Effect of Antithymocyte Serum on Experimental Toxoplasmosis in Mice

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Received for publication 6 January 1972

The effect of antithymocyte serum (ATS) on mice infected with high-virulent or low-virulent strains of Toxoplasma gondii was studied. Treatment with ATS reduced the survival time of mice infected with high-virulent Toxoplasma and aggravated the disease; treatment prolonged parasitemia in animals infected with low-virulent Toxoplasma. Although ATS adversely affected the humoral antibody response, its main effect on toxoplasmosis was probably due to depression of cell-mediated immunity. ATS appeared to be able to activate chronic Toxoplasma infection, but this effect was much less pronounced than that on acquisition of immunity. The results suggest that Toxoplasma infection should be routinely looked for in patients treated with antilymphocyte serum.

In recent years toxoplasmosis has been observed to occur as a complication of malignant disease (1, 20). Toxoplasma gondii has been considered an "opportunistic invader" i.e., relatively harmless in normal individuals but pathogenic when the defense mechanisms of the host are impaired. The cell-mediated immunity seems particularly important in the defense against Toxoplasma infection (5). Thus, human diseases, such as Hodgkin's disease, which are characterized by impairment of the cell-mediated immunity seem to be prone to be complicated by toxoplasmosis (12, 20).

Antilymphocyte serum (ALS) is extensively used as an immunosuppressant in transplantation. Treatment with ALS which may activate and aggravate a variety of infections (Fed. Proc. 29:167-168) has also been associated with cases of toxoplasmosis (13, 18). In the present study, the effect of ALS on experimental toxoplasmosis in mice was studied to get further information about the immune mechanisms important in toxoplasmosis.

MATERIALS AND METHODS

Animals. Swiss mice, 3- to 4-weeks-old, were used for studying immunity to infection and New Zealand White rabbits, weighing 3 to 5 kg, for preparation of antisera.

ATS. Antithymocyte serum (ATS) was prepared in rabbits as described by Levey and Medawar (11). Each batch of ATS, as well as the normal rabbit serum (NRS) used, was absorbed twice with an equal volume of mouse erythrocytes. The sera were thereafter tested for residual anti-mouse erythrocyte agglutinins and hemolysins to ascertain the effectiveness of the absorption. It is very difficult to find rabbits free of serum antitoxoplasma activity in Sweden, and no such rabbits were available at the time when the experiments were initiated. However, no serum used in the experiments had a dye-test titer of more than 1/50, and, for each experiment, ATS and NRS having identical dye-test titers were selected. The sera were also tested in thymocyte agglutination tests (3). The ATS used in the experiments had all agglutinin titers of 1/28 to 1/512. All sera employed were sterilized by filtration before use and were administered by the intraperitoneal (ip) route.

Toxoplasma antibodies. Toxoplasma antibodies were assayed by a modified dye test as described previously (19). In essence, the test is performed by mixing one part of serum dilution with one part of Toxoplasma-containing peritoneal exudate and two parts of normal human serum (activator). The percentage of Toxoplasma parasites showing morphological alterations is determined for each serum dilution after incubation for 1 hr at 37 C. The dye-test titer is expressed as the serum dilution evoking morphological alterations in 50% or more of the parasites.

Parasites. Three strains of Toxoplasma gondii, RH, GBG-1, and Beverley, were used. The RH strain is highly virulent for mice. The GBG-1 strain, isolated in our laboratory from the placenta of a case of congenital toxoplasmosis, is less virulent for mice than the RH strain. The Beverley strain of Toxoplasma is low-virulent, causing nonlethal infections with numerous cysts.

Peritoneal exudate. Peritoneal exudate was collected by washing the peritoneal cavity; 0.5 ml of Hanks salt solution was injected and the abdomen of each mouse was gently rubbed and then opened for aspiration of fluid. The parasites were counted in a hemocytometer.

Blood specimens. Blood specimens were obtained
from the retroorbital plexus of the mice. For assays of parasitemia, the blood was diluted in Hanks solution immediately after the bleeding. Groups of 3 to 5 mice were thereafter inoculated ip with 0.25-ml volumes of the blood specimens, diluted $10^{-2}$ to $10^{-3}$. When high-virulent Toxoplasma was used, parasitemia was determined by calculation of median lethal dose titers. In infections with low-virulent Toxoplasma, parasitemia was determined by testing sera of the inoculated mice for presence of Toxoplasma antibodies 3 weeks after injection of the blood specimens. In this way median infective dose titers of the blood specimens were assayed.

