[Jap. J. Parasit., Vol. 27, No. 4, 321-329, 1978]

Effects of Heterologous Antithymocyte Serum on *Toxoplasma gondii* Infections in Mice 2. Reactivation of Latent Infection

WALTER STAHL, GREGORY TUREK AND HASSAN A. GAAFAR
Division of Laboratories and Research, New York State Department of Health, Albany, New York, U.S.A.

(Received for publication; April 3, 1978)

Introduction

In the preceding paper (Stahl *et al.*, 1978), it was shown that heterologous antithymocyte serum (ATS), a potent and specific suppressant of cell-mediated immunity (CMI), markedly exacerbated primary, nonlethal *Toxoplasma* infections in non-immune mice. Presumably, potentiation was achieved by interference with the sequence of cellular events leading to acquired resistance, permitting the infection to progress into severe and clinically apparent disease.

The present investigation was designed to extend these findings by studying the effects of ATS on immune mice i.e., chronically infected mice possessing an acquired resistance to reinfection. Our objectives were: a) to note if ATS could overcome established resistance to reinfection; and b) to possibly explain the conditions necessary for reactivation of chronic, latent toxoplasmosis.

Materials and Methods

Parasites

The maintenance, quantitation, and infection procedures employing the nonlethal Cornell (CS) strain of *Toxoplasma* were as previously described (Stahl *et al.*, 1978). The lethal RH strain, obtained from Dr. A. Kimball, Cornell University School of Medicine, New York, NY, was maintained by intraperitoneal (ip) passage of trophozoites to clean mice every three or four days. Trophozoites were obtained from the peritoneal washings of acutely infected mice, and were counted in a Spencer Hemacytometer. One mouse-infection dose was 1×10^4 trophozoites in 0.5 ml of saline, given by ip injection.

Chronically infected mice

Normal, female, highly inbred Nya: NYLAR albino mice, 8 to 10 weeks of age, were obtained from an animal colony maintained by the New York State Department of Health. The mice were infected with the nonlethal CS strain of *T. gondii* by ip inoculation of 12 cysts, in 0.5 ml of brain emulsion. The infected mice were sorted into groups of 25, housed in large metal cages, and allowed unlimited food and water.

After a waiting period of at least 4 weeks, the CS infection was considered to have progressed from an acute phase into a chronic, latent phase, and the infected mice were suitable for experimental use. Mice incorporated into an experiment were sorted into groups of 10 and removed from the large communal cages to smaller plastic cages.

Procedures

Animal autopsy, tissue examination, *Toxoplasma* cyst counts, and ATS preparation and characterization were performed as described in detail in the preceding paper

(Stahl *et al*, 1978).

Experimental objectives and design

The initial experiments were designed first to confirm that chronically infected mice do indeed develop an immunity to reinfection, and then to determine if ATS can nullify this state of heightened resistance. We investigated the effect of reinfection alone, and the effect of normal rabbit serum (NRS) and ATS treatment on reinfection. The format and analysis of individual experiments are presented in the results.

The final experiments were to determine if ATS can reactivate latent infection. Toward this end, chronically infected asymptomatic mice were subjected to several different regimens of ATS treatment and observed for signs of acute infection. The effects of ATS treatment were categorized as clinical observations, percentage mortality, mean time to death, tissue examination, and cyst counts in the brain. Experimental variables were: length of chronic infection, and the dose, frequency, and number of antiserum injections.

The protocols of individual experiments are presented in the results.

Results

Confirmation of enhanced resistance of chronically infected mice to CS strain reinfection

Twenty normal mice and 18 mice chronically infected with the CS strain for one month were challenged with 12 cysts of the homologous CS strain of *T. gondii*. The normal mice exhibited transient signs of illness during the second and third weeks of the primary infection, consisting of ruffled fur, huddling, diarrhea, ascites, and a gradual loss of 10 to 15 percent of their body weight. During the fourth week, these symptoms abated and the lost weight was regained. No deaths were recorded during the 35 days of close, daily observation. In contrast, the chronically infected mice were much more resistant to the CS reinfection, exhibiting neither morbid changes nor mortality (Table 1).

