

THE ROLE OF L3T4⁺ AND LYT-2⁺ T CELLS IN THE IgE RESPONSE AND IMMUNITY TO *Nippostrongylus brasiliensis*^{1,2}

ILDY M. KATONA,^{3*} JOSEPH F. URBAN, Jr.,[†] and FRED D. FINKELMAN[‡]

From the Departments of *Pediatrics and †Medicine, F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814; and the ‡United States Department of Agriculture, Agricultural Research Service, Animal Parasitology Institutes, Beltsville, MD 20705

The role of L3T4⁺ and Lyt-2⁺ T cells in protective immunity to *Nippostrongylus brasiliensis* (Nb) was studied in BALB/c mice that were depleted of either the L3T4⁺ or Lyt-2⁺ T cell population by injection with rat mAb specific for the appropriate determinant. Host responses to Nb infection including spontaneous elimination of adult worms, development of intestinal mucosal mast cell hyperplasia and the generation of a polyclonal IgE response were all completely blocked by 0.5 mg anti-L3T4 antibody administered simultaneously with Nb inoculation. However, administration of 0.5 mg of anti-Lyt-2 antibody at the same time and 7 days after inoculation with Nb had no effect on any of these responses. Injection of anti-L3T4 antibody as late as 9 days after Nb inoculation interfered with spontaneous cure of Nb infection and anti-L3T4 antibody injection 11 days after Nb inoculation inhibited serum IgE levels measured on day 13 by 50%. In addition, administration of anti-L3T4 antibody at the time of the peak serum IgE response, 13 days after Nb inoculation, accelerated the decline in serum IgE levels. Injection of previously Nb-infected mice with anti-L3T4 antibody at the time of a second Nb inoculation prevented the development of a secondary IgE response but did not affect immunity to Nb infection based on finding no adult worms in the intestines of these mice. These data indicate that 1) L3T4⁺ T cells are required for spontaneous cure of Nb infection, development of intestinal mucosal mast cell hyperplasia, and the generation and persistence of an IgE response during primary infection with Nb and 2) L3T4⁺ T cells are required for a considerable time after inoculation for optimal development of these responses. However, L3T4⁺ T cells are not required for all protective responses in

immune mice. In contrast, our data indicate that considerable depletion of the Lyt-2⁺ T cell population has no significant effect on either worm expulsion or the generation of serum IgE responses.

Intestinal helminthic infections of rodents lead to the development of intestinal mastocytosis, eosinophilia, and an IgE response (1-10). Primary infections of normal mice are generally self-limiting because adult worms are eliminated spontaneously from the intestines (spontaneous cure) (11). The protective immune responses induced by helminths are dependent on T lymphocytes; no IgE secretion or intestinal mast cell response is seen in parasite-infected congenitally athymic (nude) mice, and these mice, when infected, chronically harbor adult worms in their intestines (12-14). The function of T cell subpopulations in the spontaneous cure of adult worms, however, is less clear. Partial immunity against *Trichinella spiralis* can be transferred in vivo with immune T cells. This immunity is dependent on the presence of L3T4⁺ but not on the presence of Lyt-2⁺ T lymphocytes (15). Class II MHC restricted L3T4⁺ "helper/inducer" T cells regulate IgE responses by at least two mechanisms: 1) they secrete IgE binding factors, which, depending on their degree of glycosylation, can stimulate or suppress IgE responses (16, 17); and 2) they can secrete the lymphokine, IL-4 (18), which has a stimulatory effect on IgE responses (19, 20). Both IL-4 and another T cell-produced lymphokine, IL-3, have been implicated in the development of intestinal mastocytosis (21-26).

Herein we have used mAb that kill L3T4⁺ or Lyt-2⁺ T cells in vivo to examine IgE production, IMMC⁴ hyperplasia, and spontaneous cure in mice infected with the nematode parasite Nb. The results indicate that L3T4⁺ T cells play an important role in spontaneous cure of a primary infection with Nb, but T cells are not required to prevent the establishment of a secondary intestinal infection with Nb. T cell help is required in Nb-infected mice throughout the period of infection and until IgE secretion has commenced to generate a maximum IgE response. Furthermore, the continued presence of helper T cells is necessary for the persistence of high serum IgE levels after parasite elimination.

MATERIALS AND METHODS

Animals. Six-week-old female BALB/c and male C57Bl/mice were purchased from The Jackson Laboratory, Bar Harbor, ME and were

⁴ Abbreviations used in this paper: IMMC, intestinal mucosal mast cell; GaMe, affinity purified goat antibody specific for mouse IgE; MLN, mesenteric lymph node; Nb, *Nippostrongylus brasiliensis*; s, surface; VCU, villous-crypt unit.

Received for publication September 11, 1987.

Accepted for publication January 25, 1988.

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

¹ This work was supported by the Uniformed Services University of the Health Sciences, Protocols C08634 and R08308, the Agricultural Research Services, CRIS Unit 1205-20493-013, and the National Institutes of Health Grant R01-A121328.

