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Multiplication of *Mycobacterium leprae* in the Nude mouse, and Some Applications of Nude Mice to Experimental Leprosy

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Enhanced growth of *Mycobacterium leprae* in the congenitally athymic "nude" (*nu/nu*) mouse was reported in 1976 by Colston and Hilson ([4]), who described the results of inoculating nude mice with *M. leprae* obtained from a biopsy specimen of a patient with untreated lepromatous leprosy. Although the animals had not been maintained in an isolator, one mouse survived 322 days, at which time there were 10^9 organisms per foot pad, and dissemination of the organisms to the liver, spleen, nose, skin of the tail, forepaws and testes was evident. In that same year, Kohsaka, *et al.*, reported ([6]) enhanced multiplication of *M. leprae* in isolator-maintained nude mice that survived 22 months. Subsequently, further evidence of enhanced multiplication was reported ([7]); *M. leprae* multiplied to numbers $>10^8$–10^{10} in foot pads of nude mice that had been inoculated with 10^5–10^6 organisms. Since these original publications, work with *M. leprae*-infected nude mice has continued in the St. George's and Osaka laboratories, and has also been undertaken at Carville, in Grosset's laboratory in Paris, and at the National Institute for Leprosy Research in Tokyo.

Multiplication of *M. leprae* in the foot pad of the nude mouse

Inoculation of from 10^2 to 2.5×10^7 *M. leprae* into the hind foot pads of nude mice has produced the growth curves shown in Figure 1. These growth curves are similar, and reach a maximum of approximately 5×10^{10} organisms per foot pad. The maximum is no higher in those foot pads that had been inoculated with the largest number of *M. leprae,* perhaps because, at this stage, the foot pads are greatly swollen, and demonstrate heavy infiltration by bacteria-laden macrophages, so that the blood supply to the tissues may have been compromised. Chehl and her colleagues reported ([2]) that the morphological index of the organisms recovered from heavily infected tissues of the nude mouse was lower than that found earlier in the process, suggesting that the failure of continued multiplication of *M. leprae* resulted from killing of the organisms.

Dissemination of the infection

Dissemination of the organisms from the site of inoculation in the hind foot pad was reported by Colston and Hilson ([4]) and by Kohsaka, *et al.*, ([6]) and confirmed subsequently by other workers. Chehl, *et al.*, ([2]) observed multiplication of *M. leprae* in contralateral, uninoculated foot pads in the course of harvests performed later than 272 days after inoculation of nude mice in one hind foot pad with 10^4–10^6 organisms. Even earlier, 180 days after inoculation, numbers of organisms were found in the liver and spleen. Approximately 10^9 *M. leprae* per g of tissue were found in homogenates of carcasses performed 565 days after inoculation of the mice.

In another study in St. George's ([10]), 8×10^6 *M. leprae* were inoculated into the hind foot pads of nude mice, and the organisms were enumerated in various tissues at several intervals after inoculation. As shown in Table 1, numbers $>10^{10}$ per g were found in the snout, testes and inoculated foot pads, and numbers of *M. leprae* $>10^9$ per g tissue

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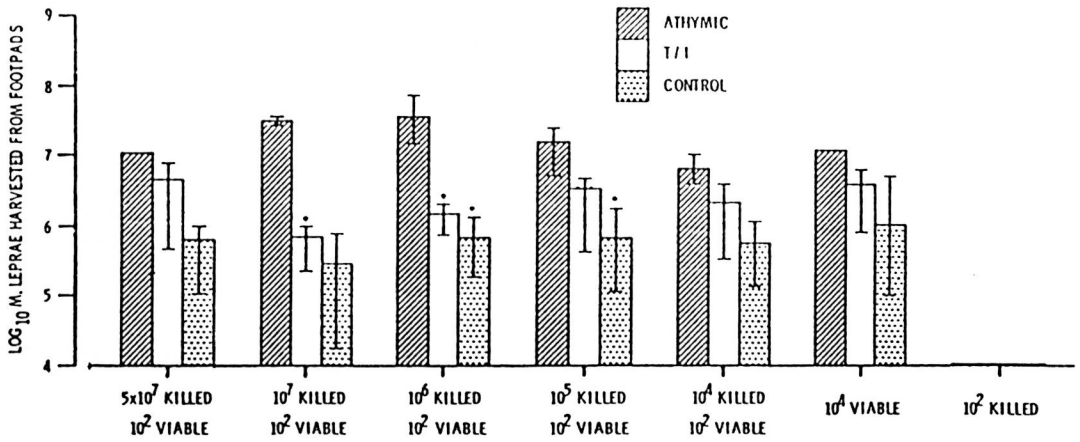

FIG. 1. Growth of *M. leprae* in nude mice (data have been taken from references nos. 1–3, 7, 9–11).

were found in lymph nodes, tail skin and ears.

