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Leprosy in Hypertensive Nude Rats (SHR/NCrj-*rnu*)[1]

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Mycobacterium leprae has not been cultured in nonliving bacteriologic culture media ([9]). Because of this, we have used nude rat strains such as Rowett-*rnu* or F344/NJcl-*rnu* (Abstract; Jpn. J. Lepr. 50, p. 20, 1981). Unfortunately, these strains were not good for the production of a large amount of the bacilli. Development of a nude rat strain carrying the nude genetic background was desired in order to harvest large amounts of *M. leprae*. Since more than a decade ago, we have attempted to develop spontaneously hypertensive rats (SHR/NCrj) carrying the nude (*rnu*) gene that permits high multiplication of *M. leprae*. The hypertensive nude rats obtained showed high susceptibility to *M. leprae* ([7, 8]) and showed a characteristic disease with a progressive pattern of leproma formation which has never been seen in the nude mouse model of *M. leprae* infection ([2, 3, 5, 17]). These results strongly suggested that these hypertensive nude rats may be a very useful animal model of human lepromatous leprosy. Although the effects of immunodeficiency caused by the lack of a thymus on hypertension are not known so far, this hypertensive nude rat strain may also be useful for an animal model to demonstrate the relationship between hypertension and cellular immunity. We have attempted to establish an inbred strain carrying both the nude and hypertensive genes.

In this paper, we report age-related blood pressure change in the congenic hypertensive nude rat (SHR/NCrj-*rnu*) of the NE12F2 generation. We also examine the susceptibility to *M. leprae* in the NE12F3 generation.

MATERIALS AND METHODS

Breeding of a SHR/NCrj-*rnu* congenic rat strain. A congenic rat strain carrying nude (*rnu*) and hypertensive genes was produced using females of the SHR/NCrj rat (Charles River Japan, Atsugi, Japan) and males of the F344/NJcl-*rnu* (NE4F2) rat which was kindly provided by the Central Institute for Experimental Animals, Kawasaki, Japan. For the first cycle, SHR/NCrj females were crossed with F344/NJcl-*rnu/rnu* males and the progeny (*rnu*/+) were then crossed to recover male rats homozygous for the *rnu* gene which were then crossed with female SHR/NCrj rats for the next cycle. Cross-intercross was carried out 12 times to establish the SHR/NCrj-*rnu* congenic rat strain. We conventionally called the hypertensive nude rats in the F2 (genotype *rnu/rnu, rnu*/+, +/+) and the F3 (genotype *rnu/rnu, rnu*/+) generations of the NE12 (SHR·F344Hfh11) rats which were used in this paper the SHR/NCrj-*rnu* rats.

Husbandry. Breeding was conducted in isolators under specific pathogen-free (SPF) conditions in the animal room of the Leprosy Research Center, National Institute of Infectious Diseases (NIID), Tokyo, Japan. Nude rats were maintained in a vinyl isolator under SPF conditions for all of their lives. Hairy litter mates were removed at the age of 4 weeks and maintained in plas-

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tic cages (CL-0108; CLEA, Kawasaki, Japan) under conventional conditions. Both nude and hairy litter mate rats were reared with autoclaved CE-2 (CLEA) and tap water.

Measurement of blood pressure. The measurement of blood pressure was performed using 7 of the female and 12 of the male NE12F1(*rnu*/+) rats and 10 nude (*rnu*/*rnu*; 4 female, 6 male) and 20 hairy litter mates (*rnu*/+ or +/+; 10 female, 10 male) NE12F2 rats 4 to 22 weeks old. Also, blood pressure was measured in 5 female SHR/NCrj rats purchased from Charles River Japan and in 5 male F344/NJcl nude rats purchased from CLEA, all of them 10 to 20 weeks old. For indirect measurements of blood pressure, we used a tail-cuff apparatus (BP-98A; Softron, Tokyo, Japan) ([6]) without anesthesia. Briefly, This apparatus consists of a tail cuff with a pair of photodiodes and a light-emitting diode, an air pump, a pressure transducer and a personal computer that controls the entire apparatus. Systolic and mean blood pressure were determined at the pressures where this curve started and became maximum, respectively. Diastolic blood pressure was calculated from the values of the systolic and mean blood pressures. Measurements of blood pressure were repeated three times, respectively, and the results of the systolic blood pressure are shown in Figure 1.

