Effect of Neonatal Thymectomy and Antithymocytic Serum on Susceptibility of Rats to *Mycobacterium leprae* Infection[1] (35908)

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The limited local infection induced in mice and rats after footpad inoculation with *M. leprae* (1–3) has led to the investigation of various methods of immunosuppression as a means of enhancing the infection.

Mice thymectomized as adults and given whole-body irradiation (900 R, followed by infusion of syngeneic bone marrow) were substantially more susceptible to *M. leprae* infection than normal mice (4–6). Moreover, in these animals *M. leprae* spread systemically to remote sites in the body (4, 6). Neither adult thymectomy nor X-irradiation alone had a significant effect on the infection (5). However, a prolonged course of antilymphocytic globulin administered to thymectomized mice significantly enhanced susceptibility to *M. leprae* infection (7).

Neonatal thymectomy alone in both mice and rats not only exerts a profound influence on subsequent immunologic responsiveness but also appears to accomplish, by more simple and direct means, the same type of impairment as that brought about by adult thymectomy accompanied by lethal irradiation or antilymphocytic serum (8, 9). Neonatal thymectomy, however, is frequently followed by severe, fatal wasting disease, especially in mice (8–10).

Rats are relatively resistant to wasting disease (9) and reportedly are as susceptible as the mouse to footpad infection with *M. leprae* (3). We have therefore investigated the course of *M. leprae* infection in neonatally thymectomized rats, as well as the additional effect of antithymocytic serum (ATS) in these animals.

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Materials and Methods. Buffalo and Lewis rats were obtained from Simonsen Laboratories, Gilroy, California. BALB/c mice were taken from our own colony.

Neonatal rats were anesthetized by hypothermia, which consisted of total immersion in crushed ice for 5–8 min. Thymectomy was carried out within 24 hr after birth, with the aid of a dissection microscope at 15× magnification. The upper half of the sternum was removed, exposing the thymus. The latter was freed from its attachments in the anterior mediastinum and removed by blunt dissection. Inspection of the area was made at higher magnification to be certain no thymic tags remained. The incision was closed with two 6-0 silk sutures and sealed with collodion. Breathing recommenced after the rats were placed under an electric bulb for a few minutes. They were then returned as a group to the mother. Mortality resulting from this procedure was about 30%.

ATS was prepared in New Zealand white rabbits from the thymuses of 5- to 6-week-old female Buffalo or Lewis rats using a method similar to that described by Gray *et al.* (11) for antilymphocytic serum. The sera were inactivated at 56° for 30 min and absorbed with homologous rat red blood cells at 37° for 30 min. Ratio of cells to serum was 1:5. Each batch of ATS had thymocyte agglutinin titers of between 1:40 and 1:80 (12), and 0.5 ml given ip reduced peripheral lymphocytes by as much as 80% in 24 hr.

The *M. leprae* used in these experiments was the Shepard mouse passaged strain obtained from Louis Levy, U.S. Public Health Service Hospital, San Francisco. The methods for growing and counting the *M. leprae,* as well as the processing of the footpads and testes, were those described by Shepard (1) and Shepard and McRae (13). All inocula

TABLE I. The Effect of Neonatal Thymectomy and Antithymocytic Serum (ATS) on *M. Leprae* Infection in Buffalo Rats.

Months after inoculation	No. of acid-fast bacilli ($\times 10^6$)/footpad after indicated treatment[a]					
	None	Weekly ATS only[b]	Weekly ATS at 4 months[c]	Thymectomy only	Thymectomy + ATS weekly[b]	Thymectomy + ATS at 4 months[c]
5	—	—	—	2.79 (2)	—	—
6	0.098 (4)[d]	0.884 (4)	0.369 (2)	9.55 (8)	2.89 (4)	4.79 (10)
7	0.218 (4)	0.043 (2)	0.214 (2)	5.87 (12)	2.34	12.9 (4)
8	0.199 (4)	0.142 (2)	0.547 (2)	8.15 (12)	1.81 (2)	3.17 (4)
10	0.089 (2)	0.182 (2)	0.042 (2)	5.66 (8)	1.28 (2)	16.02 (4)
11	0.176 (2)	0.086 (2)	—	0.146 (2)	10.8 (2)	0.283 (2)
12	0.164 (4)	0.090 (2)	0.021 (2)	1.565 (6)	6.98 (4)	7.11 (4)
13	—	—	—	—	26.4 (2)	—
14	0 (2)	0.252 (2)	—	0.685 (8)	2.00 (2)	20.6 (4)
15	—	—	—	0 (2)	—	0.093 (2)
18	0.128 (2)	—	0.183 (2)	0.960 (2)	—	0.094 (2)
19	—	—	—	0.128 (2)	—	—
Maximum increase over inoculum	43	177	109	1900	5280	4000

[a] Rats inoculated in each hind footpad with 5×10^3 *M. leprae*.

