Combined Rifampin and Dapsone Chemotherapy of *Mycobacterium leprae* Infection of the Neonatally Thymectomized Lewis Rat[1]

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We have previously reported that the neonatally thymectomized Lewis rat (NTLR) infected with large populations of *Mycobacterium leprae* responded to treatment with dapsone (4,4'-diaminodiphenylsulfone, DDS) in much the same way as do patients with lepromatous leprosy ([1]). The minimal effective dose (MED) of DDS (5×10^{-5}g%) incorporated in the diet provided the minimal inhibitory concentration (MIC) of DDS for *M. leprae* in male NTLR. However, this dose was not associated with killing of the organisms as measured by mouse foot pad inoculation. Treatment of NTLR with a 100-fold larger dose of DDS both killed *M. leprae* and reduced their numbers. The rate of killing was approximately equal to that measured in previously untreated patients with lepromatous leprosy who were undergoing initial treatment with DDS in a daily dose of 50 to 100 mg ([13]). Although the dead organisms appeared to be removed from the tissues of the NTLR at a faster rate than that encountered in patients, the results indicated that the NTLR probably would be suitable for chemotherapeutic studies relevant to man. In addition, it seemed likely that the NTLR could serve as a model for the development of treatment regimens designed to eradicate persisting *M. leprae*.

In the present experiments we have extended our earlier work to include studies of the effects of rifampin (RMP), a rapidly mycobactericidal drug ([5, 7, 12, 13]), both alone and in combination with DDS, on chronic *M. leprae* infection in the NTLR. Our primary objective has been to provide information on persistence of *M. leprae* that might be useful in the design of therapeutic regimens for patients with multibacillary leprosy.

MATERIALS AND METHODS

Animals. Pregnant inbred Wistar/Lewis rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, U.S.A. Adult male and female mice of the inbred BALB/c strain were obtained from our own colonies.

Thymectomy. This procedure was carried out in all instances between five and 16 hours after birth by the method previously described in detail by us ([1]).

Mycobacterium leprae. The strain of *M. leprae* used in these experiments was obtained from Dr. C. C. Shepard, Center for Disease Control, Atlanta, Georgia, U.S.A. It had been isolated by him from a skin biopsy specimen of a patient with lepromatous leprosy. The methods of inoculation, processing of tissues, and counting *M. leprae* have all been described previously ([1, 10, 14]).

Calculation of generation time (G). G was calculated as if the *M. leprae* multiplied in passage mice at a constant rate, without a lag phase from inoculation to harvest according to the relationship

$$G \text{ (in days)} = \frac{D}{\log_2 \frac{H}{F}},$$

where D is the time in days between inoculation and harvest, H is the number of acid-fast bacilli (AFB) harvested, and F is the number of AFB inoculated. Because F is always 5000, it is obvious that for a series of harvests performed at the same interval after inoculation, G is inversely proportional to $\log_2 H$. If viable *M. leprae* always multiply at the same rate, G will vary indirectly

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with the logarithm of the proportion of F that represents viable *M. leprae*.

Harvests of *M. leprae* from the pooled tissues of four foot pads were always performed six months after inoculation, but if too few AFB were counted, another harvest was performed at one year. G was not calculated for NTLR because harvests were performed from one to two years after inoculation, and the values obtained would have been meaningless.

***M. leprae*-infected NTLR.** During the experiments we found that some NTLR did not appear to be immunosuppressed, as evidenced by the fact that the numbers of harvested *M. leprae* were lower than expected. To eliminate such animals from experiments, we castrated male NTLR that had been inoculated at least a year earlier in both testes and foot pads. Only those animals whose testes contained at least 10^6 organisms per testis were included in an experiment. In the current experiments, the *M. leprae* in the foot pads of 13 NTLR that had yielded fewer than 10^6 *M. leprae* per testis were counted; only three NTLR yielded more than 10^7 *M. leprae* per foot pad and thus might have been usable. In other experiments in which only intravenously inoculated animals were used, the left ear was examined for AFB; those NTLR whose ears contained fewer than 10^5 *M. leprae* were excluded from the experiments. It has been our experience that when the ear contains 10^5 or more *M. leprae*, the NTLR has a heavy disseminated infection. When fewer AFB are present, the infection is quite often limited to the tail—the site of the original inoculation.