² The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the DOD or the USUHS or the Department of Agriculture. The experiments reported herein were conducted according to the principles set forth in *The Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, DHEW Pub. No. (NIH) 78-23.

³ Address correspondence and reprint requests to Dr. Ildy M. Katona, Department of Pediatrics, USUHS, Bethesda, MD 20814-4799.

used at 8 to 20 wk of age.

Parasites. Nb (the United States National Museum Helminthological Collection no. 79845, specimens on file at the United States National parasite Collection, Beltsville, MD), that had been adapted to mice, were maintained and passed in our laboratory in C57BL/6 mice (9). BALB/c mice were inoculated s.c. with 700 third-stage larvae of Nb and were killed 13 days after inoculation (primary Nb infection). Secondary Nb infections were examined in BALB/c mice that had received an inoculation with 700 third-stage larvae and were re-inoculated s.c. with 700 third stage larvae 29 to 42 days after the first inoculation. Mice were killed 7 days after re-inoculation.

T cell specific mAb. Hybridomas that secrete the rat IgG2b mAb GK 1.5 (anti-L3T4a) (27) and 2.43 (anti-Lyt-2) (28) were a gift of Dr. Frank Fitch, University of Chicago, Chicago, IL. The hybridomas were grown i.p. in pristane primed nude mice, and the antibodies were purified from ascites by $(\text{NH}_4)_2\text{SO}_4$ precipitation, DEAE-cellulose ion exchange chromatography and Sephadex G200 gel filtration. Mice received 0.5 mg of the appropriate purified antibody by i.v. injection at times indicated in the tables for different experiments.

Analysis of cell number. Single cell suspensions of MLN and spleens were depleted of erythrocytes with ammonium chloride lysing buffer (M.A. Bioproducts, Walkersville, MD) and cell numbers were determined with a Coulter Counter (9).

Immunofluorescence staining for cell surface Ag. Single cell suspensions prepared from MLN or spleens were stained with FITC-labeled antibodies. When the staining reagent was an intact Ig molecule, the rat IgG2a anti-mouse B lymphocyte IgFcR MaB, 24G2 (29) was included to prevent FcR binding. For IgER binding studies MLN or spleen cells were incubated with saturating concentrations of DIBADL (bis (α -bromacetyl- ϵ -DNP-lys-pro) $_2$ ethylenediamine) cross-linked IgE dimers (2×10^{-7} M) (30). To quantitate bound IgE, cells incubated with IgE dimers were stained with FITC-labeled affinity purified goat antibody specific for mouse IgE. Cells were then analyzed for surface fluorescence by flow microfluorometry with a FACS II (Becton Dickinson FACS Systems, Sunnyvale, CA). The percentages of specifically stained cells and the median fluorescence intensities (median brightness) of positive cells were determined (30).

Antibodies. The preparation of F(ab') $_2$ fragments of RaMc was previously described (9). Intact molecules of affinity purified goat anti-mouse IgE were prepared as was described for RaMc (9). Techniques for the preparation of F(ab') $_2$ fragments of rabbit anti-mouse IgD, rabbit anti-mouse IgM, and a control rabbit antibody have been described (31). The anti-I-A ^{d} mAb MKD6 (32) was a gift of Dr. James J. Mond, Uniformed Services University of the Health Sciences, Bethesda, MD. FITC-labeled anti-Thy-1.2 antibody was purchased from Becton Dickinson, Mountain View, CA. CBPC-101 a monoclonal mouse IgG2a that lacks known antibody activity was a gift of Dr. Michael Potter, National Institutes of Health, Bethesda, MD. Antibodies were labeled with FITC (Calbiochem-Behring, San Diego, CA) to molar ratios of 2.0 to 4.7.

Fluorescence staining for intracytoplasmic IgE. Aliquots of MLN or splenic mononuclear cells were mixed with chicken erythrocytes at a 250/1 ratio and were centrifuged onto glass slides with a Shandon Southern cytocentrifuge. Slides were air-dried, fixed in methanol, and stained with FITC-labeled F(ab') $_2$ of RaMc. The number of intracytoplasmic IgE $^+$ cells/100 chicken cells (25,000 lymphoid cells) was determined by fluorescence microscopy using a Leitz Ortholux phase contrast/fluorescence microscope (33).

Determination of serum IgE levels. Serum IgE levels were determined by ELISA (33).

Determination of intestinal worm burden. The small intestines of infected mice were split in warm saline and incubated at 37°C for 2 to 4 h. Intestinal tissue was then removed, and the number of adult worms was determined by inspection under a dissecting microscope.

Quantitation of IMMC. Pieces of small intestines (5 to 7 cm) were processed by the Swiss-roll technique, fixed in Carnoy's fixative, blocked in paraffin, and processed histologically by standard techniques. Sections prepared from these specimens were stained with alcian blue. For quantitative purposes all cells stained with alcian blue were microscopically counted on a VCU. A total of 20 VCU/mouse were counted in several fields selected at random, and the results were presented as the mean number of mast cells ($\pm$ SD)/20 VCU (34).