Confirmation of enhanced resistance of chronically infected mice to RH strain reinfection

Thirty normal mice and 53 mice chronically infected with CS for one to two months were challenged with 1×10^4 RH trophozoites. The 30 normal mice quickly developed the typical fulminating RH infection and died en masse on the sixth day, with enormous numbers of trophozoites swarming in peritoneal exudate and tissues. The chronically infected mice, on the other hand, did exhibit heightened resistance to the RH reinfection. For the first seven days after challenge, neither morbidity nor mortality was observed. However, during the second week, ruffled fur, diarrhea, precipitous weight loss and lethargy appeared in quick succession. This was followed

Table 1 Acquired resistance to reinfection in mice chronically infected with CS *Toxoplasma gondii* and challenged with the CS and RH strains

Status of Mice	Challenge[a] Reinfection	No. dead/ No. tested	Survival (days)[b]	
			average	range
Non-infected	CS	0/20	30	—
Chronic CS		0/18	30	—
Non-infected	RH	30/30	6	—
Chronic CS		53/53	17	13-23

a CS challenge: 12 cysts ip; RH challenge: 1×10^4 trophozoites ip.

b Mice observed for 30 days after challenge.

by the deaths of all the mice, on days 13 through 23. The average period of survival was 17 days (Table 1). Trophozoites were detected in the peritoneal exudate of the challenged mice, but in much smaller numbers than seen in normal mice dying of a fulminant RH infection. Histologically, *Toxoplasma* trophozoites (presumed to be RH trophozoites) were seen in all the tissues examined, including the brain. The presence of RH in the brain was verified by subinoculation of brain tissue into clean mice, causing their death 4 to 5 days later by a typical overwhelming RH infection.

Abrogation of acquired resistance to CS strain reinfection

Fifty mice, infected for 1 month with the CS strain, were challenged with a CS reinfecting dose of 12 cysts per mouse. The mice were sorted into 3 groups and then treated with either NRS or ATS, as follows: Eleven mice received 3 injections of NRS on days 0, 2, and 4 of the challenge infection; 17 mice received 1 injection of ATS on day 0; and 22 mice received 3 injections of ATS on days 0, 2, and 4. All injections of serum were 0.25 ml aliquots, given ip.

The administration of 3 injections of NRS to the first group of reinfected mice had no discernible effects in terms of morbidity or mortality. Inoculation of the second group of 17 mice with 1 dose of ATS likewise failed to produce any distinct changes in the host-parasite relationship. One mouse did die on day 25, but the cause of death is unknown. Free trophozoites were not detected in peritoneal exudate, or in tissue impression smears. However, when the third group of reinfected mice was given ATS (x3), very distinct morbid changes occurred, culminating in the death of 86% (19/22) of the treated mice in approximately 3 weeks (Table 2). Within 7 days, the mice developed ruffled fur, diarrhea, weight loss, and an increasing listlessness. These symptoms continued to intensify, and mice began dying by day 16. Free trophozoites were readily detected in peritoneal exudate, in moderate numbers. Histologically, an exeensive toxoplasmic meningoencephalitis was seen. Although many cysts were seen in the brain, clusters were not detected.

Abrogation of acquired resistance to RH strain reinfection

In this experiment, the lethal RH strain of *T. gondii* was substituted for CS as the challenge reinfection. Ninety-seven mice chronically infected with CS for one to two months were challenged with 1×10^4 RH trophozoites. The mice were sorted into three groups, and then treated with NRS (x3), ATS (x1), or ATS (x3) as before.

The administration of NRS (x3) did not potentiate the RH reinfection. The NRS-treated mice died during the third week of reinfection, the average length of survival being 17 days. The administration of one dose of ATS on the day of RH challenge did alter the course of the infection. All 36 of the reinfected mice succumbed within days 8 through 20, with the average survival being 13 days. In neither the NRS (x3) nor the ATS (x1) groups of mice did the RH reinfection fulminate. Free trophozoites were found in peritoneal exudate until the day of death, but in much smaller numbers than those seen in non-immune mice dying of fulminating infection in 5 or 6 days.