RESULTS

Effect of ATS on infection with high-virulent Toxoplasma strains. Administration of 0.25 ml of ATS 1 hr prior to injection with the RH strain ($\geq 1,000$ parasites per mouse) did not influence the survival time of the mice significantly. Thus, all animals died after an average of 6 to 7 days irrespective of whether they had been pretreated with ATS, NRS, or with saline. However, when the progress of infection was slowed down by reducing the dose of parasites, by changing the route of administration from ip to subcutaneous (sc), or by substituting the more virulent RH strain with the GBG-1 strain, the treatment with ATS decreased the survival time notably. This effect, particularly evident when the GBG-1 strain was used, is demonstrated in Fig. 1. One injection of 0.25 ml of ATS given 1 hr before the inoculation of the Toxoplasma parasites was enough to give a decrease of the survival time.

The reduction of the survival time was not influenced by injection of 0.25 ml of a rabbit antithymocyte serum (ATS) or passively administered antibody on the survival of mice infected with Toxoplasma of the GBG-1 strain. The animals, groups of 10 to 20 mice, were inoculated ip with about 1,000 Toxoplasma parasites. Serum (0.25 ml) was injected 3 hr before parasite inoculation and consisted of ATS or rabbit antitoxoplasma serum (dye-test titer: $\frac{1}{8,000}$).

![Fig. 1. Effect of antithymocyte serum (ATS) and passively administered antibody on the survival of mice infected with Toxoplasma of the GBG-1 strain. The animals, groups of 10 to 20 mice, were inoculated ip with about 1,000 Toxoplasma parasites. Serum (0.25 ml) was injected 3 hr before parasite inoculation and consisted of ATS or rabbit antitoxoplasma serum (dye-test titer: $\frac{1}{8,000}$).](image)

![Fig. 2. Effect of antithymocyte serum (ATS) and passively administered antibody on the formation of Toxoplasma dye-test antibodies. Experimental procedure is as described in legend to Fig. 1. Results of two separate experiments, both using GBG-1 Toxoplasma parasites, are illustrated. Each point represents geometric mean values obtained from four to five mice.](image)

<table>
<thead>
<tr>
<th>Day after inoculation</th>
<th>RH Toxoplasma</th>
<th>Beverly Toxoplasma</th>
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<tbody>
<tr>
<td></td>
<td>NRS-treated $^b$</td>
<td>ATS-treated $^b$</td>
</tr>
<tr>
<td>4</td>
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<td>0.7</td>
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<tr>
<td>17</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>21</td>
<td>0.5</td>
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</tbody>
</table>

$^a$ Mice treated with normal rabbit serum (NRS) or antithymocyte serum (ATS) were inoculated subcutaneously with 1,000 RH parasites or with 30 cysts of the Beverley strain. At different time intervals, five animals in each group were killed; their blood was collected, pooled, and thereafter titrated for infected parasites. The experiment with Beverley toxoplasma was repeated, this time assaying parasitemia 17 and 21 days after inoculation of parasites. Figures for parasitemia are given as log median lethal dose (RH) or log median infective dose (Beverley) per 0.25 ml of blood.

$^b$ NRS and ATS treatment: 0.25 ml of serum on days $-2, 0, +1$. antitoxoplasma serum with high dye-test titer (1/8,000). Figure 2 presents the dye-test antibody titers observed after inoculation of GBG-1 Toxoplasma parasites. The antibody formation seemed to be suppressed by ATS treatment as well as by
administration of heterologous *Toxoplasma* antibody, although injection of passive antibody in no experiment influenced the survival time.

Treatment with ATS, given as injections of 0.25 ml on days -2, 0, and +1, had no demonstrable effect on the parasitemia in experiments with RH *Toxoplasma*. The blood concentrations of infective parasites after treatment with NRS did not differ notably from those found in the ATS-treated mice (Table 1). However, antibodies against *Toxoplasma* appeared concomitantly with the parasitemia in the NRS-treated mice, whereas, in the ATS-treated group, serum antibodies were absent or occurred only in very low concentrations. The peritoneal exudate of mice treated with ATS contained more parasites than the exudate of the NRS-treated mice (Table 2).