RESULTS

Effects of anti-L3T4 antibody treatment of total cell numbers and cell surface markers in mesenteric lymph nodes of control and Nb-infected mice. BALB/c mice infected with Nb (three/group) were either not addition-

ally treated or were injected i.v. with anti-L3T4 antibody at the same time and 7 days after inoculation with Nb; control uninfected mice received either no treatment or two injections with anti-L3T4. All mice were killed concurrently 13 days after Nb inoculation. Anti-L3T4 antibody treatment caused a small decrease in the number of MLN cells detected in uninfected mice, and inhibited the increase in MLN cells that is characteristically induced by infection with Nb (Table I). Anti-L3T4 antibody had a similar effect on spleen cells (data not shown). The effect of anti-L3T4 antibody on L3T4 $^+$ spleen and MLN cells was studied by determining differences between percentages of Thy-1.2 $^+$ cells and Lyt-2 $^+$ cells. This indirect approach was necessitated by the observation that injection of mice with the rat anti-L3T4 mAb GK1.5 modulates L3T4 Ag from L3T4 $^+$ T cells before it kills these cells, so that these cells, although still present, are not stained by FITC-labeled anti-L3T4 antibody or anti-rat Ig antibody (F. D. Finkelman, unpublished data). Immunofluorescence staining and flow microfluorometry analysis demonstrated that injection of mice with anti-L3T4 antibody eliminated the great majority of L3T4 $^+$ MLN cells by 3 days after injection (data not shown), and that numbers of these cells were still greatly depleted days after a second injection (Table I). The Lyt-2 $^+$ cell population, which comprised 30% of the total MLN T cell (Thy-1.2 $^+$) population in control mice and 28% of the total MLN Thy-1.2 $^+$ population in mice infected with Nb, accounted for 91% of Thy-1.2 $^+$ MLN cells in both anti-L3T4 antibody-treated uninfected mice and in anti-L3T4 antibody-treated Nb-infected mice (Table I).

Effects of anti-L3T4 treatment on B cell expression of *sl*a and on appearance of cytophilic *sl*gE. Infection of mice with Nb leads to increases in the quantity of *sl*a and IgER expressed by B cells as well as in the percentage of MLN and spleen cells that bear cytophilic *sl*gE (9, 30). These changes were markedly inhibited by anti-L3T4 antibody treatment (Table II). Increases in the percentages of *sl*gM $^+$, *sl*a $^+$, and IgER $^+$ cells were seen in infected, anti-L3T4 antibody-treated mice and probably resulted, at least in part, from increase in the percentage of B cells secondary to the elimination of the large MLN L3T4 $^+$ lymphocyte population. However, the increase in the median fluorescence intensity (average brightness) of *sl*a $^+$ and IgER $^+$ cells, a measure of the number of *sl*a and IgER molecules per *sl*a $^+$ and IgER $^+$ lymphocytes, was inhibited in mice inoculated with Nb and treated with anti-L3T4 antibody. Thus, the increases in B cell *sl*a expression, IgER expression, and cytophilic *sl*gE that characteristi-

TABLE I
Effect of anti-L3T4 treatment on MLN cell number and cell surface markers^a

Nb Infection	Anti-L3T4 Injection	No. Cells $\times 10^{-6}$ /MLN	Thy-1.2 $^+$ (%)	Lyt-2 $^+$ (%)	<i>sl</i> gM $^+$ (%)
—	—	35.2	57.1	17.3	33.4
—	+	27.6	45.7	41.4	45.8
+	—	75.0	43.1	12.2	41.2
+	+	43.5	44.0	39.9	45.9

^a Three mice/group were inoculated with 700 third stage larvae of Nb. A group of uninfected and a group of Nb-infected mice received i.v. 0.5 mg of anti-L3T4 antibody, simultaneously with and 7 days after inoculation with Nb. All mice were killed 13 days after inoculation. Single cell suspensions prepared from the pools of MLN were counted with a Coulter Counter. Cells were labeled with FITC-anti-Thy-1.2, FITC-anti-Lyt-2, FITC-rabbit anti-mouse IgM or with FITC-control antibodies and were analyzed by FMF.

TABLE II

Effects of anti-L3T4 antibody treatment on B cell surface markers after inoculation with Nb^a

Nb Infection	Anti-L3T4 Injection	Percent of MLN Cells with (Median Fluorescence Intensity) ^b			
		sia	IgER	sigM	sigE
-	-	29.0 (115)	27.1 (178)	29.1	4.5
+	-	42.2 (391)	43.5 (312)	39.7	42.5
+	+	51.7 (162)	34.0 (169)	45.1	6.6

^a BALB/c mice (three/groups) were inoculated with 700 third stage larvae of Nb. A total of 0.5 mg of anti-L3T4 antibody was injected i.v. at the time of inoculation and mice were killed 13 days later. Single cell suspensions from pools of MLN were stained with the appropriate antibody and were analyzed by FCM.

^b Numbers in parentheses indicate on a linear scale the median fluorescence intensity (average brightness) of cells that express the indicated surface molecule.