Administering ATS (x3), however, distinctly exacerbated the RH reinfection. The 37 ATS (x3)-treated mice all died within a 5-day span, from days 5 through 9, exhibiting a clinical picture indistinguishable from that seen in normal, non-immune mice dying from RH. The infection was fulminating, with a profusion of RH trophozoites in peritoneal exudate and tissues. The average survival of the ATS (x3)-treated mice was 6 days, one-third the survival time of the NRS-treated mice (Table 2).

ATS-induced reactivation of latent toxoplasmosis

Two experiments were performed, differing with regard to the following experimental variables: single or multiple injections of serum; administration of serum at 24 or

Table 2 Effects of normal rabbit serum (NRS) and heterologous antithymocyte serum (ATS) on resistance to reinfection in mice chronically infected with CS *Toxoplasma gondii* and challenged with the CS and RH strains

Challenge[a] Reinfection (strain)	Treatment[b] of mice after reinfection	Mortality No. dead/ No. tested	Survival of mice (days)[c]	
			average	range
CS	NRS	0/11	30	—
	ATS (x 1)	1/17	30	—
	ATS (x 3)	19/22	18	16-24
RH	NRS (x 3)	24/24	17	13-22
	ATS (x 1)	36/36	13	8-20
	ATS (x 3)	37/37	6	5-9

a CS challenge : 12 cysts ip ; RH challenge : 1×10^4 trophozoites ip.
b NRS and ATS (x 3) : 3 injections, 0.25ml ip, on days 0, 2, 4 of reinfection. ATS (x 1) : 1 injection on day 0.
c Mice observed for 30 days after challenge.

Table 3 Effects of normal rabbit serum (NRS) and heterologous antithymocyte serum (ATS) on chronic, latent *Toxoplasma* infections in mice

Treatment[a] of chronically infected mice	Injection schedule (hours)	Clinical consequences of treatment	Percent mortality in 30 days (No. dead/No. tested)		Mean time to death (days)	Number of cysts in brain	
						average	range
Untreated	48	none	0	(0/20)	—	1,100	900-1,400
NRS (x 7)		none	0	(0/18)	—	1,400	1,000-1,950
ATS (x 1)		none	0	(0/18)	—	1,380	1,050-1,800
ATS (x 3)		relapse	25	(9/34)	21	3,400	2,400-4,700
ATS (x 7)		relapse	70	(17/24)	12	4,600	3,800-5,500
Untreated	24	none	0	(0/14)	—	N. D.[b]	
NRS (x 3)		none	0	(0/8)	—	N. D.	
ATS (x 3)		relapse	45	(8/18)	12	N. D.	

a NRS and ATS : 1, 3, or 7 injections of 0.25ml of serum, ip, at 48-and 24-hour intervals.
b N. D. : not determined.

48-hour intervals ; and the length of the chronic CS-infection. The results of the experiments are summarized in Table 3.

In the first experiment, five groups of chronically infected, symptom-free mice were assembled. The mice were four months old, and had been chronically infected for one month. The infected animals were well-groomed and sleek, outwardly indistinguishable from normal non-infected mice. Two of the five groups of infected mice served as controls ; the first group (20 mice) receiving no treatment, the second group (18 mice) receiving seven injections of NRS. The remaining three groups of mice received 1, 3, or 7 injections of ATS, respectively. All injections of serum were given ip, in 0.25 ml volumes, at 48-hour intervals.

The administration of seven injections of NRS or a single injection of ATS did not reactivate the latent infection. The mice remained asymptomatic, and free trophozoites were not detected either in peritoneal exudate or in tissue impression smears.

