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Toxoplasmosis in Immunoglobulin M-Suppressed Mice

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Mice challenged with a pathogenic strain of *Toxoplasma gondii* develop fatal infections. However, if such mice are initially treated with sulfadiazine (SD), they develop immunity and survive with chronic infections. The role of antibody (Ab) in establishing protective immunity against acute parasitemias and in maintaining chronic infections was investigated using B-cell-deficient (immunoglobulin M-suppressed), T-cell-deficient (athymic), and normal BALB/c mice. All mice not receiving SD treatment rapidly died (mean 7.5 days) after infection, but the majority (80%) of intact mice developed immunity during SD treatment and survived for over 5 months with chronic toxoplasmosis. Athymic mice rapidly died (mean 6.0 days) after the removal of SD treatment. Although all SD-treated immunoglobulin M-suppressed mice eventually died, they lived considerably longer (18 to 83 days) in the complete absence of antitoxoplasma Ab than unprotected mice (7 to 9 days). Histopathological sections of liver, lung, brain, and other tissues showed that toxoplasma organisms gave rise to fatal lesions in all nonsurviving animals. The injection of Ab into acutely infected and athymic mice imparted no protection, but transfer of antitoxoplasma Ab (titer > 1:8,000) to immunoglobulin M-suppressed mice after SD treatment resulted in elimination of the parasites in 50% of the mice. Results of this study suggest that Ab may not be decisive in acute infections, but may be important in controlling long-term toxoplasmosis.

Immunological mechanisms that lead to protective immunity against the intracellular parasite *Toxoplasma gondii* have not been analyzed completely. After primary experimental infection, mice die with disseminated lesions. However, if mice are treated during the first 3 weeks of infection with a subcurative dose of sulfadiazine (SD), they develop an immune response that allows them to survive with chronic infections (5, 12). That is, during SD treatment, immune responses that allow for the control, but not the elimination, of *T. gondii* are induced. T cells are involved in the development of this response, as athymic (T-cell-deficient) mice die once SD treatment is terminated (12). Although T cells may be required to provide help for antibody (Ab) production, results from several studies suggest that the effect of T cells on *T. gondii* is mediated directly through the release of soluble factors (3, 14, 19). The influence of Ab on toxoplasma immunity is also unclear. Passive transfer of antitoxoplasma Ab has little or no effect on the course of primary acute infection (6). Also transfer of Ab with immune lymphocytes does not increase the degree of protection produced by transferring lymphocytes alone (6). Based on the results of these studies, the importance of antitoxoplasma Ab appears to be minimal. However, a functional role for Ab during later stages in the infection could not be excluded.

The study reported here evaluated the effect of Ab on the course of toxoplasmosis, the development of immunity, and the prevention of pathological damage. If mice are treated from birth with anti-μ serum, they possess functional T cells but are B cell deficient (10, 20). Thus immunoglobulin M (IgM)-suppressed mice can be used to study toxoplasmosis in the absence of humoral immunity, since the mice cannot produce antitoxoplasma Ab. B-cell-deficient (IgM-suppressed), T-cell-deficient (athymic), and normal BALB/c mice were infected with *T. gondii* and treated for 3 weeks with SD. The degree of acquired immunity was assessed after the removal of SD treatment.

MATERIALS AND METHODS

Mice. BALB/c mice used in the IgM suppression study were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). For the first 9 days after birth, neonatal mice were injected intraperitoneally with 0.05 ml of either goat anti-mouse μ or normal goat serum. All mice (IgM suppressed and

controls) received 0.1 ml of serum intraperitoneally every other day for the next 9 days and 0.1 ml intraperitoneally three times a week throughout the remainder of the experiments (see below). Two groups of IgM-suppressed mice were prepared, one at the National Institutes of Health, the other at the University of Kansas. Mice were 7 to 8 weeks old at the time of experimental infection with *T. gondii*.