Preparation and administration of diets. The DDS-containing rat diets, as prepared, were assayed by Dr. J. H. Peters, SRI International, and were found to contain, on the average, 100% of the concentration of DDS theoretically present. In four NTLR bled during treatment with 5×10^{-5} g% DDS in the diet, plasma concentrations of DDS were 1.9, 4.0, 6.9, and 7.2 ng/ml and those of monoacetyldapsone (4-amino-4′-acetaminodiphenylsulfone, MADDS) were 0.4, 1.5, 1.8, and 2.0 ng/ml. These values were within the range expected for male Lewis rats ([6]). Plasma samples obtained from three NTLR one to three months after cessation of DDS treatments contained no measurable concentrations of either compound.

RMP, supplied and purified by Dr. Peters, was administered by gavage.

RESULTS

Single dose of RMP. Nineteen male NTLR inoculated intravenously with 1.89×10^7 *M. leprae* 19 months earlier were randomly placed into six groups. One group was left untreated; the NTLR of four of the groups were given single doses of RMP (1, 5, 10, or 20 mg/kg body weight) by gavage. The animals of the sixth group were given the MED of DDS in the diet for 49 days; on the 35th day of DDS administration, a single dose of RMP (10 mg/kg) was administered by gavage. Fourteen days after the dose of RMP, two NTLR from each group were sacrificed, and *M. leprae* were harvested, pooled, and inoculated into both hind foot pads of mice ("passage mice"; 5×10^3 per foot pad) and NTLR ("passage NTLR"; 10^5 to 10^7 per foot pad). The remaining treated NTLR were sacrificed for harvest of *M. leprae* and passage at longer intervals after treatment. It is interesting to note that, contrary to our earlier findings in NTLR treated with DDS after foot pad inoculation with *M. leprae*, we did not observe rapid clearing of the dead AFB from the tissues of intravenously inoculated NTLR treated with either RMP, DDS, or both drugs. Hence it was possible to inoculate many passage NTLR with 10^7 AFB. Passage mice were sacrificed for harvest six and 12 months after passage; passage NTLR were sacrificed 12 or more months after passage.

The results of harvests of *M. leprae* from passage mice and NTLR are presented in Table 1. The generation time, G ([15]), is shown for each harvest of *M. leprae* from passage mice but not for passage NTLR. In the latter case, harvests of *M. leprae* from these animals were made as early as one year and as late as two years after inoculation, and the values of G calculated for them appeared to depend more on the time of harvest than on killing or lack of killing of *M. leprae* by the drugs under test. Passage to mice from untreated NTLR yielded small values of G, suggesting that the passage inocula had contained large proportions of *M. leprae* capable of infecting mice

TABLE 1. *Results of treatment of* M. leprae*-infected neonatally thymectomized Lewis rats (NTLR) with single doses of rifampin (RMP).*

NTLR group no.	Dose of RMP (mg/kg)	Days from innoculation to: treatment[a]	Days from innoculation to: sacrifice	No. of NTLR sacrificed	Results of passage to: Mice[b] (G)	Results of passage to: NTLR[d] No. positive[c]	Results of passage to: NTLR[d] No. positive after passage to mice[e]	Results of passage to: NTLR[d] No. negative[f]
1	0	–	567	1	27.0	–	–	–
			633	1	26.8	–	–	–
2	1	584	598	2[g]	28.3	2	3	1
			745	1	32.9	–	–	–
			854	1	43.3	–	–	–
3	5	591	605	2[g]	NM[h]	3	0	0
			641	1	75.0	4	0	0
			766	1	25.1	2	2	0
4	10	598	612	2[g]	37.3	6	3	0
			704	1	34.9	1	2	0
			837	1	36.9	–	–	–
5	20	605	619	2[g]	57.6	4	2	1
6	10+ (DDS)[i]	612 (591–626)	626	2[g]	NM	0	0	3
			629	1	NM	3	1	2

[a] Test NTLR were inoculated i.v. with 1.89×10^7 *M. leprae* at the indicated time before treatment.