The group of 34 mice which received three injections of ATS gradually began to

exhibit ruffled fur and a loss of weight 12 to 14 days after treatment. Up to this time, free trophozoites were detected in small numbers in peritoneal exudate but thereafter rarely observed. During the third and fourth week of observation, neurologic symptoms appeared and 9 of the 34 treated mice died. Squash preparations and histopathology of the brain revealed numerous trophozoites, many in packets. The cause of death was diagnosed as meningoencephalitis.

In the group of 24 mice that received 7 injections of ATS, ruffled fur and diarrhea appeared within the first week of treatment, and mice began dying during the second week. The mean time to death was calculated as 12 days, as opposed to 21 days for the mice that received 3 injections of ATS. Free trophozoites were found in peritoneal exudate and tissue smears, but not in the enormous numbers indicative of a fulminating infection. Eventually 17 of the 24 mice in this group died, most within the second week of treatment. Neurological symptoms were not seen as a prelude to death.

After 30 days of observation, cerebral cyst counts were performed on representatives of the 5 groups of mice. Mice experiencing reactivation of infection possessed 2 to 3 times as many cysts as the mice in which reactivation did not occur.

In the second experiment, ATS was administered to mice chronically infected for two months. Forty infected mice were sorted into three groups; the first group (14 mice) received no treatment, the second (8 mice) received NRS, and the third (18 mice) received ATS. All injections of serum were given ip in 0.25 ml volumes at 24-hour intervals.

As in the first experiment, NRS had no visible effects on the infected mice, but the multiple injections of ATS quickly reactivated the latent CS infection. Trophozoites reappeared and persisted in peritoneal exudate for approximately 10 days, but were seen infrequently thereafter. In the brain, however, trophozoites were rarely seen before the tenth day post-treatment. Of the 18 mice that received ATS, 8 died of acute toxoplasmosis within the second week following treatment. The mean time to death was 12 days. These data show that the 3 injections of ATS at 24-hour intervals resulted in twice the percentage mortality in half the time as when the 3 injections of ATS were administered at 48-hour intervals.

Discussion

In the present study, the acquisition of resistance to reinfection was verified in mice chronically infected with *Toxoplasma* for one and two months. Tested against reinfection with the lethal RH strain, acquired resistance manifested as a threefold prolongation in host survival, from six days (non-immune) to 17 days (immune). In contrast, resistance to CS reinfection appeared to be absolute, with the challenged mice exhibiting neither morbidity nor mortality.

This clearly defined state of resistance to reinfection was quickly reversed by three injections of ATS, on days 0, 2, and 4 of the reinfection. One injection of ATS, on day 0, was ineffectual. In the ATS-treated mice, RH challenge led to a rapidly progressing, fulminant process with 100 percent mortality in five to nine days. The cause of death appeared to be a toxoplasmic pneumonitis, myocarditis, and peritonitis. Clinically and pathologically, therefore, the course of the RH reinfection in the ATS-treated immune mice was equivalent to the overwhelming RH infection seen in non-immune mice.

On the other hand, ATS treatment after reinfection with the CS strain led to a gradually evolving neurologic syndrome of some three to four weeks duration, with the eventual death of most of the ATS-treated mice. Pathologically, the cause of death was ascribed to an acute meningoencephalitis. A comparison of the pathogenesis of primary and secondary CS infections in mice given multiple injections of ATS revealed some interesting differences. The modified primary infection was a ra-

pidly progressing disease that involved all organ systems and killed most mice within *two weeks* (Stahl *et al.*, 1978). Trophozoites of the CS strain persisted in peritoneal exudate and in tissues for the length of the experiment, indicating the tissue-clearing mechanisms of the host were severely impaired. In the brain, the development of many cysts in large clusters was a constant and conspicuous finding. In contrast, the CS reinfection in the ATS-treated immune mice evolved less rapidly, and ran a more protracted course, with many mice surviving into the fourth week of the infection. Small numbers of trophozoites were detected in peritoneal exudate during the first seven to 10 days after challenge, and thereafter only rarely seen. This was interpreted as indicating that the host's tissue-clearing mechanisms were again operative after initially being suppressed by the ATS.