Serum samples were collected from all IgM-suppressed mice at the end of experimentation to verify IgM suppression. Polyethylene glycol Ouchterlony analysis (4) showed that all mice, except for one which was removed from the study, remained IgM suppressed throughout the experiment, as demonstrated by the presence of circulating goat anti-mouse μ in the serum. IgM Ab's were not detected in any of the animals. The number of immunoglobulin-positive cells in the spleen of three IgM-suppressed animals was determined to be less than 1% compared with 41.3 ± 3.5% in the age-matched controls.

Athymic nude (nu/nu) and heterozygous (nu/+) hirsute BALB/c mice were obtained from Sprague Dawley Laboratories (GIBCO Laboratories, Grand Island, N.Y.) or bred at the University of Kansas as previously described (12). Athymic nude mice and their heterozygous littermates (normal controls) were infected with *T. gondii* at 2 to 3 months of age.

Sera. Preparation of goat anti-mouse μ chain (generously supplied by Herbert J. Morse III and Richard Asofsky) has been described in detail previously (10, 11, 20). These two antisera as well as the normal goat serum used in this study were tested for the presence of goat antitoxoplasma Ab by the Sabin-Feldman dye test (18). The normal goat serum and one of the two anti-μ sera were serologically negative for toxoplasma Ab. Mice were treated with these sera during the first 120 days of the study. Since some IgM-suppressed mice survived beyond this time, it was necessary to complete the study using anti-μ serum from the remaining goat which had an antitoxoplasma titer of 1:1,000. To help determine the influence of passively transferred heterologous Ab, five mice were inoculated with 0.1 ml of this reagent. Blood samples collected 3 and 48 h later had a circulating titer of only 1:16. During analysis of the data, the possible effects of low levels of heterologous antitoxoplasma Ab's present in a few IgM-suppressed mice were considered carefully. Antitoxoplasma serum was obtained from BALB/c mice with chronic *T. gondii* infections. Ab titers ranged from 1:8,000 to 1:32,000 using the Sabin-Feldman dye test.

General experimental design. Eight groups of mice (listed in Table 1) were infected subcutaneously (s.c.) with 2×10^4 tachyzoites of the T-1 line G (passage 670) strain of *T. gondii*. Three days later, SD (30 to 60 mg/100 ml) was added to the drinking water. Mice received treatment for the following 16 days. After removal of chemoprophylactic treatment (day 0), mice were observed for 5 months or until death occurred.

The four groups used in the IgM suppression part of the study included (i) 12 untreated age-matched control BALB/c mice, (ii) 12 mice receiving normal goat serum from birth, (iii) 8 IgM-suppressed mice, and (iv) 10 IgM-suppressed mice treated with 1 ml of antitoxoplasma serum on days 0 (titer 1:8,000) and 10 (titer 1:32,000) after SD treatment. In the portion of the experiment using athymic BALB/c mice, the four groups consisted of (i) 12 untreated heterozygous (thymic) littermate controls, (ii) 6 nude mice, (iii) 5 nude mice injected with antitoxoplasma serum on day 0 (21 days after infection), and (iv) 5 nude mice each receiving one thymus equivalent (approximately 10^8 thymocytes) intraperitoneally 1 day before initiation of *T. gondii* infection.

To determine the role of Ab in reinfection, 5 IgM-suppressed and 12 control mice were infected with 2×10^4 tachyzoites, treated with SD for 18 days, and then reinfected with 2×10^4 tachyzoites.

For comparative purposes, three additional groups of BALB/c mice, infected with 2×10^4 tachyzoites, were allowed to develop acute toxoplasmosis, i.e., were not treated with SD. These three groups include (i) 6 normal BALB/c mice, (ii) 12 mice receiving normal goat serum from birth, and (iii) 12 mice receiving 1.5 ml of mouse antitoxoplasma serum (titer 1:32,000) s.c. 1 day after *T. gondii* infection. These animals were observed daily until death occurred.