TABLE III

Effects of anti-L3T4 antibody treatment on serum IgE levels, intestinal mucosal mast cells, and adult worm burdens in Nb infected mice^a

Nb Infection	Anti-L3T4 Injection	Serum IgE (μg/ml)	Mast cells/VCU	Adult Worms Recovered/Mouse
-	-	0.07 (1.21) ^b	1.2 ± 0.4**	0
+	-	22.00 (1.37)	19.9 ± 1.0	0
+	+	0.07 (1.06)	0.2 ± 0.2	161 ± 51 ^c

^a BALB/c mice (five/group) were inoculated with 700 third stage larvae of Nb. A total of 0.5 mg of anti-L3T4 antibody was injected i.v. at the time of Nb inoculation and mice were killed 13 days after inoculation. Serum IgE levels were determined by ELISA. Mast cells were identified by metachromatic staining with alcian blue. Numbers of mast cells/VCU were counted on microscopic sections. Adult Nb worms were counted in the washings of the small intestines.

^b Geometric mean (SEM).

^c Arithmetic mean ± SEM.

cally appear after infection with Nb are dependent on the presence of a functional L3T4⁺ T cell population.

The effect of anti-L3T4 antibody treatment on IgE production. Anti-L3T4 antibody blocked both the generation of cIgE⁺ MLN cells and the increase in serum IgE concentration that are induced by infection with Nb. Approximately 0.4% of the MLN cells of Nb-infected mice were cIgE⁺ 13 days after inoculation, whereas less than 0.01% of MLN cells of uninoculated mice or mice inoculated with Nb and treated with anti-L3T4 were cIgE⁺ (data not shown). Serum IgE concentrations, which averaged 70 ng/ml in uninoculated mice, increased to 22 μg/ml in mice infected with Nb but failed to increase in infected mice that were treated with anti-L3T4 antibody (Table III). A single dose of anti-L3T4 antibody, given simultaneously with Nb inoculation, was equally effective at blocking Nb-induced IgE production as two doses, given at the time of inoculation and 7 days later (data not shown).

The effect of anti-L3T4 treatment on IMMC hyperplasia. Mice infected with Nb were observed to have a 15-fold greater concentration of IMMC than uninfected mice at 13 days after inoculation (Table III). No increase in mast cells was observed, however, in infected mice that also were injected with anti-L3T4 antibody at the time of inoculation with Nb.

The effect of anti-L3T4 treatment on spontaneous cure of Nb infection. Mice inoculated with third stage larvae of Nb normally eliminate adult worms from their intestines by 10 to 11 days after inoculation. Mice that were simultaneously injected with anti-L3T4 antibody and inoculated with Nb failed to eliminate adult worms by 13 days after inoculation (Table III). Thus, L3T4⁺ T

cells are directly or indirectly important in spontaneous cure of Nb infection. The duration of intestinal infection with Nb in mice given a single injection of anti-L3T4 antibody simultaneously with inoculation or given injections of anti-L3T4 every week after inoculation was investigated. Mice that received one single dose of anti-L3T4 still had adult worms present in their intestines 14 days after inoculation. However, these mice had eliminated intestinal Nb by 21 days after inoculation at which time they once again had relatively normal numbers of L3T4⁺ MLN cells (data not shown). In contrast, mice that were injected each week with 0.5 mg of anti-L3T4 antibody continued to carry intestinal worms 42 days after Nb inoculation. These data show that L3T4⁺ T cells are critical to the induction of spontaneous cure.

The effect of anti-Lyt-2 antibody treatment on infection with Nb. Because L3T4⁺ T cells regulate the subpopulation of Lyt-2⁺ CTL (35), anti-L3T4 antibody treatment could affect spontaneous cure of infection by interfering with helminth specific Lyt-2⁺ CTL function. To test this possibility, the effect of injecting cytotoxic anti-Lyt-2 antibody into mice inoculated with Nb was studied. Inasmuch as 2.43 anti-Lyt-2 antibody, like GK1.5 anti-L3T4 antibody, modulates its ligand in vivo (F. D. Finckelman, unpublished data), it was necessary to determine the effect of anti-Lyt-2 on Lyt-2⁺ MLN cell numbers by determining the difference between percentages of Thy-1.2⁺ and L3T4⁺ MLN cells. The elimination of Lyt-2⁺ T cells after anti-Lyt-2 treatment was indicated by the fact that the percentage of Thy-1.2⁺ cells in the spleens and MLN of anti-Lyt-2 antibody-treated, Nb-infected mice was similar to the percentage of L3T4⁺ T cells (Table IV). No adult worms were present in any of the mice infected with Nb at 13 days after inoculation including those mice that received anti-Lyt-2 antibody. In addition, anti-Lyt-2 antibody had no detectable effect on the IgE responses of Nb-infected mice (Table IV).