Pathological changes

Although the degree of swelling does not always reflect the number of organisms, multiplication of *M. leprae* in the foot pad of the nude mouse is accompanied by swelling of the foot, beginning from about the ninth month ([4, 6]). There may also be ulceration of the foot pad ([6, 14]), and thickening of the ears and eyelids ([11]).

Histopathological features of the infection of the foot pad include infiltration by bacteria-laden macrophages, which eventually displace striated muscle ([2, 10]). Bacterial invasion of peripheral nerves characteristic of human leprosy has been observed ([2, 6, 10, 11]), with organisms present in perineural cells, Schwann cells, striated muscle, and the endothelial cells lining blood vessels ([2, 10, 11]).

After dissemination has occurred, *M. leprae* may be found in reticuloendothelial cells of the liver, spleen, lungs and lymph nodes ([2, 10]). During later stages of the process, macrophage granulomata full of organisms are present in the bone marrow of the tail ([2, 14]), ears, skin, nose and tongue ([10, 14]). With the single exception of the central nervous system, Chehl, *et al.*, detected organisms at some time in all tissues, including the kidneys, intestines, reproductive organs, and heart ([2]). On the other hand, Ravisse, *et al.*, reported ([14]) that it was difficult to detect *M. leprae* in the liver, spleen and lungs.

Electron-microscopic studies have demonstrated *M. leprae* both within phagosomes and free in the cytoplasm, and an electron-transparent zone ([2, 5]). In addition, Fukunishi, *et al.*, described ([5]) accumulations of small spherical droplets within the phagolysosomes of nude mouse macrophages; these droplets are responsible for the foamy appearance of the lepra cells of man ([4, 14]).

Alternative routes of inoculation

Heavy infections of nude mice have been reported to result from inoculation of *M. leprae* by the intravenous ([11]) and subcutaneous ([2]) routes. Harvests of *M. leprae* performed from foot pads, snout, testes and tongue 471 days after intravenous inoculation of nude mice with 10^7 and 10^8 organisms yielded $>10^{10}$ per g tissue (see Table 2) ([11]). Less reproducible results have been reported to follow inoculation by the intracutaneous ([12]) and intraperitoneal ([9]) routes. Nakamura and Yogi reported ([13]) that the upper lip was a suitable site for inoculation, and successful intranasal inoculation has recently been described ([1, 9]).

Applications of the *M. leprae*-infected nude mouse

Detection of persisting *M. leprae*. Because nude mice permit multiplication of *M. leprae* from a larger inoculum than do thymectomized-irradiated (TR) mice, it should be possible to detect persisting organisms, when these are present in smaller propor-

TABLE 1. *Multiplication of* M. leprae *in the tissues of nude mice.*

Tissue	No. *M. leprae* ($\times 10^8$) per g tissue on day*		
	339	628	650
Hind paws	37.1	1010	738
Tongue	4.26	1.61	7.16
Lymph node	0.077	9.75	92.9
Sciatic nerve	0.016	<0.0001**	0.42
Spleen	0.012	1.06	2.97
Liver	0.004	1.25	5.74
Ear	0.003	10.7	39.6
Lung	0.002	0.17	1.69
Testis	0.001	1.79	192
Kidney	<0.0001**	<0.0001**	0.21
Tail skin	<0.0001**	1.92	92.6
Snout	<0.0001**	13.9	194
Forepaws	ND***	4.11	ND
Eyelid	ND	0.31	121
Heart	ND	0.004	0.29
Thyroid	ND	0.002	ND

* The number of days between inoculation and harvest.
** Per ml suspension rather than per g tissue, because of the small quantities of tissue available.
*** Not done.

tions than can be detected by inoculation of normal or TR mice. To examine this possibility, a study was carried out in St. George's, in which groups of normal, TR and nude mice were inoculated in the hind foot pad with suspensions of *M. leprae* prepared by diluting 100 freshly harvested organisms with larger numbers of irradiated organisms, and performing harvests from the inoculated foot pads after 12–14 months. As shown in Figure 2, the nude mouse was more sensitive than either the normal or the TR mouse to very small proportions of viable organisms.