[b] Passage mice were inoculated with 5×10^3 AFB/foot pad. Figures given are generation times (G) as calculated from timed harvests of pools of four to six hind foot pads.

[c] Number of passage NTLR in which the inoculated AFB had multiplied unequivocally (4× or greater).

[d] Passage NTLR were inoculated with between 10^5 and 10^7 AFB/foot pad. No G values were calculated because harvest times varied up to two years after inoculation.

[e] Number of passage NTLR in which either no increase of AFB occurred or the increases were equivocal; however, subpassage of 5×10^3 organisms to intact mice resulted in unequivocal growth.

[f] Number of passage NTLR in which no growth of AFB occurred either directly or on subpassage to intact mice.

[g] Tissues from two treated NTLR pooled for passage.

[h] NM = no multiplication of *M. leprae* in mouse foot pads after observation for one year.

[i] 5×10^{-5} g% DDS given in diet for days 0 to 49, 10 mg/kg RMP by gavage on day 35.

and therefore presumed viable ([15]). Considering only those passages from treated NTLR made 14 days after RMP administration, a single dose of 1 mg/kg had little effect whereas single doses of 5, 10, and 20 mg/kg appeared to have been followed by the killing of a majority of the viable *M. leprae* initially present. When passages were made to mice from three NTLR treated with a single dose of RMP (10 mg/kg) 35 days after beginning a 49-day course of DDS (the MED) administered orally, the proportions of viable *M. leprae* in the passage inocula were too small to infect mice. In the case of passages to mice made later than 14 days after the 5 mg/kg dose of RMP, it is apparent that regrowth of *M. leprae* occurred; thus, the passage performed 175 days after the dose of RMP yielded a value for G characteristic of passages from untreated NTLR.

Multiplication of *M. leprae* after inoculation of NTLR with numbers of organisms larger than 5×10^5 per foot pad was less regular than that in mice inoculated with 5×10^3 organisms per foot pad ([2]). Therefore, the results of passages to NTLR are considered in terms of whether or not multiplication of *M. leprae* occurred. Thus, *M. leprae* multiplied in five of the six passage NTLR inoculated with organisms from NTLR treated with 1 mg/kg RMP, in all 11 passage NTLR inoculated with *M. leprae* from NTLR treated with 5 mg/kg, in all 12

TABLE 2. *Results of treatment of* M. leprae*-infected neonatally thymectomized Lewis rats (NTLR) with dapsone (DDS) and rifampin (RMP).*

NTLR group no.	Treatment	Days from inoculation[a] to:		Results of passage to:			
				Mice[b] (G)	NTLR[d]		
		treatment	sacrifice		No. positive[c]	No. positive after passage to mice[e]	No. negative[f]
7	none	–	397	22.8	–	–	–
		–	403	32.9	–	–	–
		–	517	32.5	–	–	–
		–	517	111	–	–	–
		–	768	24.9	–	–	–
		–	775	28.6	–	–	–
8	5×10^{-5} g% DDS from days 1 to 49 + 10 mg/kg RMP on day 35	404	453 (2)[g]	71.9	6	1	2
			517	NM[h]	0	0	3
			517	49.6	1	0	5
			587 (2)[g]	28.6	2	1	0
			622 (2)[g]	NM	5	1	0
			646	24.9	4	1	0
			769	31.7	–	–	–
		410	620	28.6	3	0	0
			775	32.5	–	–	–
9	5×10^{-5} DDS continuously from day 1 + 10 mg/kg RMP on day 35	410	449	71.2	5	2	2
			482	NM	0	0	2
			515	47.0	4	2	0
			676	NM	3	0	0
			691	NM	–	–	–
			775	NM	2	0	0

[a] Test NTLR were inoculated in both testes and hind foot pads with 5×10^4 *M. leprae* per organ.

[b] Passage mice were inoculated with 5×10^3 AFB/foot pad. Figures given are generation times (G) as calculated from timed harvests of pools of four to six hind foot pads.

[c] Number of passage NTLR in which the inoculated AFB had multiplied unequivocally (4× or greater).

[d] Passage NTLR were inoculated with between 10^5 and 10^7 AFB/foot pad. No G values were calculated because harvest times varied up to two years after inoculation.