Mice from each group were bled from the retroorbital sinus during the experiment (see below and Table 1), and antitoxoplasma Ab titers were determined by the Sabin-Feldman dye test. All mice were autopsied at the time of death. Impression smears of lungs, livers, spleens, brains, and the s.c. sites of parasite injection were prepared and stained with Giemsa. Histological sections also were prepared from the organs of selected animals from each group. Brains from 10 mice surviving over 5 months were inoculated into normal mice to determine if experimentally infected animals had eliminated the parasites or if they had remained chronically infected. The remaining 28 mice which survived for longer than 5 months were challenged s.c. with the RH strain of toxoplasma to assess their immune status.

RESULTS

In this study, 38 of 48 control BALB/c mice (untreated and normal goat serum recipients) were able to develop protective immunity during SD chemoprophylaxis and survived for over 5 months after *T. gondii* infection (Table 1). Surviving animals developed high titers of Ab and appeared clinically healthy at the end of the experiment. The 10 nonsurviving mice died around day 30 from toxoplasmic encephalitis, as verified by tissue sections and imprints. Of the 38 mice which survived for over 5 months, 10 were examined for the presence of cysts by subinoculating brain tissue into normal animals. Acute toxoplasmosis developed in all recipient animals. Thus, these animals had remained chronically infected without attenuation of the parasite. To test the immune status of surviving animals, the other 28 surviving mice were challenged with the highly pathogenic RH strain of *T. gondii*. Of these 28 mice, 20 survived. The eight nonsurviving mice died 1 to 4 days (mean 2) after the nonimmune controls.

IgM-suppressed mice also were able to develop some immunity during SD treatment (Table

TABLE 1. Immunization against *T. gondii* infection in B- and T-cell-deficient BALB/c mice

Treatment[a]	No. of mice studied	Animals surviving 5 mo		Average day of death of nonsurvivors[b]	Mean antitoxoplasma Ab titer of survivors on day 48	*T. gondii* present in tissues or seroconversion (No. positive/ no. tested)
		No.	%			
IgM supression study						
None	12	11	92	35.0 ± 0	1:11,000	11/11
Normal goat serum	12	10	83	29.5 ± 10.6	1:16,000	9/10
IgM suppressed	8	0	0	40.1 ± 22	<1:8	8/8
IgM suppressed plus Ab	10	5	50	21.0 ± 5	1:1,000	5/10[c]
IgM suppressed plus challenge	5	0	0	14.6 ± 0.5	<1:4	5/5
None plus challenge	12	10	83	27.0 ± 2.8	1:23,000	10/10
Athymic mouse study						
Littermate controls	12	7	58	30.6 ± 13	1:6,000	10/10
Nude	6	0	0	6.0 ± 1	—[d]	6/6
Nude plus Ab	5	0	0	5.4 ± 1	—	5/5
Nude plus thymic transplant	5	1	20	19.5 ± 5	1:16,000[e]	5/5

[a] All mice received SD.
[b] Mean ± standard deviation after removal of SD treatment (day 0).
[c] The five other mice were negative both serologically and by subinoculation after 150 days.
[d] —, Not tested.
[e] Postmortem.

1). IgM-suppressed mice survived for a period similar to that of the SD-treated controls that died; that is, IgM-suppressed mice survived for up to 83 days after removal of the SD prophylaxis (Fig. 1) in the complete absence of toxoplasma Ab. However, all IgM-suppressed mice not receiving Ab treatment eventually died. Of the eight IgM-suppressed mice, seven mice had advanced toxoplasma pneumonia (Fig. 2A), four had toxoplasmic encephalitis (Fig. 2B), and two had focal myocarditis. One mouse without pneumonia or encephalitis showed extensive toxoplasma infection of fibroblasts (Fig. 2C) involving the pancreatic stroma, the visceral and parietal peritoneum, the porta hepatis, and splenic, renal, and adrenal capsules. One mouse, which lived beyond day 60 (Fig. 1), had no detectable Ab on day 65 when IgM suppression was stopped. By day 73, this animal had developed an Ab titer of 1:2,000. It died with advanced toxoplasmic pneumonia on day 83.