The data summarized in Figure 2 suggest also that clearance of the killed organisms from the site of inoculation was most rapid in normal mice and slowest in the nude mice ([10]). Unpublished data from St. George's suggest further that the rate at which killed *M. leprae* are cleared from the foot pad also depends upon the method by which the organisms were killed. Heat-killed organisms were removed or degraded more rapidly than were organisms that had been killed by irradiation, whereas the number of organisms that had been killed by exposure to rifampin (RMP) *in vivo* decreased only slightly in the course of one year.

Experimental chemotherapy. In St. George's, the effects of treatment with RMP on "established" *M. leprae* infection of the nude mouse were studied ([9]). RMP in a dosage of 20 mg per kg body weight administered twice weekly for three weeks failed to eradicate the infection. Treatment was administered six months after inoculation, and *M. leprae* were harvested from the infected foot pads 12 months later. The number of organisms per foot pad increased from 10^6 at six months to 10^8 at 18 months. In an

TABLE 2. *Recovery of* M. leprae *from the tissues of nude mice following intravenous inoculation with (a) 1 × 10^8 or (b) 3.5 × 10^7 organisms.*

Tissue	No. *M. leprae* ($\times 10^8$) per g tissue on day*		
	460 (a)	462 (b)	650 (b)
Hind paws	19.8	142	272
Tongue	ND**	2.99	165
Inguinal lymph node	34.2	11.7	21.1
Sciatic nerve	2.09	0.031	4.76
Spleen	2.49	0.35	1.38
Liver	4.39	0.22	3.72
Ear	15.5	76.1	89.4
Testis	0.13	21.3	231
Back skin	0.043	4.03	3.51
Snout	10.3	480	183
Forepaws	ND	4.11	470

* The number of days between inoculation and harvest.
** Not done.