[e] Number of passage NTLR in which either no increase of AFB occurred or the increases were equivocal; however, subpassage of 5×10^3 organisms to intact mice resulted in unequivocal growth.

[f] Number of passage NTLR in which no growth of AFB occurred either directly or on subpassage to intact mice.

[g] Number in parentheses indicates that at the indicated time interval two NTLR were sacrificed, and their foot pads were pooled for passage.

[h] NM = no multiplication of *M. leprae* in mouse foot pads after observation for one year.

NTLR inoculated with *M. leprae* from NTLR treated with 10 mg/kg, in six of seven NTLR inoculated with organisms from NTLR treated with 20 mg/kg, and in four of nine passage NTLR inoculated with organisms from the animals treated with both RMP and DDS.

Single doses of RMP combined with DDS. In another experiment (Table 2), 18 male NTLR inoculated with 5×10^4 *M. leprae* in both hind foot pads and testes 13 months earlier were randomly divided between a group administered a single dose of 10 mg/kg RMP 35 days after beginning a 49-day course of treatment with DDS (the MED incorporated into the diet) and a group that received the same treatment except that the administration of DDS was continued indefinitely. Four male and two female NTLR inoculated at the same time, the former having shown good multiplication of *M. leprae* in the testes, served as untreated controls.

M. leprae were harvested from three

TABLE 3. *Results of treatment of* M. leprae*-infected neonatally thymectomized Lewis rats (NTLR) with dapsone (DDS) and rifampin (RMP).*

NTLR group no.	Treatment	Days from inoculation to:		Results of passage to:			
				Mice[b] (G)	NTLR[d]		
		treatment[a]	sacrifice		No. positive[c]	No. positive after passage to mice[e]	No. negative[f]
10	none	–	549	23.8	–	–	–
11	5×10^{-5} g% DDS continuously from day 1 + 10 mg/kg RMP on day 35	570	619	34.1	2	1	0
			637	67.6	–	–	–
			728	118	2	3	0
			889	NM[g]	5	1	0
			908	70.7	2	1	0
		621	929	NM	3	0	0
12	5×10^{-5} g% DDS continuously from day 1 + 10 mg/kg RMP on days 35 and 49	570	627	93.8	–	–	–
			754	NM	4	1	1
			843	NM	–	–	–
			890	NM	5	1	0
13	5×10^{-5} g% DDS continuously from day 1 + 10 mg/kg RMP on days 35 to 39 and 42 to 46	570	613	NM	3	0	0
			754	NM	0	0	2
			802	NM	4	0	2
		612	719	NM	5	0	0
14	5×10^{-3} g% DDS continuously from days 1 to 49 + 10 mg/kg RMP on day 35	570	619	35.3	2	1	0
			619	42.4	1	2	0
			754	NM	3	0	0
			886	NM	–	–	–
		612	815	NM	3	0	0
			929	64.5	2	4	0

[a] Test NTLR were inoculated i.v. with 2.07×10^7 *M. leprae* at the indicated time before treatment.

[b] Passage mice were inoculated with 5×10^3 AFB/foot pad. Figures given are generation times (G) as calculated from timed harvests of pools of four to six hind foot pads.

[c] Number of passage NTLR in which the inoculated AFB had multiplied unequivocally.

[d] Passage NTLR were inoculated with between 10^5 and 10^7 AFB/foot pad. No G values were calculated because harvest times varied up to two years after inoculation.

[e] Number of passage NTLR in which either no increase of AFB occurred or the increases were equivocal; however, subpassage of 5×10^3 organisms to intact mice resulted in unequivocal growth.

[f] Number of passage NTLR in which no growth of AFB occurred either directly or on subpassage to intact mice.

[g] NM = no multiplication of *M. leprae* in mouse foot pads after observation for one year.