Passively transferred hyperimmune serum

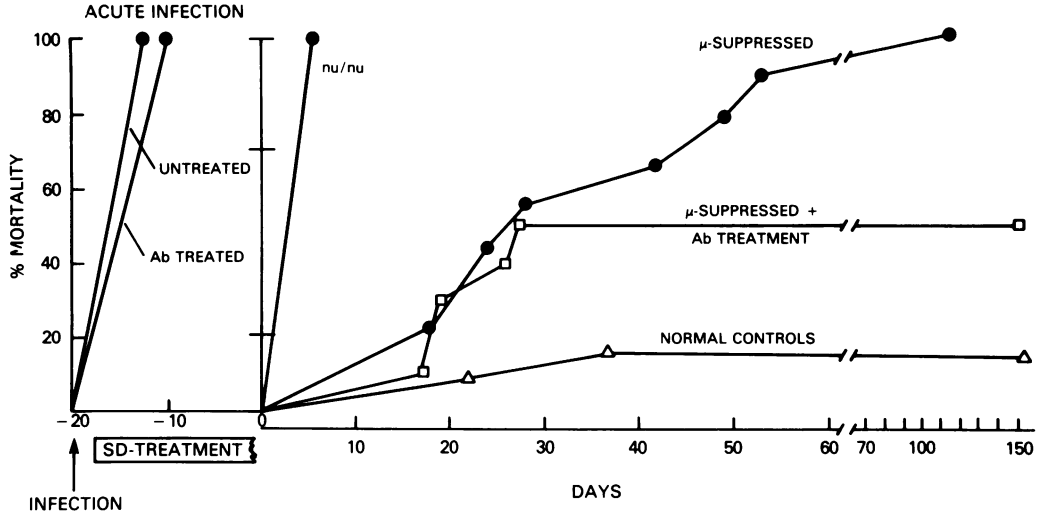

FIG. 1. Cumulative mortality of mice with toxoplasmosis. μ-Suppressed means IgM suppressed.