Infection with *M. leprae*. *M. leprae* infection was performed using four of both sexes of congenic hypertensive nude rats (SHR/NCrj-*rnu*) and their hairy, litter mate, hypertensive rats (SHR/NCrj-*rnu*/+) of the NE12F3 generation which had crossed with male nude rats and the females of their hairy litter mates of the NE12F2 generation. Also used were four F344/NJcl-*rnu* of both sexes obtained from CLEA. The leprosy bacilli, Thai-53 strain derived from foot-pad passage of nude mice, were kindly provided by Dr. M. Matsuoka, Leprosy Research Center, NIID. The suspension was prepared as described previously ([12]). The inoculum size was 1.3×10^8 bacilli/foot pad of both hind feet.

Identification. In order to confirm the background of the hypertensive nude rats, we used a general genetic monitoring test with the NE12F2 generation. Genetic testing was carried out using two nude rats and two of their hairy litter mates of both sexes. Cellulose acetate membrane electrophoresis was applied to determine the genotypes of the following eight isozyme loci: *Amy1, Es1, Es2, Es3, Es4, Es14, Hbb* and *Svp1*. The genotype of *Mup1* was determined using 15% polyacrylamide gel. The genotypes of seven isozymes, *Akp1, Alp1, Es6, Es7, Es8, Es9* and *Es10*, were determined by isoelectric focusing. Typing of both the *RT1* and *RT2* loci was carried out using hemagglutination tests with allo-antisera. Genetic testing was performed by the ICLAS Monitoring Center (Central Institute for Experimental Animals, Kawasaki, Japan). Also, for identification of *M. leprae*, an immunohistopathological stain was done with the avidin-biotin-peroxidase complex (ABC) method to confirm the presence of phenolic glycolipid-I (PGL-I). The detection of the Thai-53 strain of *M. leprae* DNA was performed with three sets of specific primers: primers C and D hybridized with part of an *M. leprae*-specific repetitive sequence, S13 and S62 for the part of DNA encoding the 36-kDa protein gene of *M. leprae* ([1]), and the R5 and R6 primers also hybridized with a repetitive sequence of *M. leprae* ([15]).

Histopathological study. Tissues obtained from the infected site of tested rats 12 months after inoculation with *M. leprae* were fixed with 10% buffered formalin solution and were processed for paraffin sections. Then Fite-Faraco, hematoxylin and eosin (H&E) and Giemsa staining was done. For the dissemination of the infection, other tissues [fore foot pad, lip, ear, heart, lung, liver, spleen, pancreas, kidney, popliteal-iliac-lumbar and mandibular lymph nodes, the female (ovary and uterus) and male (testis, epididymis and others) reproductive system] were examined.

RESULTS

Blood pressure in NE12F1 and NE12F2 generations of rats. Blood pressure was measured from week 4 to week 22 after birth in the NE12F1 (*rnu*/+) rats. It began to increase from 6 weeks of age (female, 147 ± 2.3 mm Hg; male, 159 ± 9.9 mm Hg) and reached the highest level at around 20 weeks. The highest blood pressures in female and male rats were 183 ± 2.4 and 200 ± 5.8 mm Hg, respectively. Blood pressure readings of the NE12F2

