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The Neonatally Thymectomized Rat as a Model of the Lepromatous Patient

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In the preceding paper of this collection ([6]), infection of the neonatally thymectomized rat (NTR) was discussed, in terms of its application to the detection of viable *Mycobacterium leprae* present in small proportions in the bacterial populations of patients with lepromatous leprosy undergoing effective antimicrobial chemotherapy. Because it sustains relatively large bacterial populations, and is partially immunosuppressed, the NTR has also been employed as a model of the lepromatous patient, for studies of chemotherapy and immunotherapy. The purpose of this paper is to describe some of these applications.

The model. Preparation of the NTR was described in the preceding paper ([6]). Fieldsteel found ([5]) that, after inoculation in the hind foot pad with 5000 *M. leprae,* the organisms multiplied to a maximum of 10^7–10^8 organisms per foot pad in the course of one year, and that organisms capable of multiplying in mice (i.e., the proportion of viable organisms was $\geq$1:5000) could be recovered by harvest conducted as late as two years after inoculation. Following intravenous inoculation of $\geq 10^7$ *M. leprae,* a disseminated infection occurs ([1]), characterized by the absence of macroscopic findings. *M. leprae* are found to have multiplied to levels of 10^7–10^8 per hind foot pad and tail. Microscopically, lesions are noted to be confined to the distal portions of the body—snout, ears, tail, testes, and, especially, the pads of both hind- and forefeet. The principal changes are those of edema and of large macrophage granulomata. The macrophages possess a foamy cytoplasm, and contain large numbers of acid-fast bacilli. In the foot pads, large numbers of organisms are found within macrophages, that have accumulated deep to a subepidermal clear zone; the small nerves demonstrate degenerative changes, and the perineural cells are found to contain small numbers of *M. leprae*; the deeper striated muscle fibers are vacuolated, and contain large numbers of organisms.

Application of the NTR to experimental chemotherapy of leprosy. Clinical trials of therapy of lepromatous leprosy are very costly in money and time. Moreover, ethical considerations bar some investigations in patients, if, for example, the drugs are toxic or the question under examination is not sufficiently consequential, so that the value of the results may be considered not to justify the risks of an experimental chemotherapy. The role to be filled by an animal model of the lepromatous patient is clear. Some characteristics of the NTR appear to make the animal suitable for such studies: the NTR sustains reasonably large populations of *M. leprae,* and does not rapidly kill the organisms, once the maximum of multiplication has been achieved; the NTR is immunosuppressed, although by a mechanism different from that operating in the lepromatous patient; finally, the NTR is easily maintained and long lived.

Earlier studies had shown ([14]) that, in rats administered dapsone (DDS) continuously in the diet, the minimal inhibitory concentration (MIC) of DDS for *M. leprae* was in the range 1.5–4.0 μg per ml plasma, although the concentrations of DDS required in the diet to achieve these plasma concentrations were different in male and female rats. Subsequent studies, in which the proportions of surviving organisms were measured by passage of *M. leprae* from treated NTR to the foot pads of untreated mice, showed ([2–4]) that administration to infected NTR of the concentration of DDS required to achieve the MIC in the plasma [i.e., the minimal effective dosage (MED)] failed to kill *M. leprae,* although the organisms were inhibited from multiplying, whereas administration of DDS in a 100-fold greater concentration resulted in killing of the organisms at a rate similar to that observed in

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TABLE 1. *Chemotherapy of* M. leprae *infection of NTR.*

Treatment	No. NTR in which organisms multiplied / No. NTR examined	
	During therapy	After therapy
DDS 0.005% in diet + 10 doses RMP 10 mg/kg	2/7	4/5
DDS 0.005% in diet + RMP 0.01% in diet	3/6	4/9
RMP 0.01% in diet	0/4	2/7
CLO 0.01% in diet + RMP 0.01% in diet	0/3	0/9
DDS 0.005% in diet + ETH 0.2% in diet	1/3	3/9
RMP 0.01% in diet + ETH 0.2% in diet	0/4	0/9

patients under treatment with DDS in full dosage.

Although the bactericidal activity against *M. leprae* of DDS appeared to be of the same order in the NTR as in man, that of rifampin (RMP) was considerably weaker in the NTR than in the patient. Single doses to NTR of RMP as large as 20 mg per kg body weight failed to render the *M. leprae* consistently non-infective for normal mice inoculated with 5000 organisms per foot pad (i.e., failed to reduce the proportion of viable organisms to $<1:5000$) ([4]), although a similar dose (600 mg) was almost always effective in the patient ([8]). On the other hand, administration of a single 10-mg-per-kg-dose of RMP to NTR continuously administered DDS in the MED rendered the RMP more strongly bactericidal. [In accompanying papers, similar results are reported from studies in both normal and nude mice ([7, 10]). Yet the combinations of continuous dietary dapsone 0.00005% in the diet (MED) together with a single dose of rifampin 10 mg/kg, a single dose of rifampin 10 mg/kg together with continuous dapsone 0.005% in the diet, and up to 10 doses of rifampin, 10 mg/kg, combined with continuous dapsone 0.005% in the diet were unable to totally eliminate surviving persistent *M. leprae* when viability was assessed by subpassages of large numbers (10^6) of *M. leprae* to NTR.]

