacity of *T. vivax*-infected C3H/He mice to

synthesize antibody may not be related to this form of immunodepression, because antibody responses rapidly recovered after drug-mediated trypanosome destruction, i.e., at a time when a large number of organisms had been destroyed and when the host was exposed to a quantity of trypanosome membrane equivalent to the total trypanosome load in the body. This was likely to be between 5×10^8 and 10^9 organisms and far exceeds the amount of trypanosome membrane used by other investigators (8, 34, 44) to elicit immunodepression.

We have no information on the actual mechanism leading to the development of parasite-specific and TNP-specific plasma cells with diminished capacity to synthesize and secrete immunoglobulin in *T. vivax*-infected C3H/He mice. Preliminary studies with *T. brucei*-infected C57BL/6 mice, however, demonstrates that a similar phenomenon can arise in the second and third parasitemic waves (S. M. Mahan, L. Hendershot, and S. J. Black, unpublished data). Curiously, the phenomenon appears to cycle in the infected C57BL/6 mice with phases of depressed immunoglobulin production followed by periods of recovery, without any detectable cycling in the proportion of cIg^+ cells. This observation suggests that the trypanosome-induced suppression is also subject to some form of contrasuppression. Resolution of the mechanisms involved requires that more information be collected on the surface phenotypes and intracellular morphology of secreting and nonsecreting plasma cells in infected mice and that the phenomenon can be reproduced in vitro. We are conducting investigations in these areas.

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