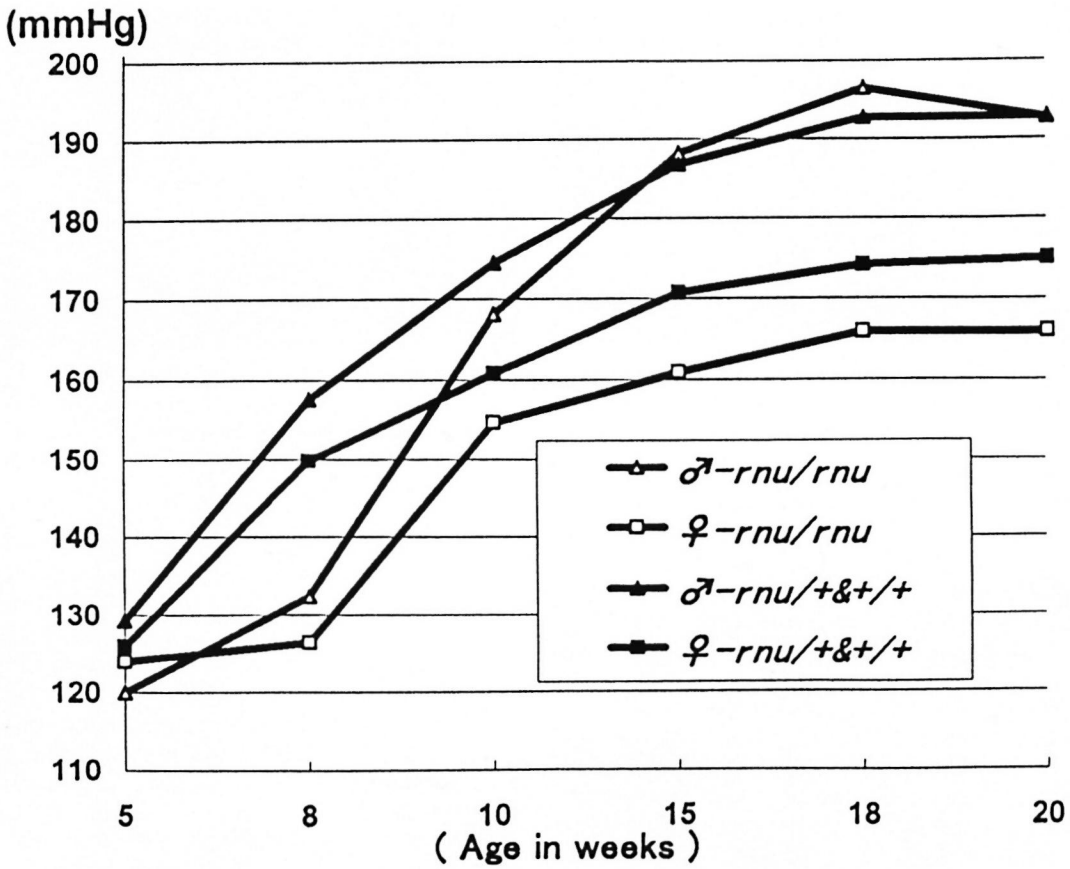

FIG. 1. Age-related changes in blood pressure of SHR/NCrj-*rnu* (*rnu-rnu*) and their litter mates (*rnu*/+ or +/+) from 5 to 20 weeks old. Each value represents the mean of 6 males-*rnu/rnu* (△), 4 females-*rnu/rnu* (□), 10 each of males-*rnu*/+ (▲) and females-*rnu*/+ (■).

(*rnu/rnu*, *rnu*/+ or +/+) rats are shown in Figure 1. In the hairy litter mate female and male rats, blood pressure began to increase to over 150 mm Hg at around week 8 (female, 150 ± 7.0; male, 158 ± 4.4 mm Hg) while those of the nude rats were 126 ± 10 mm Hg in females and 132 ± 4.5 mm Hg in males. In nude female and male rats, blood pressure began to increase at 10 weeks (*rnu/rnu*: female 155 ± 6.2 mm Hg, male 168 ± 19 mm Hg; *rnu*/+ or +/+: female 161 ± 9.7 mm Hg, male 174 ± 13 mm Hg). In both nude rats and their hairy litter mates blood pressure reached a peak at around week 20. The highest blood pressures in the female and male nude rats were 166 ± 1.4 and 197 ± 11 mm Hg, respectively, and in their hairy litter mates 175 ± 11 and 193 ± 3.2 mm Hg, respectively. Additionally, blood pressure for five 10-week old SHR/NCrj rats was 169.9 ± 3.1 mm Hg; by 20 weeks old, 188.6 ± 4.7 mm Hg. Whereas five 10-week old F344/NJcl nude rats had blood pressures of 124.4 ± 3.4 mm Hg and 118.4 ± 4.6 mm Hg at 20 weeks old. Subsequently, most blood pressure changes in the congenic hypertensive rats showed minor deviations from the average at 20 weeks, and were 171 ± 1.4 and 181 ± 11 mm Hg in females and males of the nude rats and 174 ± 26 and 188 ± 4.7 mm Hg in females and males of their hairy litter mates, respectively, although SHR/NCrj female blood pressure was 195.5 ± 12.3 mm Hg and for F344/NJcl male nude rats was 118.2 ± 6.8 mm Hg at 60 weeks of age.