The effect of anti-L3T4 treatment on secondary infection with Nb. To study the role of the L3T4⁺ T cell population in mice given a secondary infection with Nb, no antibody or 0.5 mg of anti-L3T4 antibody was injected into mice simultaneously re-inoculated with Nb larvae 33 days after primary inoculation. Whereas anti-L3T4 treatment did not ablate protective immunity because no adult worms became established in the intestine, it did prevent the large increase in serum IgE levels that normally accompanies a second inoculation with Nb (Table V).

TABLE IV

Effect of anti-Lyt-2 treatment on T lymphocyte subsets and serum IgE levels in mice infected with Nb^a

Nb Infection	Antibody Injection	Serum IgE (μg/ml)	L3T4 ⁺ (%)	Thy-1.2 ⁺ (%)
-	—	0.06 (1.32) ^b	54.6 ± 1.7 ^c	71.7 ± 1.1 ^c
+	—	27.4 (1.16)	39.7 ± 0.6	53.8 ± 0.2
+	Rat IgG 1×	19.7 (1.27)	39.2 ± 0.4	54.3 ± 1.2
+	a-Lyt-2 1×	18.2 (1.16)	47.1 ± 1.0	50.9 ± 0.7
+	Rat IgG 2×	33.2 (1.27)	40.6 ± 1.7	57.2 ± 1.1
+	a-Lyt-2 2×	26.2 (1.25)	47.5 ± 1.3	48.4 ± 1.4

^a BALB/c mice (five/group) were inoculated with 700 third stage larvae of Nb and injected i.v. with 0.5 mg of normal rat IgG or anti-Lyt-2 antibody simultaneously with inoculation (1×), or both simultaneously and 7 days after inoculation (2×). Five mice served as uninfected controls. Mice were bled and killed 13 days after inoculation. Serum IgE levels were determined by ELISA. Single cell suspensions from MLN were stained with the appropriate antibody and were analyzed by FCM.

^b Geometric mean (SEM).

^c Arithmetic mean ± SEM.

TABLE V
Effect of anti-L3T4 treatment on the IgE response induced by secondary infection with Nb^a

Nb Infection	Anti-L3T4 Injection	Serum IgE (μg/ml)
—	—	0.15 (1.70) ^b
1×	—	9.87 (1.12)
2×	—	103.00 (1.21)
2×	+	15.80 (1.25)

^a BALB/c mice (five/group) were inoculated with 700 third stage larvae of Nb. Mice in two groups received a second inoculation of 700 third stage Nb larvae 33 days after the first inoculation, whereas a third group was not re-inoculated. One group of mice received 0.5 mg of anti-L3T4 treatment i.v. 7 days before and on the day of the second inoculation with Nb. These mice and a group of uninfected mice were killed concurrently 40 days after the first and 7 days after the second inoculation. Serum IgE levels were determined by ELISA.

^b Geometric mean (SEM).

TABLE VI
Effect of anti-L3T4 treatment on serum IgE response given late during an infection with Nb^a

Nb Infection	Anti-L3T4 Injection	Serum IgE (μg/ml)
—	—	0.2 (1.31) ^b
+	—	55.5 (1.20)
+	Day 10	1.9 (1.56)
+	Day 11	13.1 (1.74)
+	Day 12	37.0 (1.39)

^a BALB/c mice (five/group) were inoculated with 700 third stage larvae of Nb. Mice in the infected groups received a single i.v. injection of 0.5 mg anti-L3T4 10, 11, or 12 days after inoculation with Nb. All mice were bled 13 days after inoculation and serum IgE levels were determined by ELISA.

^b Geometric mean (SEM).

TABLE VII
Effect of anti-L3T4 treatment on spontaneous cure of adult Nb^a

Nb Infection	Anti-L3T4 Injection	No. of Mice with Nb	Mean Worm Count/Mouse ^b
+	—	0/5	0
+	Day 0	5/5	340 ± 40
+	Day 7	5/5	150 ± 59
+	Day 8	4/5	81 ± 48
+	Day 9	2/5	2 ± 1
+	Day 10	0/5	0

^a BALB/c mice (five/group) were inoculated with 700 third stage larvae of Nb. Mice in the infected groups received a single i.v. injection of 0.5 mg of anti-L3T4 antibody, simultaneously with inoculation (day 0) or 7, 8, 9, or 10 days later. All mice were killed 13 days after inoculation. Adult worms were counted in small intestine.

^b Arithmetic mean ± SEM of mice with adult worms.

How long do L3T4⁺ T cells contribute to generation of IgE production, and spontaneous cure in mice infected with Nb? To determine how long L3T4⁺ T cells must be present to contribute to the generation of an optimal IgE response and adult worm elimination from the intestines, mice were given a single injection of anti-L3T4 antibody at various times after inoculation with Nb. Injection of anti-L3T4 antibody as late as 10 days after inoculation inhibited day 13 serum IgE levels by more than 95%; injection of anti-L3T4 on day 12, 1 day before mice were bled also reduced the magnitude of that response by 34% (Table VI). Some mice that were injected with anti-L3T4 antibody 7, 8, or 9 days after inoculation had adult worms in their intestines at day 13 (Table VII). These data demonstrate that T cells are required close to the time of worm elimination from the intestines or the initiation of IgE secretion for optimal expression of these events.