In more recent experiments, we have studied the effects on *M. leprae* in heavily infected NTR of treatment by a variety of regimens. Our goal was to determine if regimens which we had reason to believe might be more potent than those studied previously could more nearly sterilize the NTR during therapy and prevent regrowth of persisters after therapy had been discontinued. NTR were treated for four months. Organisms were harvested from the NTR after two and four months of treatment, and two and four months after treatment had been terminated, organisms were enumerated, and the proportion viable was determined by passage of 5000 *M. leprae* into the foot pads of mice, and of $\geq 10^6$ into the foot pads of new NTR. The incomplete results, presented in Table 1, suggest that the combinations of RMP plus clofazimine (CLO), and RMP plus ethionamide (ETH), all drugs administered continuously in the diet were consistently effective and more active than RMP administered alone or in combination with DDS. In fact, the results of treatment with the combination RMP and DDS in a dosage 100-fold the MED suggest antagonism.

Application of the NTR to experimental immunotherapy. Although the mechanism of the immune defect characteristic of patients with lepromatous leprosy is currently the subject of much controversy, and, in any case, is probably not the same as that of the immune defect of the NTR, the *M. leprae*-infected NTR appears to offer a suitable model for some studies of immunotherapy. Evidence suggests, for example, that, in lepromatous patients, production of interleukin 2 (IL-2) ([9, 11]) or its leukocyte receptor ([12]) is inadequate, or that release of gamma interferon is aberrant ([13]). Because recombinant IL-2 and gamma interferon are now available, study of the effects of these agents, either singly or in combination with antimicrobial chemotherapy, in *M. leprae*-infected NTR appears of interest. We have recently begun such studies.

REFERENCES

1. Dawson, P. J., Ringus, J. C. and Fieldsteel, A. H. Neonatally thymectomized Lewis rats infected with *Mycobacterium leprae*. 2. Histopathologic and electron microscopic observations. Int. J. Lepr. **47** (1979) 561.
2. Fieldsteel, A. H. and Levy, L. Dapsone chemotherapy of *Mycobacterium leprae* infection of the neonatally thymectomized Lewis rat. Am. J. Trop. Med. Hyg. **25** (1976) 854.
3. Fieldsteel, A. H. and Levy, L. Combination dapsone-rifampin therapy in neonatally thymectomized Lewis rats (NTLR) chronically infected with *Mycobacterium leprae*. Int. J. Lepr. **47** (1979) 108.
4. Fieldsteel, A. H. and Levy, L. Combined rifampin and dapsone chemotherapy of *Mycobacterium leprae* infection of the neonatallly thymectomized Lewis rat. Int. J. Lepr. **45** (1980) 267.
5. Fieldsteel, A. H. and McIntosh, A. H. Effect of neonatal thymectomy and antithymocyte serum on susceptibility of rats to *Mycobacterium leprae* infection. Proc. Soc. Exp. Biol. Med. **138** (1971) 408.
6. Gelber, R. H. and Levy, L. Detection of persisting *Mycobacterium leprae* by inoculation of the neonatally thymectomized rat. Int. J. Lepr. Submitted herewith.
7. Grosset, J. H. and Guelpa-Lauras, C.-C. Activity of rifampicin in infections of normal mice with *Mycobacterium leprae*. Int. J. Lepr. Submitted herewith.
8. Levy, L., Shepard, C. C. and Fasal, P. Rapid bactericidal effects of rifampin on *M. leprae* in man: a) single doses of 600, 900 and 1200 mg; and b) daily doses of 300 mg. Int. J. Lepr. **44** (1976) 183.
9. Longley, J., Haregowoin, A., Yemaneberhan, T., *et al.* *In vitro* responses to *Mycobacterium leprae*: antigen presentation, interleukin-2 production, and immune cell phenotypes in naturally occurring leprosy lesions. Int. J. Lepr. **53** (1985) 385.
10. McDermott-Lancaster, R. D., Ito, T., Kohsaka, K., Guelpa-Lauras, C.-C. and Grosset, J. H. Multiplication of *Mycobacterium leprae* in the nude mouse, and some applications of nude mice to experimental leprosy. Int. J. Lepr. Submitted herewith.
11. Modlin, R. L., Hofman, F. M., Horwitz, D. A., *et al.* In situ identification of cells in human leprosy granulomas with monoclonal antibodies to interleukin-2 and its receptor. J. Immunol. **132** (1984) 3085.
12. Mohagheghpour, N., Gelber, R. H., Larrick, J. W., Sasaki, D. T., Brennan, P. J. and Engleman, E. G. Defective cell-mediated immunity in leprosy: failure of T cells from lepromatous leprosy patients to respond to *Mycobacterium leprae* is associated with defective expression of interleukin-2 receptors, and is not reconstituted by interleukin-2. J. Immunol. **135** (1985) 1443.
13. Nogueira, N., Kaplan, G., Levy, E., *et al.* Defective G-interferon production in leprosy: reversal with antigen and interleukin 2. J. Exper. Med. **158** (1983) 2165.
14. Peters, J. H., Gordon, G. R., Murray, J. F., Jr., Fieldsteel, A. H. and Levy, L. Minimal inhibitory concentration of dapsone for *Mycobacterium leprae* in rats. Antimicrob. Ag. Chemother. **8** (1975) 551.