It is of interest to note that the time of reinfection, the chronically infected mice were seropositive for *Toxoplasma* antibodies (Stahl *et al.*, 1978). Although ATS may depress the formation of *Toxoplasma* antibodies to a primary infection (Strannegard and Lycke, 1972), it does not have any known effects upon antibodies already present in the circulation. Therefore, the ability of ATS to effectively abrogate resistance to reinfection illustrates convincingly the minor, if any, role of humoral antibodies in protective immunity to *Toxoplasma*.

Much of present-day knowledge concerning CMI and acquired resistance to intracellular parasitism comes from the contributions of Mackaness and North. These workers have shown conclusively that resistance to the intracellular bacterial parasite, *Listeria monocytogenes*, is mediated by specifically sensitized thymus-dependent lymphocytes (T-cells), but is expressed by activated macrophages (Mackaness, 1962, 1969; Mackaness and Hill, 1969; North, 1969 a, b; 1974)- In the *Listeria* system, activated macrophages achieved peak numbers on day 6 of an initial infection, and declined in number over the succeeding two weeks (North and Deissler, 1975). Neither the sensitized T-cells nor the activated macrophages are long-lived; neither cell, therefore, persisted indefinitely. However, a long-lasting state of heightened resistance to reinfection remained in effect and was related to the capacity of immune mice to generate mediator T-cells faster and in larger numbers than non-immune mice (North, 1975).

In the present study, using ATS as a sensitive and specific eradicator of T-cells, we obtained data corroborating the above interpretation. The population of cells that mediate immunity to *Toxoplasma* reinfection apparently has many of the same properties as those mediating immunity to primary infection, i.e., thymus-dependent lymphocytes, as evidenced by their sensitivity to ATS, and the resultant loss of acquired resistance to reinfection.

Having thus established that mice chronically infected with *Toxoplasma* possess an ATS-labile acquired resistance to reinfection, we can now logically proceed to consider activation of latent infection. It is evident from our experimental results that heterologous ATS is capable of reactivating latent toxoplasmosis, transforming chronic, clinically inapparent infections into acute, disseminated disease. The ability of ATS to provoke relapse was related to the amount of antiserum administered, and to the number and frequency of injections. The greater the amount and the more frequent the injections, the more severe the clinical relapse, in terms of morbidity and mortality.

Reactivated infections evolved slowly compared to ATS-modified primary infections, and ran a protracted course in mice receiving three injections of ATS. Although 30 percent of the relapsed mice eventually died, few of the animals ever appeared critically ill. In all cases, free *Toxoplasma* trophozoites reappeared in the tissues and peritoneal exudate following multiple injections of ATS, but not in the enormous numbers seen in ATS-modified primary infections. Typically, small numbers of trophozoites

were detected in the peritoneal exudate for about seven to ten days after treatment, but thereafter were seen only rarely. In the brain, numerous packets of intra and extracellular trophozoites and wide areas of necrosis and inflammation were seen. The cause of death was thought due to acute toxoplasmic meningoencephalititis.

These histopathologic findings are similar to those described by Frenkel (1957, 1975), who showed that cortisone and radiation treatment induced relapse in hamsters chronically infected with *Toxoplasma*. Parasite proliferation resumed, and the hamsters died of a fatal encephalitis or pneumonia. An active retinochoroiditis was also detected in some hamsters. Pathologically, the animals developed multifocal necrotic CNS lesions comparable to the toxoplasmic encephalitis seen in immunologically compromised humans (Frenkel, 1975). Frenkel visualized each CNS lesion as being the histologic correlate of a single ruptured cyst from which trophozoites emerged, parasitized, and destroyed neighboring cells, thereby creating a large lesion with a necrotic center. The rupture of previously latent cysts and the renewed multiplication of trophozoites were related by Frenkel to defects in host immunity. It was reasoned the lesions of relapse materialized in the CNS rather than in visceral organs because immunity to reactivation may have remained relatively intact in the peripheral tissues.