[b] ATS, 0.5 ml ip weekly for 10 months from day of inoculation of *M. leprae*.

[c] ATS, 0.5 ml ip weekly for 6 months, starting 4 months after *M. leprae* inoculation.

[d] Number of footpads counted is given in parentheses.

were tested in appropriate culture media to confirm that no cultivable acid-fast organisms were present. They also were inoculated into the footpads of intact BALB/c mice to be certain they remained localized and had the growth patterns typical of *M. leprae* in that host. In addition, organisms harvested from the footpads of rats were inoculated into footpads of BALB/c mice to test for viability.

In experiments, between 5×10^3 and 2.78×10^5 *M. leprae* were inoculated into each hind footpad of all rats and into each testis of the male rats. Each footpad and testis was processed, and the organisms were enumerated, separately. Results are reported as average for each pair.

Results. The combined results of 2 experiments in which we compared the multiplication of *M. leprae* in the foot pads of normal, thymectomized, and thymectomized–ATS-treated Buffalo rats are summarized in Table I. The intact Buffalo rats had a low susceptibility to infection with *M. leprae* since there was only a 43-fold maximum increase of the initial inoculum of 5×10^3 organisms. Weekly administration of ATS for 6 to 10 months to intact Buffalo rats resulted in a maximum increase only 4-fold greater than that found in untreated animals. However, neonatal thymectomy resulted in significantly greater susceptibility to *M. leprae* infection. The maximum increase to a mean of 9.55×10^6 organisms/footpad was 44 times greater than that seen in the intact rats. The peak was reached 6 months after inoculation, and the numbers remained at a high level until the tenth month after which a sharp drop occurred. However, by the ninth month after inoculation, the organisms were no longer viable.

Although higher levels of multiplication were reached in footpads of both the thymectomized–ATS-treated groups, the differences could not be considered to be significantly greater than in the thymectomized group. It is worth noting, however, that viable *M. leprae* were recovered 14 months after inoculation from both the footpads and the testes of rats in the thymectomized group treated with ATS starting 4 months after inoculation.

In general, growth of *M. leprae* in the testes of Buffalo rats was poor, rarely approaching or exceeding 10^5 organisms/testis, irrespective of the treatment given. However, the only 2 instances in which the numbers of organisms reached or exceeded 10^7/testis were in thymectomized–ATS-treated rats.

The greatest number of *M. leprae* found in an individual footpad of a nonthymectomized Buffalo rat after inoculation with 5×10^3 organisms was 4.3×10^5. This appeared to be the maximum replication that would occur in the footpads of intact Buffalo rats, because when 2.8×10^5 *M. leprae* were inoculated, no multiplication occurred over a period of 13 months. The same was true of thymectomized Buffalo rats in that the upper limit of multiplication of *M. leprae* remained constant irrespective of the size of the original inoculum. The maximum number of organisms found in a single footpad after inoculation of 2.8×10^5 was 2.9×10^7; whereas after inoculation with 5×10^3, the ceiling was 1.7×10^7.

Shepard and Habas (14) had shown that different strains of mice varied widely in their susceptibility to infection with *M. leprae*. We therefore also investigated Lewis rats since this strain, when thymectomized within 24 hr of birth, was known to develop immunological impairment (15). The results of footpad inoculation with 5×10^3 *M. leprae* are summarized in Table II. Lewis rats appeared to be more susceptible to *M. leprae* infection than Buffalo rats, although multiplication proceeded at a slower rate, reaching a peak at about 14 months. The level of multiplication in untreated Lewis rats was 10-fold greater than in comparable Buffalo rats, and thymectomy alone resulted in about another 10-fold increase. The results in the thymectomized group that received ATS for 10 months beginning with the day of infection appeared to be similar to those of the group that only were thymectomized. On the other hand, the thymectomized group in which ATS was started 4 months after infection and continued for 6 months consistently had higher counts than any of the other groups, reaching a maximum of 9.81×10^7 acid-fast bacilli/footpad at 15 months. The one rat

TABLE II. The Effect of Neonatal Thymectomy and Antithymocytic Serum (ATS) on *M. Leprae* Infection in Lewis Rats.