**Effect of ATS on infection with low-virulent *Toxoplasma* strain.** Three types of experiments were performed. In the first experiment the Beverley strain of *Toxoplasma* was inoculated sc into 4-week-old mice, 30 cysts per mouse. Before and after the infection, the mice were treated with ATS in doses of 0.25 ml of undiluted serum per mouse. Thus, ATS was administered on days -4, -3, -1, +1, +4, and +7. Control mice received NRS instead of ATS but were otherwise identically treated. In a second set of experiments, the dosage of ATS was increased to 0.5 ml and injections were given on day 0 (i.e., when the mice were infected with parasites) and on days +4, +11, and +18.

In contrast to 15 NRS-treated, parasite-infected controls and 7 uninfected but ATS-treated mice, all surviving the treatment, 3 of 15 parasite-infected, ATS-treated mice died within a period of 3 weeks. Weight curves demonstrated a clear-cut difference between infected and uninfected animals. An inability to gain weight or, in many cases, loss of weight, was more pronounced when

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**TABLE 2. Influence of antithymocyte serum (ATS) on number of parasites in peritoneal fluid**

<table>
<thead>
<tr>
<th>Day after infection</th>
<th>Number of parasites X 10^5/ml</th>
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<tr>
<td></td>
<td>ATS-treated</td>
</tr>
<tr>
<td>4</td>
<td>0.02</td>
</tr>
<tr>
<td>5</td>
<td>1.33</td>
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<td>6</td>
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<td>7</td>
<td>14.33</td>
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<tr>
<td>8</td>
<td>5.69</td>
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</tbody>
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* Mice inoculated intraperitoneally with 1,000 RH *Toxoplasma* parasites were treated with 0.25 ml of normal rabbit serum (NRS) or ATS on days -2, 0, and +1. Mean values of determinations on four to five mice are given.

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Toxoplasma-infected mice received treatment with ATS (Fig. 3).

In the third set of experiments 3-month-old mice, which had been infected with *Toxoplasma* for 8 weeks, were given eight injections of 0.25 ml of ATS or NRS within an 11-day-period. Three out of 12 mice in the ATS group and 1 of 12 in the NRS group died within 2 weeks. Between the second and third week after the beginning of treatment, all mice in the ATS-treated group lost weight (mean, 2.3 g), whereas the NRS-treated mice gained some weight (mean, 0.3 g). Thus, in all three sets of experiments, *Toxoplasma*-infected mice treated with ATS showed impaired growth ability or loss of weight.

To study the effect of ATS on parasitemia, seven mice in each of the groups of NRS- or ATS-treated, chronically infected animals were bledd before the treatment and then 3 days after the treatment was finished. The blood from each mouse was titrated for infective parasites. Before treatment, two mice in each group showed parasitemia, and after the treatment parasitemia was detectable in three mice in the ATS group and in two mice in the NRS group. Thus ATS had no significant ability to provoke parasitemia in chronically infected mice.

To study the effect of ATS on parasitemia, also during acquisition of infection, mice were injected with 0.25 ml of ATS or NRS on days -2, -1, and 0 when they were inoculated with 30 cysts of the Beverley strain in addition. Blood specimens were drawn 7, 10, and 14 days and, in a second experiment, 17 and 21 days after initiation of the
infection and titrated for infectivity. As seen in Table 1, parasitemia was demonstrable in the NRS-treated mice during the first 10 days after infection but was not detectable in the first experiment after 2 weeks; in the second experiment, parasitemia was found after 17 days but not after 3 weeks. In contrast, the ATS-treated mice showed parasitemia for the whole period of observation. During the initial period after infection, however, parasitemia was more marked in the NRS-treated mice.

Infection with low-virulent and challenge with high-virulent Toxoplasma in ATS-treated mice. Sixty mice were inoculated sc with about 30 cysts from brains of mice infected with the Beverley strain. Half the number of mice were injected with ATS (0.5 ml of a 1:2 dilution) 1 hr before inoculation with parasites and then after 1, 5, 6, and 8 days, respectively. Control mice were injected with NRS instead of ATS. On days 5, 7, and 11 after inoculation with the Beverley strain, 10 ATS-treated and 10 NRS-treated mice were challenged, each with about 1,000 parasites of the RH strain. No serum was injected following challenge. An additional group of controls received parasites of the RH strain only. The effect on infection by treatment with NRS or ATS under the conditions described is illustrated in Fig. 4. The experiment was repeated, this time challenging the mice 3, 6, and 10 days, respectively, after the inoculation with Beverley Toxoplasma.