NTLR during the DDS administration soon after the dose of RMP—one that was found dead four days after the dose of RMP (Group 9, day 449) and two sacrificed 14 days after RMP administration (Group 8, day 453, tissue pooled). In both instances, G in mice was found to be prolonged—71.2 and 71.9 days, as compared to 42.1 days, the mean value for the six untreated NTLR of Group 7. For the remaining eight NTLR in Group 8 that were sacrificed at longer intervals after the DDS administration had been stopped, the results were variable. No multiplication was detected in passages to mice from one of the NTLR sacrificed on the 517th day and from the NTLR sacrificed on

the 622nd day (78 and 183 days, respectively, after RMP). In the former instance, it was also not possible to detect viable *M. leprae* in passage NTLR; in the latter instance, however, viable organisms were detected in all six passage NTLR. On passage of *M. leprae* from the remaining six NTLR in Group 8 sacrificed from the 517th to the 769th day (78 to 330 days after the dose of RMP), the organisms multiplied in mice and the G was similar to that for controls. The organisms also multiplied in passage NTLR in each instance tested.

Group 9 is of interest in that multiplication of *M. leprae* could not be detected in passage mice from four of the five remaining NTLR sacrificed during continuous DDS administration 482 to 775 days after inoculation (37 to 330 days after the dose of RMP). The mouse passage from the NTLR killed on the 515th day (70 days after RMP) yielded a G of 47 days, representing a considerable delay in growth of the organisms. However, in two of the instances in which *M. leprae* failed to grow in passage mice, they did grow in passage NTLR.

Multiple doses of RMP combined with DDS. Because the regimen involving a single dose of RMP on the background of the MED of DDS was not always effective, we also used other regimens involving either multiple doses of RMP plus the MED of DDS or a single dose of RMP in combination with 100-fold the MED of DDS. The results are given in Table 3. Group 11 received the same treatment as Group 9 in Table 2; in this experiment, multiplication could be detected in four of the six groups of passage mice, but G varied from 34.1 to 118 days, representing a considerable degree of killing. Multiplication was also detected in passage NTLR in the five instances tested. In the two instances in which no multiplication of *M. leprae* was detected in passage mice, multiplication was detected in passage NTLR.

When the regimen included two doses of RMP (10 mg/kg on the 35th and 49th day of continuous treatment with the MED of DDS), it was almost totally effective. Multiplication of *M. leprae* could not be detected in three of four groups of passage mice. Although organisms were detected in the passage mice of the treated NTLR killed on day 627 (8 days after the second dose of RMP), the G was 93.8 days, indicating almost complete killing of the *M. leprae*. In the two instances in which no multiplication was detected in passage mice and simultaneous passage had been made to NTLR, multiplication was detected in the latter.

The only regimen that was totally effective in that *M. leprae* did not multiply in passage mice was that administered to Group 13, which consisted of ten doses of 10 mg/kg RMP on the 35th to 39th and 42nd to 46th days of continuous treatment with the MED of DDS. In no instance was *M. leprae* detected in passage mice from treated NTLR killed 719 to 802 days after inoculation (61 to 186 days after the tenth dose of RMP). Another NTLR died accidentally on day 613 immediately after the seventh dose of RMP; no *M. leprae* could be detected in passage mice. Passages from treated NTLR made simultaneously to NTLR, however, yielded multiplication in three of the four groups.

It is of interest that the regimen in which DDS was administered in 100-fold the MED (5×10^{-3} g%) in combination with the one dose of 10 mg/kg RMP (Group 14) did not appear to be significantly more effective than the regimens using the MED of DDS (Groups 6 and 8). Multiplication could not be detected in three of six groups of passage mice, but in two instances simultaneous passage to NTLR revealed the presence of viable *M. leprae*.

DISCUSSION

One of the most important obstacles to effective chemotherapy of lepromatous leprosy today is the capacity of *M. leprae* to "persist," persistence being defined as the survival of a small fraction of a drug-susceptible population of organisms despite apparently adequate chemotherapy [3, 8, 9, 16]. Although it has not been established that persisting *M. leprae* pose a threat of relapse to lepromatous leprosy patients whose chemotherapy has been stopped, there is concern that some of these patients who lack an efficient immune response to the infecting organisms [4] will experience such a relapse. The alternative to effective chemotherapy for a limited period of time—chemotherapy that is prolonged indefinitely—is clearly not optimal.