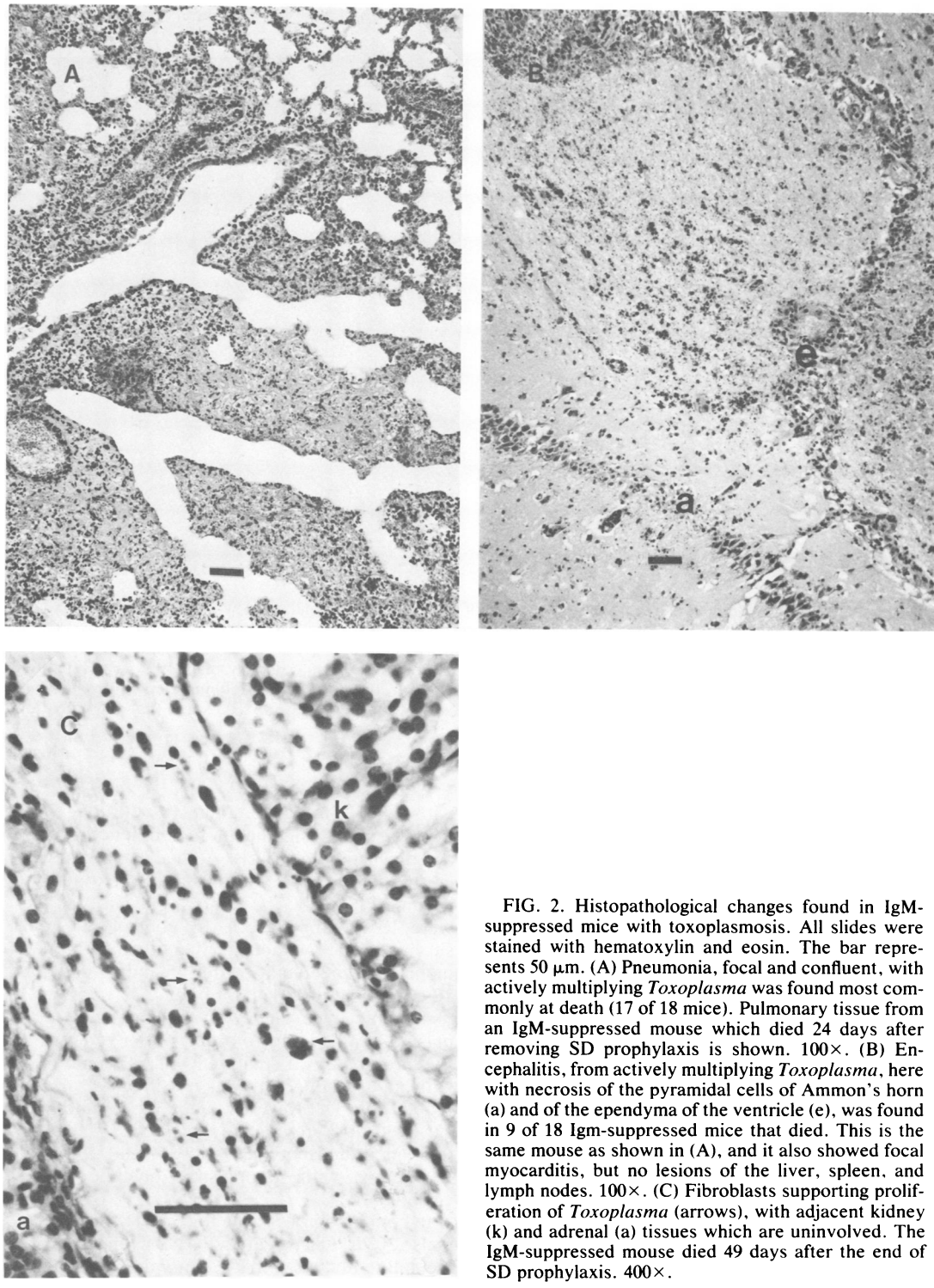

FIG. 2. Histopathological changes found in IgM-suppressed mice with toxoplasmosis. All slides were stained with hematoxylin and eosin. The bar represents 50 μm. (A) Pneumonia, focal and confluent, with actively multiplying *Toxoplasma* was found most commonly at death (17 of 18 mice). Pulmonary tissue from an IgM-suppressed mouse which died 24 days after removing SD prophylaxis is shown. 100×. (B) Encephalitis, from actively multiplying *Toxoplasma*, here with necrosis of the pyramidal cells of Ammon's horn (a) and of the ependyma of the ventricle (e), was found in 9 of 18 Igm-suppressed mice that died. This is the same mouse as shown in (A), and it also showed focal myocarditis, but no lesions of the liver, spleen, and lymph nodes. 100×. (C) Fibroblasts supporting proliferation of *Toxoplasma* (arrows), with adjacent kidney (k) and adrenal (a) tissues which are uninvolved. The IgM-suppressed mouse died 49 days after the end of SD prophylaxis. 400×.