T cell help is needed to sustain elevated serum IgE levels after inoculation with Nb. To investigate whether the continuous presence of L3T4⁺ T cells is needed to sustain the increased serum levels of IgE induced by

infection with Nb, infected mice were bled 13 days after inoculation (the peak of the serum IgE response) and were then injected with 0.5 mg of GK1.5 anti-L3T4 mAb or with 0.5 mg of normal rat IgG. Seven days later the mice were re-bled. Serum IgE levels were determined by ELISA. Day 20 serum IgE levels decreased substantially more from day 13 levels in the anti-L3T4 antibody injected mice than in the other groups of mice infected with Nb (Table VIII). These results indicate that the continuing presence of T cell help is required to sustain a chronic IgE response.

DISCUSSION

The data presented herein indicate that induction of a polyclonal IgE response, IMMC hyperplasia, and the spontaneous cure of Nb infection are all prevented in mice inoculated with Nb and given a single injection of a anti-L3T4 mAb, which kills T cells of the helper phenotype and inhibits their function. This confirms previous studies performed in congenitally athymic (nude) mice that demonstrated the importance of thymus-derived T lymphocytes in the immune response to infection with Nb (36). Our studies, however, eliminate the possibility that the defects seen in nude mice result from abnormal B lymphocyte development associated with the chronic absence of T cells, and identify the L3T4⁺ subset as the T cell population crucial to several host response to a primary infection with Nb. Indeed, elimination of the MHC class I-restricted Lyt-2⁺ lymphocyte population, which is most strongly associated with CTL and suppressor function from spleens and MLN, had no effect on either the elimination of intestinal Nb or on the generation of a polyclonal IgE response. The former response suggests that a CTL response may not be important in the spontaneous cure of a primary Nb infection. This possibility is not totally eliminated, however, because 1) cytotoxic T cells in the intestinal mucosa might be Lyt-2⁺ just as some CTL function has been identified in the L3T4⁺ T cell subset (37); 2) in vivo antibody treatment might be less effective at eliminating Lyt-2⁺ T cells in the intestinal mucosa than in MLN or spleen. The failure of elimination of the Lyt-2⁺ T cell subset to enhance serum IgE levels in Nb-infected mice was somewhat surprising. T lymphocytes that express Lyt-2⁺, but not L3T4⁺, have been shown to produce a lymphokine, glycosylation-inhibiting factor, that stimulates the production of IgE binding factors that inhibit rather than enhance IgE production (16). Thus, we expected that Nb-infected mice that had been depleted of Lyt-2⁺ T cells might generate a larger IgE response than Nb-infected mice that received

TABLE VIII
Effect of anti-L3T4 treatment of Nb infected mice 13 days after inoculation on serum IgE^a

Nb Infection	Antibody Injection	Day 13 Serum IgE (μg/ml)	Day 20 Serum IgE (μg/ml)
—	—	0.11 (1.26) ^b	0.13 (1.25)
+	—	46.7 (1.35)	15.7 (1.17)
+	Rat IgG	25.8 (1.49)	5.26 (1.23)
+	Anti-L3T4	25.6 (1.31)	0.80 (1.45)

^a BALB/c mice (five/group) were inoculated with 700 third stage larvae of Nb. Mice were bled 13 days after inoculation along with uninfected controls and given no further treatment or injected i.v. with 0.5 mg of normal rat IgG or 0.5 mg of anti-L3T4 antibody. All mice were re-bled 7 days later. Serum IgE levels were determined by ELISA.

^b Geometric mean (SEM).

control antibody treatment. Our results therefore support the possibility that T cell regulation of the IgE response in Nb-infected mice is mainly the property of the L3T4⁺ subset and suggest that the glycosylation inhibiting factor either is 1) normally made in such excess that the few Lyt-2⁺ cells that remain after anti-Lyt-2⁺ antibody treatment are capable of producing a sufficient amount of this factor to maximally down-regulate IgE secretion; 2) that an Lyt-2⁻ T cell population can produce glycosylation inhibitory factor; 3) that Lyt-2⁺ T cells persist and produce this factor at sites other than spleen and MLN; or 4) that the glycosylation inhibitory factor has no importance in this system.

The use of anti-L3T4 antibody rather than nude mice to study T cell functions in parasite-infected mice has made it possible to eliminate the Th cell subset at specified time points during the infection. This has enabled us to demonstrate that intact Th cell function contributes to both spontaneous worm elimination from the intestines and to the generation of an IgE response up until the times that worms are expelled or serum IgE is being produced in maximum quantity. In addition, our studies demonstrate that both the persistence of increased serum IgE levels in mice infected with Nb after the peak period of IgE secretion and the development of a secondary IgE response to re-infection are dependent on the continuing presence of L3T4⁺ T cells. Although we did not study specific IgE responses, these observations may be relevant to the control of IgE responses in atopic individuals, inasmuch as they suggest that suppression of some T cell functions may induce rapid declines in serum levels of IgE antibody specific for clinically important allergens.