There is a great need therefore for chemotherapeutic regimens with an acceptably small rate of relapse to be administered during a finite, if not a short period of time. The great expense and the ethical requirements of clinical trials of chemotherapy of patients with lepromatous leprosy limit the number and variety of drugs and combinations of drugs that may be tested for antimicrobial activity against *M. leprae*, particularly for their ability to minimize or eradicate persisting *M. leprae*. The availability of a suitable animal model would greatly expedite these studies. However, the immunologically normal mouse does not harbor a population of organisms large enough to permit studies of the actions of drugs on persisting *M. leprae* ([10]). We have attempted to exploit the *M. leprae*-infected NTLR as a model of the lepromatous patient so that such studies could be carried out.

We first established the concentration of DDS in the diet that provided the MIC of DDS for *M. leprae* ([6]) and demonstrated that this dosage does not result in killing of *M. leprae*. However, a 100-fold higher dose kills the organisms with considerable efficiency ([1]). We then examined the effects of single doses of RMP and of a single effective dose of RMP superimposed on treatment with DDS (the MED administered in the diet). In the first experiment described above, single doses of 5, 10, or 20 mg/kg RMP administered to NTLR killed significant proportions of the viable *M. leprae*. The 5-mg/kg dose appeared to be most effective, reducing the number of viable *M. leprae* so that mice inoculated with 5×10^3 organisms per foot pad were not infected, but passage NTLR inoculated with 3.24×10^5 organisms per foot pad were infected. This was probably due to variability among treated NTLR rather than greater effectiveness. The effect was much greater when 10 mg/kg RMP was administered to NTLR after five weeks' treatment with the MED of DDS, the administration of DDS being continued for the two weeks between administration of RMP and sacrifice of the animals. The organisms harvested from the NTLR of this last group were noninfectious for mice inoculated with 5×10^3 organisms per foot pad and for three passage NTLR inoculated with either 1.1×10^5 or 1.28×10^6 *M. leprae* per foot pad. On the other hand, *M. leprae* multiplied in two passage NTLR inoculated with 5.14×10^5 organisms and in two passage NTLR inoculated with 1.28×10^6 *M. leprae* per foot pad.

To capitalize on these results, a second type of experiment was planned with the aim of answering two questions. First, was the combined treatment likely to be eradicative? That is, would it reduce the proportion of viable *M. leprae* to such a low level that they could not be detected even by inoculating passage NTLR with the largest possible inocula? Certainly, this combined treatment did not eradicate the viable *M. leprae* in one of the three NTLR treated in the first experiment (Group 6). The number of organisms recovered from the remaining two NTLR was not sufficient to permit inoculation of passage NTLR with more than 1.1×10^5 *M. leprae* per foot pad. To answer this question then, we planned to administer the combined treatment, stop treatment 14 days after the dose of RMP, and sacrifice NTLR at intervals thereafter to learn whether regrowth of *M. leprae* had occurred.

The second question dealt with the possibility that by continuing the low dose of DDS indefinitely after the single dose of RMP, we could produce a situation in the treated NTLR akin to that in patients harboring persisting *M. leprae*. That is, could the proportion of viable organisms be maintained for some period of time at a level too low to be detected by inoculation of normal mice but detectable by inoculation of passage NTLR with numbers of *M. leprae* greater than 5×10^3?

The immediate effect of a single 10 mg/kg dose of RMP together with the MED of DDS for a limited period of time was not as great as it had been in the first experiment. Although a large proportion of the viable *M. leprae* were killed in the NTLR studied two weeks after the dose of RMP (Group 8), the results of the first experiment were not duplicated.

Additional NTLR of Group 8 were studied at intervals of 78 to 330 days after the dose of RMP (64 to 316 days after stopping administration of DDS in low dose). In one NTLR (sacrificed on the 517th day, 78 days after RMP), viable *M. leprae* appeared to have been eradicated, in the sense that no