The actual mechanism of relapse is obscure. Frenkel (1953, 1956, 1961, 1975) has speculated repeatedly that relapse begins in the CNS with cyst rupture, and the release and proliferation of the trophozoites therein. Although this hypothesis is logical and attractive, we are unable to confirm it. Numerous gross and microscopic examinations of infected mouse brains have not uncovered either ruptured cysts or cyst-wall debris (Waaij, 1959 ; Stahl *et al.*, 1965, 1966a, b, 1976, 1978). To Frenkel's hypothesis we now add our own thoughts as to the origin of the proliferative forms seen in relapse : i) a continuous escape of trophozoites from any cyst in any tissue, and/or ii) the persistence of small, localized foci of active infection in various tissues, with frequent spillover.

Regardless of source, *Toxoplasma* trophozoites do reappear periodically in the blood of chronically infected hosts (Remington, 1961). The immunologically intact host is able to quickly contain these flare-ups because it is in a state of heightened resistance to reinfection. The host with diminished cell-mediated immunocapacity, rendered so by the administration of immunosuppressive drugs or by underlying lymphoreticular disorders, loses the power to contain the parasite. This is followed by proliferation, dissemination, and recolonization of the host. Via infected macrophages and leukocytes, trophozoites are again transported to the CNS. We believe that the reappearance of proliferating forms (trophozoites) in the brain is a sequel of relapse, rather than its cause.

By using graded doses of ATS, to achieve less than maximum cell-mediated immunosuppression, we were able to follow the course of reactivated *Toxoplasma* infections in hosts of differing immuno-capacity. Vigorous immunosuppression quickly led to a rapidly progressing, fulminant disease, where all organ systems were involved and death appeared due to visceral organ failure rather than encephalitis. With moderate immunosuppression, however, a protracted neurological syndrome evolved, with encephalitis a prominent pathological finding.

To sum up, our view is that ATS does not actively provoke relapse through induced cyst rupture, but rather, through its highly selective effects on CMI, permits reactivation to occur by providing the parasite an immunologically less hostile environment.

Summary

Mice chronically infected with nonlethal CS strain of *Toxoplasma gondii* were confirmed resistant to reinfection with either

the homologous CS strain or the lethal RH strain. It was shown that this state of acquired resistance could be effectively abrogated by the administration of ATS, and that reactivation of the chronic, latent infection would occur. Reactivated infections ran a milder, more protracted course than did ATS-modified primary infections, clearly indicating that acqusition of resistance was more sensitive to the effects of ATS than was established immunity.

The possible sequence of events leading to reactivation of a latent infection was discussed. Our interpretation was that ATS did not *cause* relapse through any direct effects on the parasite, but rather *permitted* reactivation to occur by its suppressive effects on the cell-mediated immune capacity of the host.