The use of anti-L3T4 antibody to interrupt Th cell function at defined times during the infection with Nb also provides a model in which experiments can be performed to see if specific lymphokines can replace the requirement for Th cells during infection. In particular, the increasing availability of recombinant lymphokines should make it possible to study whether T cell help for the generation of an IgE response can be replaced by IL-4 and/or stimulatory IgE-binding factor, and whether T cell help for the induction of IMMC hyperplasia can be replaced by IL-2 and/or IL-3 at any time point after inoculation with Nb.

Finally, the observations that L3T4⁺ T lymphocytes are not required for the expulsion of Nb during a second infection, and that considerable depletion of Lyt-2⁺ lymphocytes does not interfere with spontaneous cure of a primary infection suggest that T cell effector mechanisms are not in all circumstances required for parasite killing. Instead, T cell-dependent antibody production and mast cell differentiation may be sufficient to prevent Nb infection of the intestines during a secondary infection.

Acknowledgments. The authors express their gratitude to Drs. K. Ishizaka, Z. Ovary, and J. G. Thornbecke for their helpful comments and criticism; to Steve Kang for excellent technical assistance, to Neil Hardegen for FACS analysis, and to Amy Hankins for preparing the manuscript.

REFERENCES

1. Befus, A. D., M. Johnston, and J. Bienenstock. 1979. *Nippostrongylus brasiliensis*: Mast cells and histamine levels in tissues of infected and normal rats. *Exp. Parasitol.* 48:1.
2. Askenase, P. W. 1980. Immunopathology of parasitic disease: involvement of basophils and mast cell. *Semin. Immunopathol.* 2:417.
3. Butterworth, A. E. 1977. The eosinophil and its role in immunity to helminth infection. *Current Top. Microbiol. Immunol.* 77:128.
4. Jarrett, E. E. E., and H. Bazin. 1974. Elevation of total serum IgE in rats following helminth infection. *Nature (Lond.)* 251:613.
5. Kojima, S., and Z. Ovary. 1975. Effect of *Nippostrongylus brasiliensis* infection on anti-hapten IgE antibody response in the mouse. I. Induction of carrier specific helper cells. *Cell. Immunol.* 15:274.
6. Kojima, S., and Z. Ovary. 1975. Effect of *Nippostrongylus brasiliensis* infection on anti-hapten IgE antibody response in the mouse. II. Mechanism of potentiation of the IgE antibody response to a heterologous hapten-carrier conjugate. *Cell. Immunol.* 17:383.
7. Watanabe, N., S. Kojima, and Z. Ovary. 1976. Suppression of IgE antibody production in SJL mice. I. Nonspecific suppressor T cells. *J. Exp. Med.* 143:833.
8. Ishizaka, T., J. F. Urban, Jr., and K. Ishizaka. 1976. IgE formation in the rat following infection with *Nippostrongylus brasiliensis*. I. Proliferation and differentiation of IgE-forming cells. *Cell. Immunol.* 22:248.
9. Katona, I. M., J. F. Urban, Jr., I. Scher, C. Kanelopoulos-Langevin, and F. D. Finkelman. 1983. Induction of an IgE response in mice by *Nippostrongylus brasiliensis*: characterization of lymphoid cells with intracytoplasmic or surface IgE. *J. Immunol.* 130:350.
10. Urban, J. F., Jr., I. M. Katona, D. A. Dean, and F. D. Finkelman. 1984. The cellular IgE response of rodents to infection with *Nippostrongylus brasiliensis*, *Trichinella spiralis* and *Schistosoma mansoni*. *Vet. Parasitol.* 14:193.
11. Miller, H. R. P., and H. F. W. Jarrett. 1971. Immune reactions in mucous membrane. I. Intestinal mast cell response during helminth expulsion in the rat. *Immunology* 20:277.
12. Mitchell, G. F. 1980. T cell dependent effects in parasite infection and disease. *Prog. Immunol.* 4:794.
13. Ishizaka, K. 1976. Cellular events in the IgE antibody response. *Adv. Immunol.* 23:1.
14. Ito, K., T. Ogita, M. Suko, M. Mori, K. Kudo, T. Hayakawa, H. Horiuchi, and Y. Horiuchi. 1979. IgE levels in nude mice. *Int. Arch. Allergy Appl. Immunol.* 58:474.
15. Grecis, R. K., J. Riedlinger, and D. Wakelin. 1985. L3T4-positive T lymphoblasts are responsible for transfer of immunity to *Trichinella spiralis* in mice. *Immunology* 56:213.
16. Ishizaka, K., T. F. Huff, P. Jarden, K. W. Moore, and C. L. Martens. 1985. IgE-binding factors. Selective regulation of the IgE response by T cell factors. *Int. Arch. Allergy Appl. Immunol.* 77:13.
17. Ishizaka, K. 1985. Twenty years with IgE: from the identification of IgE to regulatory factors for the IgE response. *J. Immunol.* 135:1.
18. Howard, M., J. Farrar, M. Hilfiker, B. Johnson, K. Takatsu, T. Hamaoka, and W. E. Paul. 1982. Identification of a T-cell derived B cell growth factor distinct from interleukin 2. *J. Exp. Med.* 155:914.
19. Coffman, R. L., J. Ohara, M. W. Bond, J. Carty, A. Zlotnik, and W. E. Paul. 1986. B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. *J. Immunol.* 136:4538.
20. Finkelman, F. D., I. M. Katona, J. F. Urban, Jr., C. M. Snapper, J. E. Ohara, and W. E. Paul. 1986. Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine BSF-1. *Proc. Natl. Acad. Sci. USA* 83:9675.
21. Smith, C. A., and D. M. Rennick. 1986. Characterization of a murine lymphokine distinct from interleukin 2 and interleukin 3 (IL-3) possessing a T-cell growth factor activity and a mast-cell growth factor activity that synergizes with IL-2. *Proc. Natl. Acad. Sci. USA* 83:1857.
22. Mosmann, T. R., M. W. Bond, R. L. Coffman, J. Ohara, and W. E. Paul. 1986. T cell and mast cell lines respond to B cell stimulatory factor-1. *Proc. Natl. Acad. Sci. USA* 83:5654.
23. Razin, E., C. Cordon-Cardo, and R. A. Good. 1981. Growth of a pure population of mouse mast cells in vitro with conditioned medium derived from concanavalin A-stimulated splenocytes. *Proc. Natl. Acad. Sci. USA* 79:2559.
24. Nabel, G., S. J. Galli, A. M. Dvorak, H. F. Dvorak, and H. Cantor. 1981. Inducer T lymphocytes synthesize a factor that stimulates proliferation of cloned mast cells. *Nature (Lond.)* 291:332.
25. Yung, Y. P., R. Eger, G. Teritan, and M. A. S. Moore. 1981. Long term in vitro culture of murine mast cells. II. Purification of a mast cell growth factor and its dissociation from TCGF. *J. Immunol.* 127:784.
26. Nagao, K., K. Yokoro, and S. T. Aaronson. 1981. Continuous lines of basophil/mast cells derived from normal mouse bone marrow. *Science* 212:333.
27. Wilde, D. B., P. Marrack, J. Kappler, D. P. Dialynas, and F. W. Fitch. 1983. Evidence implicating L3T4 in class II MHC antigen reactivity: monoclonal antibody GK 1.5 (anti-L3T4a) blocks class II MHC antigen-specific proliferation, release of lymphokines, and binding by cloned murine helper T lymphocyte lines. *J. Immunol.*