If the mice were challenged 6 days or later after the inoculation with the Beverley strain, a gradually growing resistance was observed. This partial immunity was not demonstrable when challenge was given with virulent parasites 3 or 5 days after the inoculation with cysts. Immunity to challenge was markedly reduced, although not eliminated, by the treatment with ATS. No Toxoplasma antibodies occurred at the time of challenge but high titers (1/1,280) were observed in NRS-treated mice 2 weeks after infection with the Beverley strain.

**DISCUSSION**

The present results indicate that administration of ATS to mice infected with *T. gondii* will aggravate the disease, prolong the period of parasitemia, and shorten the survival time. ATS may depress both humoral and cell-mediated immunity, but it is especially effective as a depressor of the latter (10). The present results indicate that the formation of *Toxoplasma* antibodies may be depressed by ATS. In concordance with earlier findings of minor protection conferred by passively administered antibody (4–6), heterologous humoral antibodies were not found to give any evident protective effect in the present studies. Therefore, probably suppression of antibody formation does not account for the observed influence of ATS on the course of disease. Also, the effect of ATS on the development of immunity to challenge was discernible before antibody formation was noted. G. Huldt (Ph.D. thesis, Tryckeri Balder AB, Stockholm, 1967) has proposed that interferon might be responsible for the
early development of immunity to *Toxoplasma*; interferon has been shown to protect cells from *Toxoplasma* infection (14). However, an early development of cell-mediated immunity is indicated by the results of Krahenbuhl et al. (9), who found positive delayed-hypersensitivity skin tests and positive macrophage migration-inhibition tests as early as 1 week after infection in *Toxoplasma*-infected guinea pigs. In view of the depressive effect of ATS on cell-mediated immunity (16) and the importance of such immunity in toxoplasmosis (5), the influence of ATS on the infection observed in the present experiments seems to be best explained by this effect.

Not even intense treatment with ATS invariably resulted in death of mice infected with the low-virulent *Toxoplasma* strain, although the treatment seemed to aggravate the disease as evidenced by an impaired weight gain. In chronically infected mice, treatment with ATS also appeared to adversely affect resistance to infection, as shown by weight curves, but in these cases, too, the ATS treatment did not always result in parasitemia or death of the animals. The latter observation has also been made by others (Beverley, *personal communication*). Thus, the effect of ATS was more evident on acquisition of immunity than on established immunity. This finding is in concordance with the recent results of Wilson and Frenkel (21) who studied the effect of ATS on infection in hamsters with *Besnoitia jellisoni*, a parasite which is closely related to *T. gondii*.

The effect of ATS on infection with a high-virulent strain of *Toxoplasma* was more difficult to demonstrate than that on infection with the low-virulent strain used. This is not surprising considering that animals infected with high-virulent *Toxoplasma* died so early after infection that immunity would not have had much chance to influence the course of the disease. In infection with low-virulent *Toxoplasma*, parasitemia was evidently prolonged by ATS. During the early stages of infection, however, there appeared to be more parasites in the blood in the NRS- than in ATS-treated animals. This finding should be compared with the results of Jandasek (8) who found a reduced number of deaths in vaccinia-infected rats treated with ALS. The latter results could be explained by a depletion of the free peritoneal cells serving as host cells for viral multiplication. The observation of a decreased initial parasitemia in ATS-treated mice might thus be due to a lymphopenia caused by the treatment with ATS, since *Toxoplasma* parasites multiply in lymphoid cells (7).

Treatment of rats with ATS virtually abolishes resistance to infection with *Plasmodium berghei* (17). Another protozoan, *Pneumocystis carinii*, is particularly prone to cause infection in "compromised" hosts, e.g., in hosts having a suppressed immune response (16), including patients treated with ALS (18). It thus seems that, in protozoan diseases, cell-mediated immunity should provide one of the most important defense mechanisms. For evaluation of vaccine efficacy, for example, in these diseases, a test for cell-mediated, instead of humoral, immunity might therefore be a good choice.

The present results suggest that treatment with ALS may activate a latent *Toxoplasma* infection or aggravate the course of the disease. Indeed, it is known that *Toxoplasma* infection in patients with compromised immunological functions may result in much more severe disease than in previously healthy subjects (7, 12, 13, 15, 20). Since the symptoms of toxoplasmosis are often nonspecific, a clinical diagnosis is usually difficult to achieve. Therefore, it seems advisable that patients treated with ALS should be routinely tested serologically for toxoplasmosis.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI05074 from the National Institutes of Allergy and Infectious Diseases. The skillful technical assistance of Inga-Mai Lindberg is gratefully acknowledged.

LITERATURE CITED