multiplication occurred in passage NTLR inoculated with 5×10^5 organisms per foot pad despite a drug-free interval of 64 days during which some regrowth could have occurred. The organisms from the second NTLR killed the same day yielded a G of 49.6 days in mice, and there was multiplication of the organisms in one of six passage NTLR. Passage from NTLR (sacrificed on the 622nd day after inoculation, 183 days after the dose of RMP) after a drug-free interval of 169 days, during which time considerable regrowth of *M. leprae* could have occurred, revealed the proportion of viable organisms to be insufficient to infect normal mice but large enough to produce infection in passage NTLR inoculated with 5×10^5 or more organisms. Passages to mice from the remaining NTLR of Group 8 revealed values of G in the range of 24.9 to 32.5 days, consistent with high proportions of viable *M. leprae*. Thus, regardless of the immediate effect of the combined treatment, considerable regrowth of the organisms had occurred during drug-free intervals ranging from 134 to 322 days. These results demonstrated that a single 10 mg/kg dose of RMP on the background of the MED of DDS was only occasionally capable of reducing the proportion of viable *M. leprae* to below that detectable in mice and rarely reduced this proportion below that detectable by inoculation of passage NTLR with at least 5×10^5 organisms.

Considering the effect of continuous DDS administration, the results of passage from the six NTLR of Group 9 are pertinent. Passages from four of the six animals failed to produce multiplication in mice. Passage to NTLR, made from three of these four animals, resulted in multiplication in two instances but not in the third. In these two instances, we appear to have succeeded in achieving and maintaining a proportion of viable *M. leprae* too small to infect mice but sufficient to infect passage NTLR inoculated with 5×10^5 or more *M. leprae*. However, in a repeat experiment (Group 11) using this regimen the results were reversed. Passages from four of six NTLR produced multiplication in mice. In the two NTLR of this group killed 273 and 284 days after the dose of RMP and in which multiplication of *M. leprae* was not detected in passage mice, multiplication was detected in all nine passage NTLR.

Thus, it appeared that the combination of a single dose of 10 mg/kg RMP and daily treatment with the MED of DDS was not quite sufficient to achieve the goal of regular reduction of the proportion of viable *M. leprae* to a level not detectable by inoculation of mice but regularly detectable in NTLR inoculated with large numbers of organisms. Therefore, we investigated the regimens in which multiple doses of RMP were given on the background of the MED of DDS as well as one dose of RMP on the background of 100 times the MED of DDS. The only regimen that we considered completely effective with respect to the elimination of infectivity for intact mice was that in which ten doses of RMP were given on the background of the MED of DDS. No viable organisms were detected in passage mice, but multiplication of *M. leprae* was detected in 12 of 16 passage NTLR representing three of the four groups to which passages were made.

In these experiments it is notable that in no instance did we fail to detect organisms in passage NTLR when we detected them in passage mice. On the other hand, we demonstrated multiplication in passage NTLR in 14 instances in which the *M. leprae* failed to multiply in passage mice. In only four instances did multiplication of *M. leprae* fail to occur in both passage mice and NTLR. These results confirm that the NTLR, because of its high degree of immunosuppression, can detect a small proportion of surviving *M. leprae* in inocula containing up to 5000 times as many organisms as can be inoculated into intact mice. In addition, the *M. leprae*-infected NTLR appears to provide a model for the study of microbial persistence in leprosy. Additional experiments designed to exploit this model are now in progress.

SUMMARY

Neonatally thymectomized Lewis rats (NTLR) were shown to be highly susceptible to infection with *Mycobacterium leprae*. We have used them in chemotherapeutic studies as models of human lepromatous leprosy. NTLR chronically infected with *M. leprae* were treated with various regi-

mens combining a background of the minimal effective dose (MED) of dapsone (4,4′-diaminodiphenylsulfone, DDS) or 100 times this dose in the diet with one to ten doses of rifampin (RMP) of 10 mg/kg. To test for persisting viable *M. leprae* passage of 5×10^3 organisms was made to intact mice, and 10^5 to 10^7 acid-fast bacilli were passaged to NTLR. The only regimen that appeared to be completely effective in eliminating infectivity for intact mice was ten doses of RMP given on the background of the MED of DDS. No viable organisms were detected in any passage mice, but multiplication of *M. leprae* was detected in 12 of 16 passage NTLR, representing three of the four groups in which passage was made. In no instance did we fail to detect organisms in passage NTLR when we detected them in passage mice, and multiplication was demonstrated in passage NTLR in 14 instances in which *M. leprae* failed to multiply in passage mice. Because of its high degree of immunosuppression, the NTLR was able to detect a small population of viable *M. leprae* in inocula containing up to 5000 times the number of organisms that can be inoculated into intact mice. The NTLR appears to provide a model for the study of microbial persistence in leprosy.