- 131:2178.
28. Sarmiento, M., A. L. Glasebrook, and F. W. Fitch. 1980. IgG or IgM monoclonal antibodies reactive with different determinants on the molecular complex bearing Lyt2 antigen block T cell-mediated cytotoxicity in the absence of complement. *J. Immunol.* 125:2665.
 29. Unkeless, J. C. 1979. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. *J. Exp. Med.* 150:580.
 30. Katona, I. M., J. F. Urban, Jr., J. A. Titus, D. A. Stephany, D. M. Segal, and F. D. Finkelman. 1984. Characterization of murine lymphocyte IgE receptors by flow microfluorometry. *J. Immunol.* 133:1521.
 31. Hunter, K. W., F. D. Finkelman, G. T. Strickland, P. C. Sayles, and I. Scher. 1979. Defective resistance to *Plasmodium yoelii* in CBA/N mice. *J. Immunol.* 123:133.
 32. Kappler, J. W., B. Skidmore, J. White, and P. Marrack. 1981. Antigen-inducible, H-2 restricted, interleukin-2 producing T cell hybridomas. Lack of independent antigen and H-2 recognition. *J. Exp. Med.* 153:1198.
 33. Finkelman, F. D., C. M. Snapper, J. D. Mountz, and I. M. Katona. 1987. Polyclonal activation of the murine immune system by a goat antibody to mouse IgD. IX. Induction of a polyclonal IgE response. *J. Immunol.* 138:2826.
 34. Alizadeh, H., J. F. Urban, Jr., M. Katona, and F. D. Finkelman. 1986. Cells containing IgE in the intestinal mucosa of mice infected with the nematode parasite *Trichinella spiralis* are predominantly of a mast cell lineage. *J. Immunol.* 137:2555.
 35. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Lyt cells in the generation of killer activity. *J. Exp. Med.* 141:1390.
 36. Jacobson, R. H., and N. D. Reid. 1974. The immune response of congenitally athymic (nude) mice to the intestinal nematode *Nippostrongylus brasiliensis*. *Proc. Soc. Exp. Biol. Med.* 147:667.
 37. Mizouchi, T., H. Golding, A. S. Rosenberg, L. H. Glimcher, T. R. Malek, and A. Singer. 1985. Both L3T4⁺ and Lyt-2⁺ helper T cells initiate cytotoxic T lymphocyte responses against allogeneic major histocompatibility antigens but not against trinitrophenyl-modified self. *J. Exp. Med.* 162:427.