Months after inoculation	No. of acid-fast bacilli ($\times 10^6$)/footpad after indicated treatment[a]				
	None	Weekly ATS only[b]	Thymectomy only	Thymectomy + ATS weekly[b]	Thymectomy + ATS at 4 months[c]
5	—	—	1.55 (2)	—	—
6	0.83 (2)[d]	2.79 (2)	3.76 (4)	4.47 (2)	7.85 (2)
7	0.69 (2)	0 (2)	6.07 (4)	5.37 (2)	8.40 (2)
8	0.07 (2)	0.50 (4)	5.34 (4)	2.93 (2)	5.28 (2)
12	0.16 (2)	2.37 (2)	16.46 (8)	3.40 (2)	16.96 (4)
14	2.30 (2)	1.71 (2)	23.30 (2)	56.10 (2)	70.80 (2)
15	0.91 (2)	0.49 (2)	4.78 (2)	7.55 (2)	98.10 (2)
16	0.39 (2)	0.97 (2)	24.70 (2)	—	—
18	0.99 (2)	—	4.22 (2)	—	61.40 (2)
Maximum increase over inoculum	460	558	4940	11,220	19,620

[a] Rats inoculated in each hind footpad with 5×10^3 *M. leprae.*
[b] ATS, 0.5 ml ip given weekly for 10 months from day of inoculation of *M. leprae.*
[c] ATS, 0.5 ml ip weekly for 6 months, starting 4 months after *M. leprae* inoculation.
[d] Number of footpads counted is given in parentheses.

from this group that was killed at 18 months is of additional interest even though the level of 6.14×10^7 organisms/footpad is below the peak. At 17 months, the ears of this animal were examined for acid-fast bacilli, and none were found. However, at the time the animal was killed, the left front footpad, which had not been inoculated, contained 3.62×10^5 acid-fast bacilli. None were found in the right front foot.

The results of inoculating the male Lewis rats in the testes are shown in Fig. 1. Although only a small number of rats were examined (each point on the curve represents the testes from one animal), the results are impressive. The largest number of *M. leprae* found in the testes of normal rats was 6.73×10^4. At the termination of the experiment, the growth of *M. leprae* in the testes of the rats thymectomized and treated with ATS at 4 months appeared still to be in the logarithmic phase of growth. The 2.9×10^8 organisms in each testis represented a generation time of 29.1 days, which is not incompatible with that found by Shepard and Habas (14) in intact mice.

Just before termination of the experiment, a rat from the latter group died 448 days after inoculation, and although the testes were atrophied, they contained a total of 4.8×10^8 acid-fast bacilli. Both hind footpads from this animal contained 3.4×10^7 organisms. The rat representing the highest point on the curve was killed 459 days after inoculation. The testes contained a total of 5.8×10^8 organisms and both hind or inoculated footpads had a total of 1.96×10^8 organisms. No acid-fast bacilli were found after an extensive search of the front foot pads and the right ear. However, the left ear did contain small numbers of acid-fast bacilli, amounting to 1.73×10^4.

Discussion. Intact Buffalo and Lewis rats were found to have a low order of susceptibility to footpad infection with *M. leprae,* although the latter were slightly more susceptible. When ATS was administered weekly to intact rats for periods up to 10 months, susceptibility was increased slightly in Buffalo, but not at all in Lewis rats. However, either neonatal thymectomy or neonatal thymectomy combined with ATS for up to 10 months had a great enhancing effect on footpad infection. In Buffalo rats, the maximum number of *M. leprae* increased more than 120 times that found in intact rats.