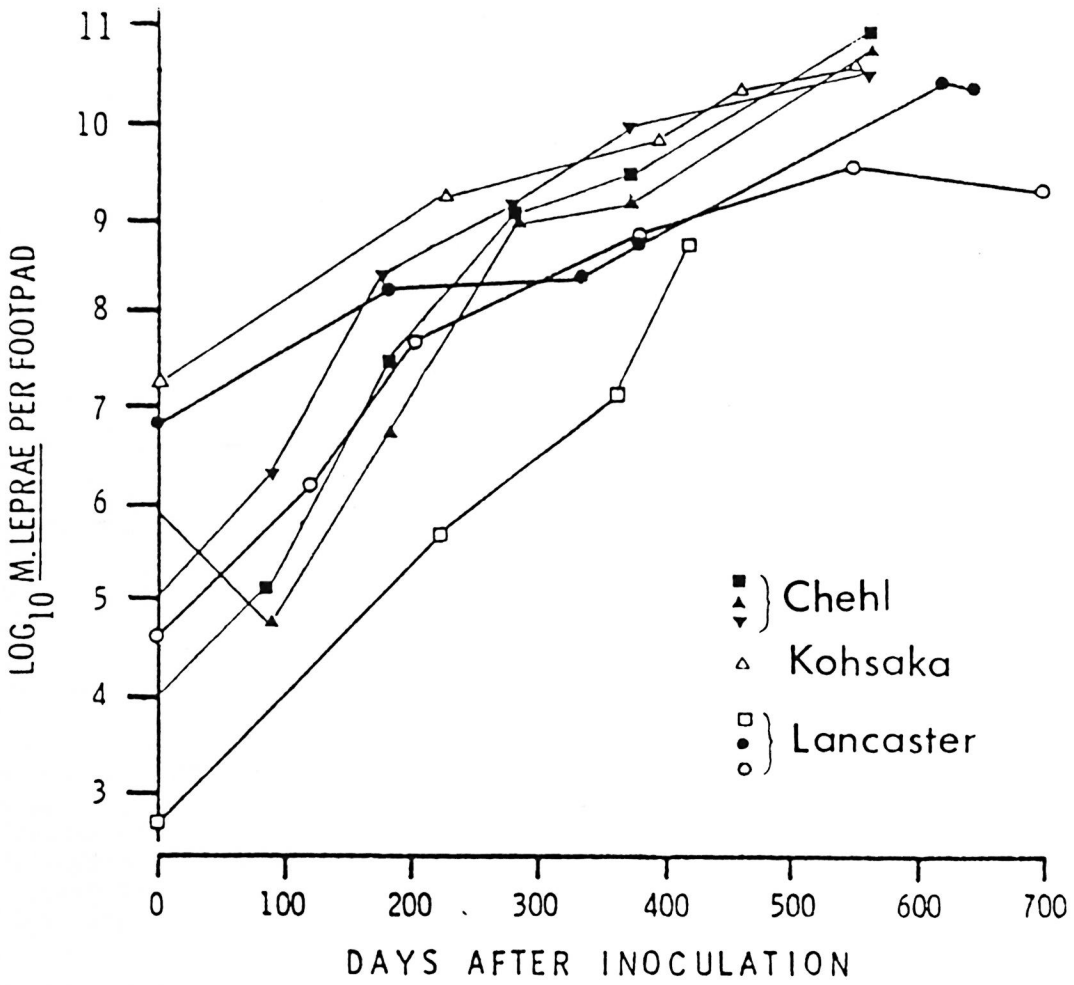

FIG. 2. Multiplication of *M. leprae* in nude, thymectomized-irradiated and normal mice. Groups of five mice were inoculated with mixtures of freshly harvested and ^{60}Co-irradiated organisms. Bars show the range of counts; * indicates that harvested organisms were sub-inoculated into normal mice; in all four instances, *M. leprae* were found to have multiplied in the sub-inoculated mice (adapted from references nos. 11 and 12).

additional experiment, RMP administered in a weekly dose of 30 mg per kg for four months also failed to eradicate the infection.

In Paris, two experiments have thus far been carried out. The first experiment employed five nude mice with swollen foot pads that had been inoculated into the hind foot pads 14 months earlier with 8×10^5 *M. leprae* per foot pad. Initially, two of the animals (untreated, "control" mice) were sacrificed, and 10^7 organisms per mg were harvested from the swollen foot pads and inoculated into mice as follows. Twenty nude mice were inoculated with 3×10^7 *M. leprae* per hind foot pad, and 20 normal mice were inoculated into a hind foot pad with 5×10^3 organisms per foot pad. Two days later, the remaining three nude mice were administered RMP by gavage in a single dose of 10 mg RMP per kg body weight. Four, seven and 21 days later, one nude mouse was sacrificed, and the inoculated foot pad harvested; on each occasion, the harvested organisms were employed to inoculate 10 nude passage mice with 3×10^7 organisms per foot pad, and 10 normal passage mice with 5000 *M. leprae* per foot pad. The nude passage mice were examined monthly, and the thickness of the inoculated hind foot pads was measured by means of a dial-gauge caliper 8, 9 and 10 months after passage. At intervals of eight and nine

TABLE 3. *Numbers of* M. leprae *harvested from the foot pads of normal mice sub-inoculated with 5 × 10^3 organisms passaged from nude mice administered a single 10 mg/kg dose of RMP or left untreated.*

Time of sub-inoculation (days)	Log_{10} *M. leprae* per foot pad	
	8 mo	12 mo
0	4.9	5.3
	5.3	5.3
	5.4	5.7
	5.6	5.8
	5.7	5.9
4	4.6	5.3
	5.0	5.3
		5.5
		5.5
		5.6
7	4.3	5.3
	5.6	5.5
		5.5
		5.6
		6.0
21	4.9	5.3
	5.5	5.7
		5.7
		5.7
		5.8

months after passage, *M. leprae* were harvested from the inoculated foot pads of normal passage mice.

As shown in Tables 3 and 4, no differences were observed between those passage animals inoculated with organisms harvested from the untreated nude mice, and those inoculated with *M. leprae* that had been harvested from the nude mice to which RMP had been administered.