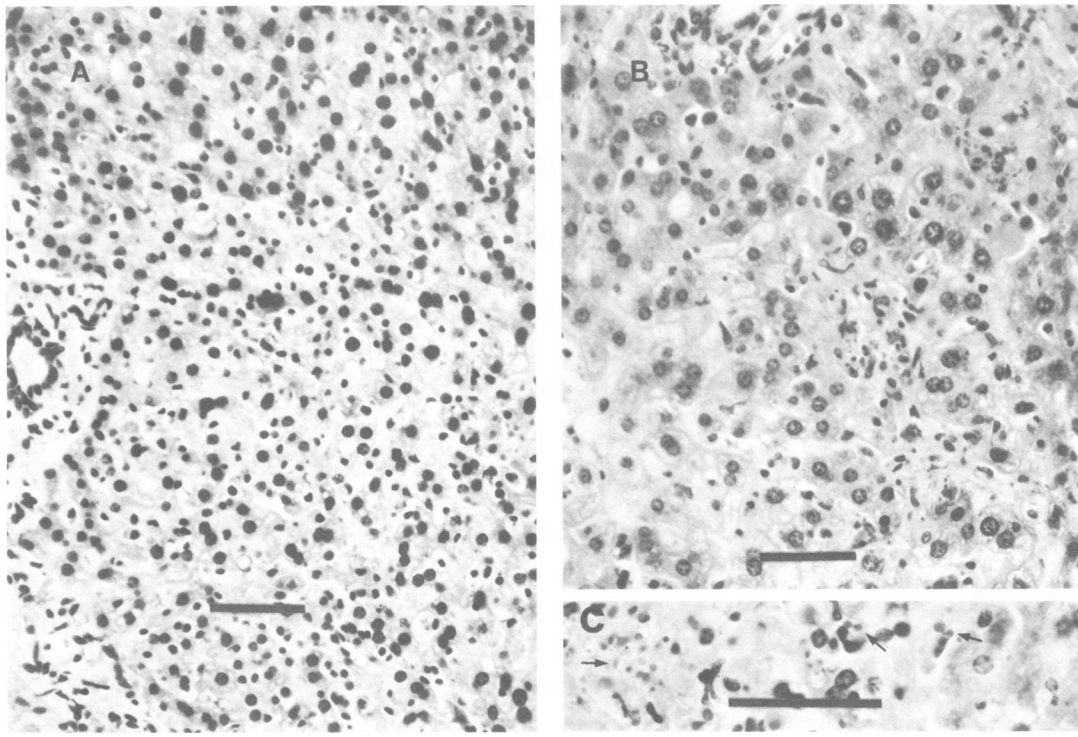

FIG. 3. Histopathological changes found in livers of immune and normal mice with toxoplasmosis. Slides were stained with hematoxylin and eosin. The bar represents 50 μm. (A) Regenerating liver suggests that *Toxoplasma* had proliferated and destroyed liver cells, but was immunologically cleared. This IgM-suppressed mouse died 14 days after *Toxoplasma* challenge, with extensive toxoplasmic pneumonia. 250×. (B) Hepatitis, with active toxoplasmic proliferation in hepatocytes which are destroyed. This acutely infected control mouse, treated with normal goat serum, died 8 days after s.c. infection. 250×. (C) Same as (B) except that magnification is 400×. Arrows point to *Toxoplasma*.

permitted 5 of 10 IgM-suppressed mice to survive *Toxoplasma* infection for longer than 150 days after SD treatment (Fig. 1). Ab titers in the 10 mice ranged from 1:256 to 1:1,000 4 weeks after passive transfer of Ab, and Ab was detected in the serum of individual mice for 75 to 96 days. Five mice died with toxoplasmic pneumonia, and two of these also had encephalitis. The five surviving mice did not have demonstrable levels of toxoplasma Ab by day 152. Thus, passively transferred antitoxoplasma Ab was no longer present in these animals, and they failed to produce anti-*T. gondii* Ab after the removal of IgM suppression. Passage of brain tissue from these mice did not produce toxoplasmosis in naive mice. Also, *Toxoplasma* organisms were not observed in histological sections. Thus, these five animals apparently eliminated their *Toxoplasma* infections.

The effect of challenge with tachyzoites immediately after chemoprophylaxis was studied in 5 IgM-suppressed and 12 control mice. All IgM-suppressed mice died (mean 14.6 ± 0.5 days) with toxoplasma pneumonia. In addition, three mice also had significant foci of encephalitis, and one had myocarditis. All had actively regenerating liver tissue without active toxoplasmic lesions (Fig. 3A). The antitoxoplasma Ab titers were less than 1:4. Of 12 control mice, 10 survived. The two nonsurvivors died with pneumonia and encephalitis after 25 and 29 days; all 12 controls developed antibody.

Athymic mice died with disseminated toxoplasmosis an average of 6 days after removal of SD prophylaxis. Treatment of athymic mice with mouse antiserum did not modify survival time (Table 1). However, one of five nude mice survived after thymic replacement. The other four mice died between days 14 and 25 (mean 19.5 days) with toxoplasmic pneumonia and encephalitis.