Susceptibility of SHR/NCrj-*rnu* to *M. leprae*. Following inoculation with *M. leprae* in both hind foot pads of both sexes of the SHR/NCrj-*rnu* rats, a slight swelling in

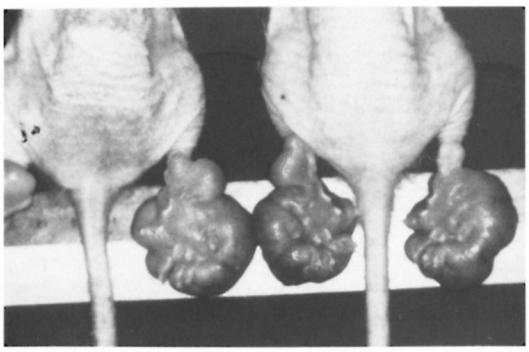

FIG. 2. Enlargement of both *M. leprae*-inoculated hind foot pads of hypertensive nude rats (SHR/NCrj-*rnu*) 12 months after inoculation.

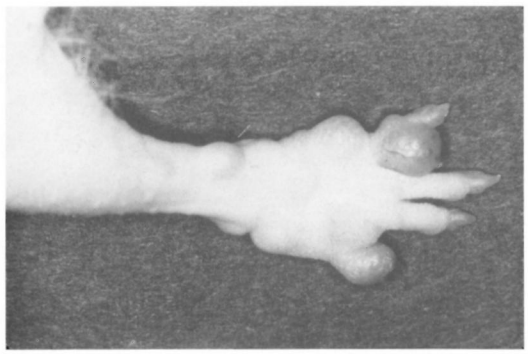

FIG. 3. Fore foot of an SHR/NCrj-*rnu* rat 12 months after inoculation with *M. leprae* to both hind foot pads. Macroscopic nodular lesions began to show on uninoculated fore foot about 10 months after inoculation with *M. leprae*.

each of the injected foot pads was seen at 4 months. No swelling was seen in the foot pads of F344/NJcl-*rnu* rats at that time. In both sexes of the F344/NJcl-*rnu* rats, a slight swelling was found at the inoculated site only after 6 months of infection. Approximately 10 months after infection, the swelling at the injected sites of both male and female SHR/NCrj-*rnu* rats became larger, and macroscopic nodular lesions were seen on the uninoculated fore foot and then on the lip. However, these lesions were not seen in the F344/NJcl-*rnu* rats. Massive swelling due to multiplication of *M. leprae* was seen in SHR/NCrj-*rnu* rats of both sexes 12 months postinfection (Fig. 2). The average weight of the inoculated hind foot below the ankle joint in SHR/NCrj-*rnu* rats was 14.6 ± 0.98 g per three rats of both hind feet while that in male F344/NJcl-*rnu* rats used as controls was 2.1 ± 0.13 g per two rats. Nodular lesions in the uninoculated fore foot (Fig. 3) and lip (Fig. 4) were more progressive. A 12 months postinfection mild nodular lesions were observed on the lower legs above the inoculation sites on the hind limbs and on the uninoculated fore feet in the F344/NJcl-*rnu* rats (Fig. 5). These changes were seen only in males, however, and not in females. The hairy litter mates of the SHR/NCrj-*rnu*/+ rats had only mildly swollen foot pads until 12 months after inoculation.