In the second experiment, 20 nude mice, which had been inoculated in the left hind foot pad with 1.5 × 10^7 *M. leprae* per foot pad, were assigned randomly to one of four groups of five mice each. Two groups were held without treatment, whereas the mice of two groups were administered RMP by gavage in a dose of 10 mg per kg, one group eight months, and the second group 12 months after inoculation. Four days after administration of RMP, *M. leprae* were harvested from the inoculated foot pads of five untreated and five treated nude mice, counted, pooled, serially diluted, and sub-inoculated into the hind foot pads of normal mice, to determine the proportions of viable organisms [in terms of the most probable number (MPN)], of the *M. leprae* that had been harvested from the nude mice.

TABLE 4. *Proportions of nude mice with swollen feet that had been sub-inoculated with organisms passaged from nude mice administered a single 10 mg/kg dose of RMP or left untreated.*

Time of sub-inoculation (days)	Proportion of mice with swollen feet		
	8 mo	9 mo	10 mo
0	5/16	14/16	13/14*
4	2/8	6/8	8/8
7	3/8	7/8	7/7**
21	2/9	3/9	6/9

* Two mice died between the 9- and 10-month measurements.

** One mouse died between the 9- and 10-month measurements.

As shown in Table 5, *M. leprae* multiplied between eight and 12 months in the foot pads of the nude mice, increasing from median values of 2.4 × 10^8 and 4.0 × 10^8 after eight months in untreated and treated mice, respectively, to median values of 1.7 and 2.6 × 10^9 after 12 months. As shown in Table 6, the single dose of 10 mg RMP per kg did not render the *M. leprae* non-infective for normal mice at either interval. However, the proportions of viable organisms were smaller in the passage inocula derived from treated than in those derived from untreated mice, and smaller in the inocula derived from mice treated after 12

TABLE 5. *Numbers of* M. leprae *harvested from the inoculated foot pads of nude mice 8 or 12 months after inoculation of 1.5 × 10^7 organisms per foot pad.*

Log_{10} *M. leprae* harvested per foot pad	
8 mo	12 mo
Untreated mice	
7.2	9.0
7.7	9.0
8.4	9.2
8.5	9.5
8.5	9.7
Treated mice	
7.6	8.9
7.8	9.2
8.6	9.4
8.7	9.5
8.9	9.6

TABLE 6. *Infective* M. leprae *harvested from nude mice treated with a single dose of 10 mg RMP/kg or left untreated.*

Time of sub-inoculation (mo)	No. mice showing multiplication / No. mice harvested / No. *M. leprae* inoculated			MPN per 4500 *M. leprae*
	4500	45	0.45	
8				
Untreated mice	8/8	6/8	1/8	163
Treated mice	2/2	2/7	0/5	33.3
12				
Untreated mice	7/7	5/5	3/7	231
Treated mice	4/5	1/7	0/7	2.06

months than in those derived from mice that had been administered RMP eight months after inoculation.

In Osaka, experiments showed that RMP, administered orally in a single dose of 20 mg per kg body weight, or in a daily dose of 8 mg per kg for two weeks, prevented multiplication of *M. leprae* in nude mice.

Model of reversal reactions. Reversal reactions are manifestations of delayed-type hypersensitivity to *M. leprae* that appear to represent a return of cell-mediated immune responsivity, and, in patients, are characterized by swelling of lesions and infiltration of mononuclear cells. These reactions can be reproduced in *M. leprae*-infected nude mice by transfer of allogeneic spleen cells from *nu/+* mice; although transfer of cells from unimmunized mice will produce the response, cells from immunized heterozygote mice are more effective ([3]). Reversal reactions may also be reproduced in nude mice by subcutaneous or intraperitoneal transplants of thymus tissue ([8]).

Summary

Two aspects of the immune deficiency of nude mice make these animals particularly useful tools for leprosy research. Nude mice are capable of supporting multiplication of *M. leprae* to levels approaching 10^{10} per g in peripheral body tissues. In addition, nude mice may be inoculated with $>10^4$ (in fact, with as many as 10^8) organisms per foot pad, without provoking an immune response that prevents multiplication of the organisms. Thus, the nude mouse should be particularly suitable for detecting persisting *M. leprae* in treated patients, and as a model of the patient for evaluating chemotherapeutic regimens.

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