In comparison with the above SD-treated animals, 30 of 30 untreated BALB/c mice experimentally infected with *T. gondii* died with acute infections. Mice that were infected s.c. died after 7.5 ± 0.6 days (Fig. 1), and neither treat-

TABLE 2. Distribution of toxoplasma lesions at the time of death

Type of mice	Day of death	Treatment	No. of mice examined	No. of mice with:					
				Hepatitis/ Splenitis	Fibrositis		Myocarditis	Pneumonia	Encephalitis
					At injection site	Generalized			
With acute infections	7.5 ± 0.6	None	6[a]	6	6	0	0	6	0
	8.5 ± 0.5	Normal goat serum	12[a]	12	12	0	0	10	0
	9.4 ± 0.5	Mouse antitoxoplasma serum	12[a]	12	12	0	0	10	0
SD treated	6.0 ± 1.0	Athymic	7	7/6	6	0	1	4	0
	21–53	Control	7	1[b]	0	0	1	3	5
	14–15	IgM suppressed plus challenge	5	0	0	0	4	5	3
	17–27	IgM suppressed plus antiserum	5	0	0	0	0	5	2
	18–83	IgM suppressed	8	0	0	1[c]	2	7	4

[a] Results are based on sections and impression smears. All other results are based on tissue sections only.
[b] Evidence of subsiding hepatitis/splenitis.
[c] Generalized toxoplasma infection was observed in fibroblasts in a mouse that died on day 49.

ment with normal goat serum (8.5 ± 0.5 days) nor antitoxoplasma Ab (9.4 ± 0.4 days) significantly prolonged survival. These mice showed extensive toxoplasmic hepatitis (Fig. 3B), splenitis, and lymphadenitis with, as yet, little pneumonia and no encephalitis.

The distribution of lesions found at death is shown in Table 2. Of special interest is that mice that died within 10 days of infection or shortly after removal of SD treatment died with extensive toxoplasmic hepatitis, splenitis, and lymphadenitis, whereas mice that died later showed subsiding inflammation or no inflammation in these organs. The mice that died after 2 weeks with apparent partial immunity had toxoplasmic pneumonia and encephalitis and sometimes myocarditis. One IgM-suppressed mouse that died after 49 days showed parasitization of the fibroblasts in the renal capsules, the hilum of the spleen and liver, the pancreas, and the s.c. site of injection. Patchy epicarditis, often with calcification, was found in 16 of 38 mice that died. It was also present in three of five IgM-suppressed mice.

DISCUSSION

The importance of T and B cells in establishing and maintaining immunity to toxoplasmosis was evaluated in this study. As reported previously for outbred hamsters (6), BALB/c mice died from acute toxoplasmosis in 7 to 10 days, even in the presence of passively transferred antitoxoplasma Ab (Table 2). Subcurative prophylaxis with SD for 16 days allowed approximately 80% of BALB/c mice to develop protective immunity. These animals survived for over 5 months (until the experiment was terminated), and most remained chronically infected.

Athymic BALB/c mice rapidly died after removal of SD (Table 1). This finding again demonstrates the requirement for functional T cells in establishing immune protection (12).

Although all IgM-suppressed mice eventually died, they survived for a considerable period (mean 40.0 days) in the absence of demonstrable antitoxoplasma Ab's. Previous studies have shown that IgM-suppressed mice have normal T-cell functions, including T-cell help (9), allograft rejection (13), and response to allogenic cells in vitro (20). The failure of IgM-suppressed mice to survive indefinitely in the presence of T-cell immunity suggests a role for Ab in maintaining immunity with chronic infections.

Nonimmune mice died with s.c. lesions and pneumonia after 6 to 9 days. IgM-suppressed mice rechallenged after the period of chemoprophylaxis died after 14 to 15 days with lesions at the s.c. challenge site and with indications of hematogenous spread to the lung, brain, and heart. This suggests that absence of Ab permitted organisms to reach vital organs earlier and in greater numbers, from which fatal lesions developed. By comparison, the unchallenged IgM-suppressed group exhibited a similar spectrum of lesions, but at a later time.