Histopathological findings of the infected hind foot pads of the SHR/NCrj-*rnu* rats 12 months postinfection with *M. leprae* are shown in Figure 6. Bacilli were observed over the entire field, including inside the bone marrow. As shown in Figure 6B (Giemsa stain), a mild lymphoid infiltration

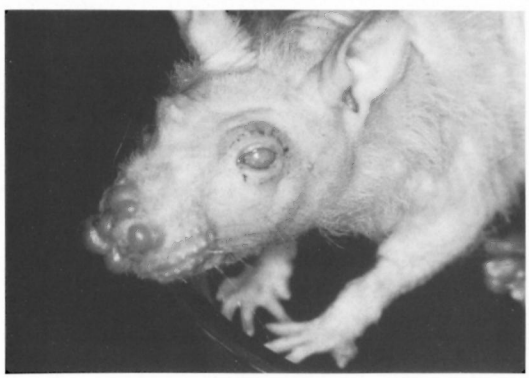

FIG. 4. Lips of an SHR/NCrj-*rnu* rat 12 months after inoculation with *M. leprae* to both hind foot pads. Multiple nodular lesions also began to show on the lips at about the same time as fore foot.

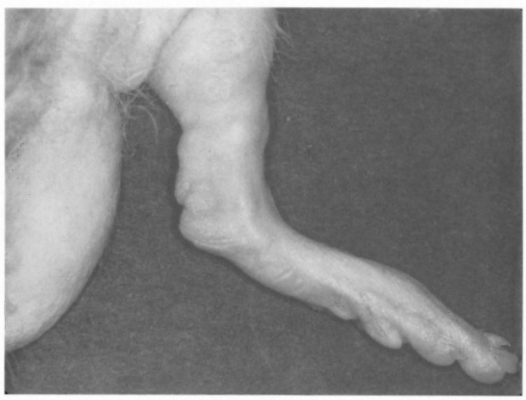

FIG. 5. *M. leprae*-inoculated hind foot of a male F344/NJcl-*rnu* rat 12 months postinfection.