RESUMEN

Las ratas Lewis timectomizadas al nacimiento (RLTN) resultaron ser muy susceptibles a la infección con el *Mycobacterium leprae*. Nosotros las usamos en un estudio quimioterapéutico como modelo de la lepra lepromatosa humana. Las RLTN crónicamente infectadas con *M. leprae* se trataron con varias combinaciones de dapsona y rifampina. Las combinaciones fueron desde la mínima dosis efectiva (MDE) de dapsona (4,4′-diaminodifenilsulfona, DDS) hasta 100 veces esa dosis, con uno a diez dosis de rifampina (RMP) de 10 mg/kg por dosis, adicionadas a la dieta. Para probar la persistencia de *M. leprae* viables, se hicieron pases de 5×10^3 organismos a ratones intactos, y de 10^5 a 10^7 bacilos ácido-resistentes a RLTN. El único tratamiento que pareció ser completamente efectivo en eliminar la infectividad en los ratones intactos fue el consistente en 10 dosis de RMP adicionadas a la mínima dosis efectiva (MDE) de DDS. No se encontraron organismos viables en ninguno de los passes a los ratones, pero la multiplicación del *M. leprae* se demostró en 12 de 16 pases a las RLTN, representando tres de los cuatro grupos en los cuales se hicieron pases. Siempre que se demostraron microorganismos en los pases a los ratones se encontraron también en los pases a las RLTN. La multiplicación del *M. leprae* se demostró en los pases a las RLTN en 14 casos en los cuales el microorganismo no se multiplicó en los pases a los ratones. Debido al alto grado de inmunosupresión, las RLTN fueron capaces de permitir el crecimiento de el pequeño número de *M. leprae* viables presente en inóculos conteniendo hasta 5000 veces el número de organismos que pueden ser inoculados en los ratones intactos. La RLTN parece ser un buen modelo para el estudio de la persistencia microbiana en la lepra.

RÉSUMÉ

On a démontré que des rats Lewis nouveau-nés et thymectomisés (NTLR) étaient hautement susceptibles à l'infection par *Mycobacterium leprae*. Ces rats ont été utilisés pour mener des études chimiothérapiques, en les prenant comme modèles de la lèpre lépromateuse humaine. Ces rats NTLR infectés de manière chronique par *M. leprae* ont été traités par différents types de traitement combinant une dose effective minimale (MED) de dapsone (4,4′-diaminodiphenylsulfone, DDS), ou une dose 100 fois plus élevée, dans la ration, à laquelle on avait ajouté de une à dix doses de rifampine (RMP) de 10 mg/kg. Afin d'étudier la persistance de *M. leprae* viable, on a procédé à des transferts d'organismes, à raison de 5000 bacilles acido-résistants à des souris intactes, et de 100.000 à 10.000.000 bacilles acido-résistants à des rats NTLR. Le seul régime qui est apparu entièrement efficace pour éliminer l'infectiosité pour les souris intactes, était celui consistant en dix doses de RMP données en supplément à une dose effective minimale (MED) de DDS. Aucun organisme viable n'a été détecté chez aucune souris inoculée, mais une multiplication de *M. leprae* a été par contre détectée chez 12 des 16 rats NTLR inoculés, ce qui représente trois parmi les quatre groupes soumis à ce passage. On a toujours pu détecter les organismes chez les rats NTLR inoculés, lorsqu'on pouvait les détecter chez les souris intactes inoculées, et une multiplication a été démontrée chez les rats NTLR inoculés dans 14 cas chez lesquels *M. leprae* n'avait pu se multiplier chez les souris intactes inoculées. A la suite du degré élevé d'immunosuppression, les rats NTLR permettaient de détecter une population faible de *M. leprae* viable dans des inoculats contenant jusqu'à 5000 fois le nombre d'organismes que l'on pouvait inoculer à des souris intactes. Les rats NTLR semblent fournir un modèle pour l'étude de la persistance microbienne dans la lèpre.

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