Non-Ab-mediated mechanisms undoubtedly contributed to the prolonged survival of IgM-suppressed mice in the absence of Ab. One such possible mechanism is interferon which is known to inhibit the growth of *T. gondii* in mouse cells in vitro (15). However, homologous interferon has proven ineffectual in altering *Toxoplasma* in human cells in vitro (1) and in

hamsters in vivo (7). Immunity in mice has been shown to be mediated, in part, specifically by a lymphokine derived from T cells (14, 19). This mediator of *Toxoplasma*, similar to a 4000- to 5,000-molecular-weight polypeptide produced by hamster lymphocytes, is effective not only on macrophages, but also on other somatic cells (3, 14). Because toxoplasma grows primarily in nonmacrophage somatic cells, mediation of immunity to such cells is of special interest. This immunity appears to be conveyed unequally to different cells. In the experiments presented, the liver, spleen, and lymph nodes, which were demonstrably susceptible during primary infection, appeared solidly immune at a time when immunity failed at least focally in the lungs, brain, and myocardium and in one instance, in fibroblasts throughout the body.

Passive transfer of serum from chronically infected mice to IgM-suppressed mice (on days 0 and 10) permitted half of them to survive. The other half of the mice did not benefit by receiving antiserum and died at the same rate as IgM-suppressed mice without Ab (Fig. 1). The surviving mice, however, failed to synthesize Ab after removal of IgM suppression, remained without Ab after the decline of passively transferred Ab, and failed to transfer toxoplasmosis upon subinoculation. Because all of the other 199 mice used in this study, including cagemates, developed either toxoplasmic lesions or high Ab titers (Table 1), it is highly unlikely that these animals had not become infected initially. It is probable that these mice cleared their infections early after the passive transfer of Ab and SD treatment. Similarly, it appears that some of the control mice, which possessed high Ab titers 48 days after infection (Table 1), also cleared their infections and ultimately lost their immunity. Of 28 control mice which survived for over 150 days, 8 were not able to resist challenge with the pathogenic RH strain. Unfortunately, the Ab titer in these mice was not determined immediately before challenge. It appears that high Ab titers present with T-cell immunity during certain stages of infection may sometimes lead to sterile immunity in some animals. However, in the absence of Ab, IgM-suppressed mice failed to develop sterilizing immunity, whereas Ab in the absence of cellular immunity merely prolonged the survival of mice by 2 days (Fig. 1).

The changing pattern of lesions in mice with fatal toxoplasmosis over a period of 3 months is of interest (Table 2). After infection, there was toxoplasma proliferation with focal necrosis at the injection site, in the regional and some distant lymph nodes, and in the liver, with slight diffuse pneumonia. This was observed in mice that received no treatment, normal goat serum, or mouse serum containing toxoplasma Ab and all of which died between 7 and 10 days after infection. Even in athymic mice that died 6 ± 1 days after cessation of SD therapy, the distribution of lesions was similar, although more extensive and advanced, because the mice developed no immunity (12). Mice that had acquired some immunity during the first 3 weeks of SD-controlled infection died with toxoplasmic pneumonia and encephalitis, and occasionally with myocarditis. These necrotizing lesions were generally focal, with infection extending peripherally. There was no difference in the lesions of mice with and without Ab. The lesions resembled those of corticosteroid-treated animals and humans, but their small size and the presence of few organisms (5, 8) suggested imperfect cell-mediated immunity. The exclusive parasitization of fibroblasts in many sites has not been described previously and suggests a defect in mediation or expression of immunity. In fact, all of the lesions in the partially immune mice can be so interpreted if one adds the concept of focal overloading of cellular immunity once multiplication of organisms has started.

The role of B cells in immunity to several protozoan parasites has been assessed using IgM-suppressed mice. B cells clearly are essential for establishing protection to the erythrocytic stages of the rodent malarial parasite, *Plasmodium berghei yoelii* (20), but immunized mice may resist the sporozoite stage in the absence of Ab (2). IgM-suppressed rats infected with *Trypanosoma cruzi* have accelerated parasitemias and decreased periods of survival (16). However, mice survive babesiasis better in the absence of Ab (17). Thus the role of Ab appears to differ considerably among various species and even stages of protozoa.

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