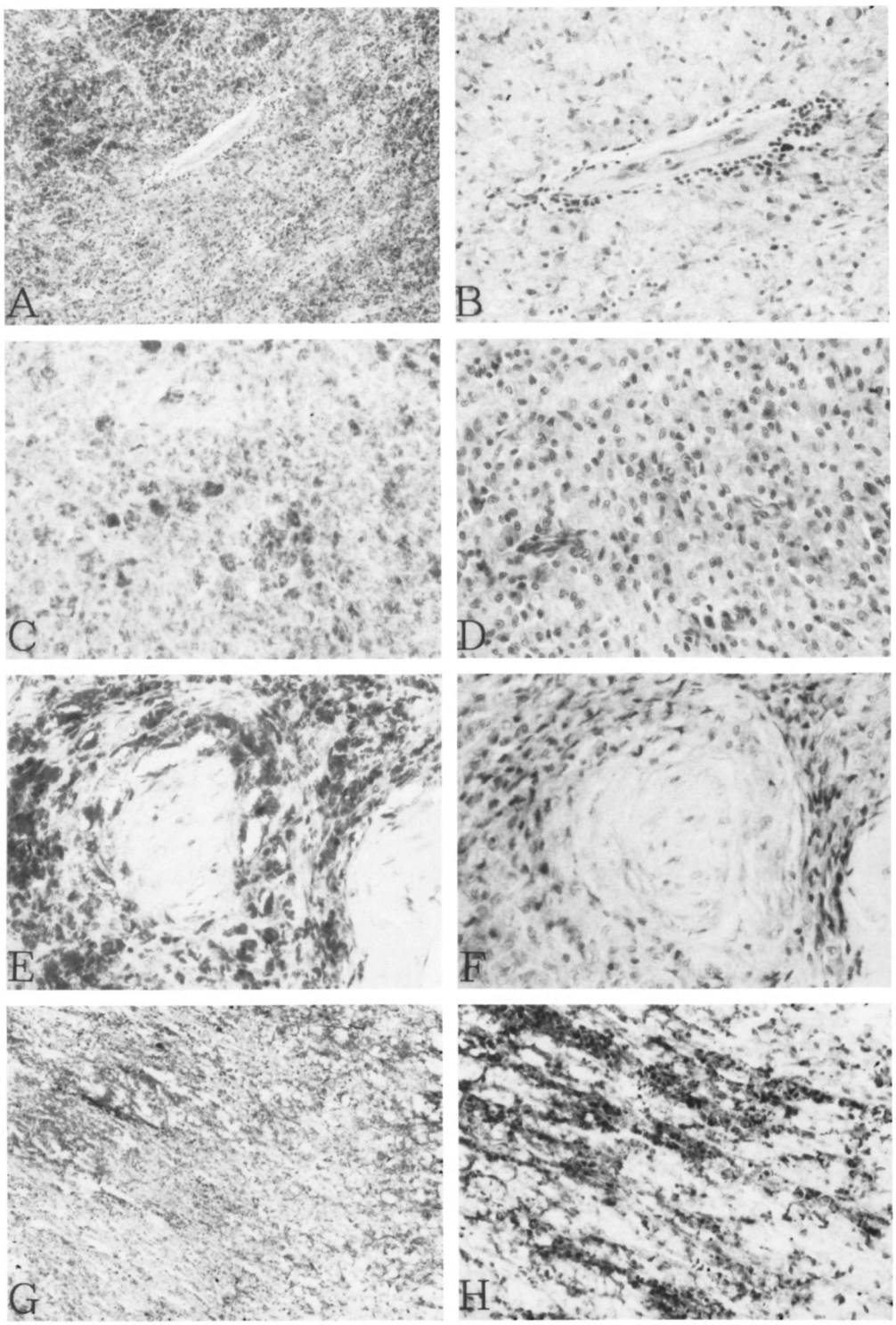

FIG. 6. Histopathological findings of SHR/NCrj-*rnu* rat 12 months postinfection with *M. leprae*. **A** = Inoculated hind foot pad (Fite-Faraco ×200); **B** = a serial section of **A** (Giemsa ×400); **C** = nodular lesion of uninoculated fore foot (Fite-Faraco ×400); **D** = serial section of **C** (H&E ×400); **E** = lips (Fite-Faraco ×400); **F** = serial section of **E** (Giemsa ×400); **G** = bone marrow of an inoculated hind foot pad (Fite-Faraco ×200); **H** = serial section of **G** (H&E ×400).

was apparent in the surrounding blood vessels in the inoculated hind foot pads. At the uninoculated sites, such as the fore foot pads (Fig. 6D), lips (Fig. 6F), or ears, no lymphoid infiltration was seen and only cells of the mononuclear phagocyte system, containing *M. leprae,* were present. Sites located at the intermuscular layer of the inoculated hind foot pad, inside the bone marrow, and in the popliteal lymph nodes of the hypertensive SHR/NCrj-*rnu* nude rats showed necrosis in the presence of numerous *M. leprae* (Fig. 6G, 6H). Additionally, *M. leprae* dissemination in the hypertensive nude rats reached throughout the internal organs, such as the liver, spleen and lungs, but it did not involve the heart, kidneys or pancreas. In the liver of the SHR/NCrj-*rnu* rats, almost all of the branches of the hepatic artery had disappeared, and leprosy bacilli were seen adjacent to the portal vein and bile duct and also in the Kupffer cells. Lymphocyte proliferation was never seen in the liver or at the uninoculated site of multiplication of *M. leprae.* The male reproductive system, especially in the interstitial tissue of the epididymis (Fig. 7) or ductus deferens and vas deferens, had many leprosy bacilli in contrast to the female reproductive system which did not. As shown in Figure 8, *M. leprae* were observed surrounded by many lymphoid infiltrates with heavy necrosis in the inoculated hind foot pads of the SHR/NCrj-*rnu*/+ litter mates. On the other hand in the F344/NJcl-*rnu* rates more multiplication of leprosy bacilli was observed at or near the surface of the skin, i.e., the dermis (Fig. 9A, 9B), than in the intermuscular layer (Fig. 9C, 9D) surrounded by lymphocytes and a few polymorphonuclear cells. *M. leprae* growth with lymphocytes and a few neutrophils was also seen in the uninoculated fore foot pad (Fig. 9E, 9F), lips and ears of F344/NJcl-*rnu* rats of both sexes. Dissemination in the F344/NJcl-*rnu* rats was similar in the hypertensive nude rats; however, a smaller number of bacilli was seen in them compared to the SHR/NCrj-*rnu* rats.