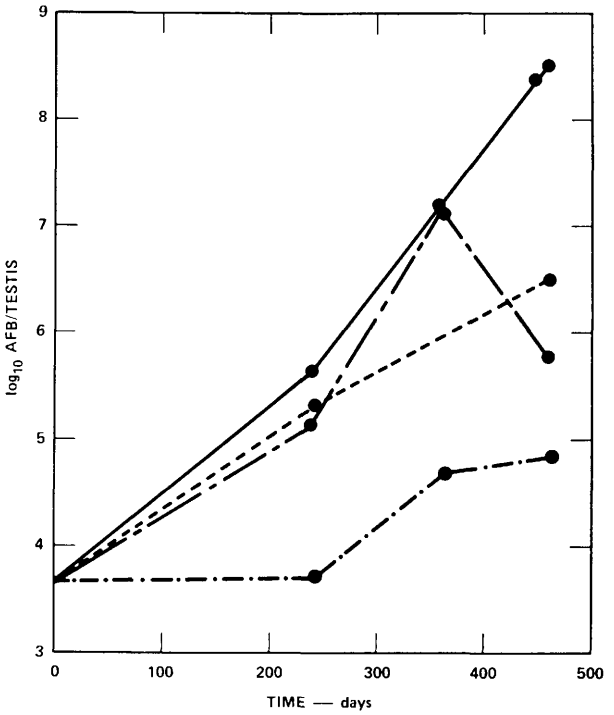

FIG. 1. Effect of neonatal thymectomy and antithymocytic serum (ATS) on testis infection with *M. leprae* in Lewis rats. Inoculum was 5×10^3 *M. leprae*/testis, and each point on the curves represents the mean count of the testes from one rat. Normal rats (—●—); thymectomized rats (● - -); thymectomy + 0.5 ml of ATS weekly for 10 months from day of inoculation of *M. leprae* (●— - —); thymectomy + 0.5 ml of ATS weekly for 6 months starting 4 months after *M. leprae* inoculation (●—).

The differences between intact and immunologically supressed Lewis rats were not so striking due to their somewhat greater susceptibility to *M. leprae* infection. However, much greater levels of multiplication were reached in the thymectomized and the thymectomized–ATS-treated Lewis rats than in comparable groups of Buffalo rats. It is also interesting to note that ATS treatment of thymectomized Lewis rats appeared to be more effective in enhancing infection when it was started 4 months after infection rather than at the time of infection. The reason for this is unknown, but at each time interval between 6 and 15 months after inoculation, the number of organisms in the footpads of Lewis rats in the former group was consistently greater. Although not so striking, the results were somewhat similar in comparable groups of Buffalo rats.

The testes of the intact Lewis rats, like those of the intact Buffalo rats, showed a very low order of susceptibility to *M. leprae* infection. However, in the former, both thymectomy and thymectomy plus weekly ATS had a strong enhancing effect. Again, the most effective treatment was thymectomy plus weekly ATS started 4 months after inoculation. The number of organisms in this group increased almost logarithmically until the last male was killed 15 months after inoculation. The increase of 57,600-fold to 2.88×10^8 organisms/testis is most significant compared with the 13-fold increase which occurred in the number of acid-fast bacilli in the testes of normal rats.

We have seen only a limited spread of *M. leprae* to uninoculated sites, rather than the massive spread described by Rees and Waters (16) in thymectomized–irradiated CBA mice. However, the results in Lewis rats reported herein are comparable to those de-

scribed by Gaugas in both thymectomized–irradiated (5) and thymectomized–antilymphocytic serum (ALS)-treated mice (7); as well as by Shepard and Congdon (6) in thymectomized-irradiated mice. The use of the rat for the study of leprosy may offer an advantage in that, compared with mice, they are relatively free of adventitious agents that might cause overt disease only in chronically immunosuppressed animals. Thus, Gaugas *et al.* (17) reported the premature termination of an experiment with *M. leprae* because all of 48 thymectomized–ALS-treated mice developed polyoma virus-induced tumors. None of 48 thymectomized or 24 intact controls developed tumors. In our studies to date, the neonatally thymectomized–ATS-treated rat has proven to be a remarkably healthy animal.

Summary. Neonatal thymectomy and neonatal thymectomy plus ATS resulted in a marked increase in susceptibility of both Buffalo and Lewis rats to infection with *M. leprae.* In Buffalo rats, increase in susceptibility was limited to footpad infection. In Lewis rats, this was extended to include testis infection where the organisms in thymectomized–ATS-treated rats were found to be still in the logarithmic phase of growth 15 months after inoculation.

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