References

1) Frenkel, J. K. (1953): Host, strain and treatment variations as factors in the pathogenesis of toxoplasmosis. Amer. J. Trop. Med. Hyg., 2, 390-416.
2) Frenkel, J. K. (1956): Pathogenesis of toxoplasmosis and of infections with organisms resembling *Toxoplasma*. Ann. N. Y. Acad. Sci., 64, 215-251.
3) Frenkel, J. K. (1957): Effects of cortisone, total body irradiation, and nitrogen mustard on chronic, latent toxoplasmosis. Amer. J. Path., 33, 618-619.
4) Frenkel, J. K. (1961): Pathogenesis of toxoplasmosis with a consideration of cyst rupture in *Besnoitia* infection. Survey Opthal., 6 (Part II), 799-825.
5) Frenkel, J. K., Nelson, B. M., and Arias-Stella, J. (1975): Immunosuppression and toxoplasmic encephalitis. Human Path., 6, 97-111.
6) Mackaness, G, B. (1962): Cellular resistance to infection. J. Exp. Med., 116, 381-406.
7) Mackaness, G. B. (1969): The influence of immunologically committed lymphoid cells on macrophage activity *in vivo*. J. Exp. Med., 129, 973-992.
8) Mackaness, G. B., and Hill, W. C. (1969): The effect of antilymphocyte globulin on cell-mediated resistance to infection. J. Exp. Med., 129, 993-1012.
9) North, R. J. (1969a): Cellular kinetics associated with the development of acquired cellular resistance. J. Exp. Med., 130, 299-314.
10) North, R. J. (1969b): The mitotic potential of fixed phagocytes in the liver as revealed during the development of cellular immunity. J. Exp. Med., 130, 315-326.
11) North, R. J. (1974): Cell-mediated immunity and the response to infection, in "Mechanisms of cell-mediated immunity" (McCluskey, R. T., and Cohen, S., ed), 185-220. Wiley and Sons, New York.
12) North, R. J. (1975): Nature of "memory" in T-cell-mediated antibacterial immunity: anamnestic production of mediator T cells. Infect. Immunity, 12, 754-760.
13) North, R. J., and Deissler, J. F. (1975): Nature of "memory" in T-cell-mediated antibacterial immunity: cellular parameters that distinguish between the active immune response and a state of "memory". Infect. Immunity, 12, 761-767.
14) Remington, J. S., Melton, M., and Jacobs, L. (1961): Induced and spontaneous recurrent parasitemia in chronic infections with avirulent strains of *Toxoplasma gondii*. J. Immunol., 87, 578.
15) Stahl, W., Matsubayashi, H., and Akao, S. (1965): Effects of 6-mercaptopurine on cyst development in experimental toxoplasmosis. Keio J. Med., 14, 1-12.
16) Stahl, W., Matsubayashi, H., and Akao, S. (1966a): Murine toxoplasmosis: development of cysts in bizarre clusters. Jap. J. Parasit., 15, 44-47.
17) Stahl, W., Matsubayashi, H., and Akao, S. (1966b): Modification of subclinical toxoplasmosis in mice by cortisone, 6-mercaptopurine and splenectomy. Amer. J. Trop. Med. Hyg., 15, 869-874.
18) Stahl, W., Collins, D. N., Benitez, P., Turek, G., and Gaafar, H. (1976): *Toxoplasma gondii:* disease patterns in mice treated with the folate antagonist methotrexate. Exp. Parasit., 39, 135-142.
19) Stahl, W., Turek, G., and Gaafar, H. (1978): Effects of heterologous antithymocyte serum on *Toxoplasma gondii* infections in mice. 1. Potentiation of primary, nonlethal infection. Jap. J. Parasit., 27, 231-238.
20) Strannegard, O., and Lycke, E. (1972): Effect of antithymocyte serum on experi-

mental toxoplasmosis in mice. Infect. Immunity, 5, 769-774.
21) Waaij, D. van der. (1959): Formation, growth and multiplication of *Toxoplasma gondii* cysts in mouse brains. Trop. Geogr. Med., 11, 345-360.

マウスにおける *Toxoplasma gondii* 感染に対する異種 Antithymocyte Serum の効果について
2. 不顕性感染よりの再発

WALTER STAHL, GREGORY TUREK AND HASSAN A. GAAFAR
(ニューヨーク州保健局)

Toxoplasma gondii の弱毒 CS 株を感染させた慢性感染マウスは同じ CS 株，または強毒 RH 株に対する再感染に抵抗をもつことが確認された．この獲得した抵抗性は，ATS の投与により明らかに阻害され，そして慢性不顕性感染の状態から再び発症することが示めされた．再発した感染は ATS によって変化させられた初めの感染よりも軽症で，より長い経過をたどる．このことは明らかに，ATS の効果は免疫の確立に対するよりも抵抗性を獲得することに対して感受性がより高いことを示していた．不顕性感染の再発症に至る過程についての著者の解釈は，ATS が虫体に対して直接的な影響を及ぼして再発を起させるものではなく，むしろ宿主の細胞性免疫能に対する ATS の抑制効果によって再発が起ることを示唆していると考えた．