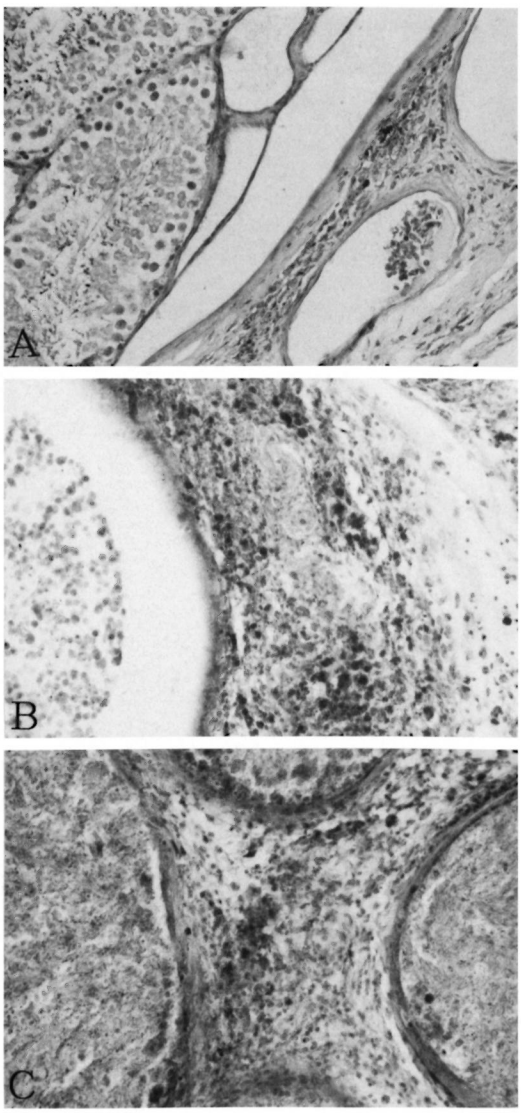

FIG. 7. Male reproductive system of an SHR/NCrj-*rnu* rat 12 months after inoculation with *M. leprae.* **A** = The testis; multiplication of bacilli are observed only near the blood vessels in the capsule (Fite-Faraco ×200); **B** = the epididymis; many leprosy bacilli are seen in the interstitial tissue of epididymis cauda (Fite-Faraco ×200); **C** = the ductus deferens; acid-fast bacilli are seen in the smooth muscle and connective tissue surrounding the ducts (Fite-Faraco ×200).

Identification. As shown in The Table, results of the genetic profile of the congenic SHR/NCrj-*rnu* NE12F2 generation rats was the same as that of the SHR/NCrj rats. The *RT1* locus in the hypertensive nude rat was *k* and the *RT2* locus was *b* in the SHR/NCrj rats; whereas the *RT1* locus was *l* and the *RT2* locus was *a* in the F344/NJcl-*rnu* rats. This result demonstrated that the genes of the F344/NJcl rat are not contributing to the genetic background of the congenic strain, except for the *rnu* gene, although for identification of *M. leprae* we carried out an immunohistopathological stain with the ABC

method and the presence of PGL-I was demonstrated at the site of multiplication of the bacilli in the inoculated hind foot pads. Also, specific *M. leprae* DNA from the Thai-53 strain (the strain of *M. leprae* inoculated into the hind foot pads of the SHR/NCrj-*rnu* rats) was detected and *M. leprae* DNA was seen with the three primers, namely, the C and D had a band with 372 bp, the S13 and S62 primers with 530 bp, and the R5 and R6 primers with 447 bp.

DISCUSSION

In order to develop an animal model which shows high susceptibility to *M. leprae,* we produced F2 progeny between the nude rat and several other inbred rat strains, that is, Rowett-*rnu,* F344/NJcl-*rnu* (NE4), WKY/NCrj-*rnu* (NE2), SHR/NCrj-*rnu* (NE4), LOU/N-*rnu* (NE2), LEW-*rnu* (NE2), ACI-*rnu* (NE3), and WM-*rnu* (NE2). We found that F2 rats (*rnu/rnu*) obtained using the SHR/NCrj strain were highly susceptible to *M. leprae* ([7, 8]). We attempted to establish a congenic strain, SHR/NCrj-*rnu*, which will be a good animal model for leprosy research.

It is well known that the SHR strain was established from Wistar Kyoto (WKY) rats by selection of blood pressure ([10]) and has been used as an animal model for human essential hypertension for a long time ([16]). We show age-related blood pressure changes in the congenic hypertensive nude rats (NE12F2 generation) (Fig. 1). An increase in the blood pressure in nude rats was found to begin at a slightly delayed age when compared with their hairy litter mates. The curve in both sexes of nude rats was similar to that of their hairy litter mates. Both female and male rats showed the highest blood pressure at around 20 weeks of age. The blood pressure of male rats was higher than female rats. The SHR/NCrj-*rnu* rats manifested hypertension as did their hairy litter mates. Additionally, as a result of the genetic monitoring test shown in The Table the genetic profile of the SHR/NCrj-*rnu* rat was the same as that of the SHR/NCrj rat except for the *rnu* gene. We have successfully developed the congenic hypertensive SHR/NCrj nude rat strain (SHR·F344Hfh11:SHR/ NCrj-*rnu*).

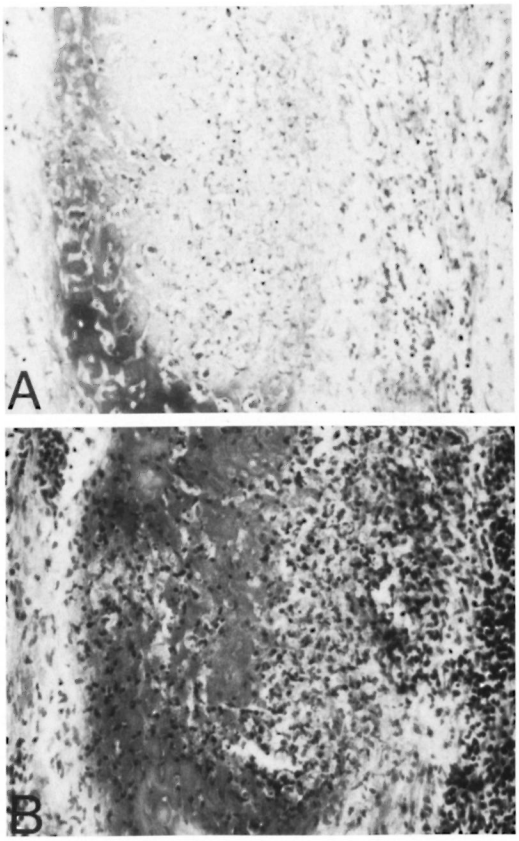

FIG. 8. Inoculated hind foot pad of a hairy litter mate of the SHR/NCrj-*rnu* rat 12 months after inoculation with *M. leprae.* **A** = Acid-fast bacilli are found in the intermuscular layer with necrosis (Fite-Faraco ×200); **B** = serial section of **A** (H&E ×200).

In the present study, comparisons were made on the susceptibility of *M. leprae* in hypertensive SHR/NCrj-*rnu* and normotensive F344/NJcl-*rnu* rats. We have reconfirmed that congenic hypertensive nude rats (NE12F2 generation) were highly susceptible to *M. leprae* with leproma formation. In the SHR/NCrj-*rnu* rats, both sexes developed massive swellings due to multiplication of *M. leprae* and also nodular lesions were produced in the uninoculated fore foot pads and in the lip (Figs. 2, 3, 4), while in the F344/NJcl-*rnu* rat only a slight swelling of the inoculated foot with a mild nodular lesion was seen (Fig. 5). As previously reported ([7, 8]), the formation of nodular lesions may be a disorder peculiar to nude rats. At noninoculated sites lymphocyte proliferation and necrosis were not seen; the leprosy bacilli at these sites were numerous and

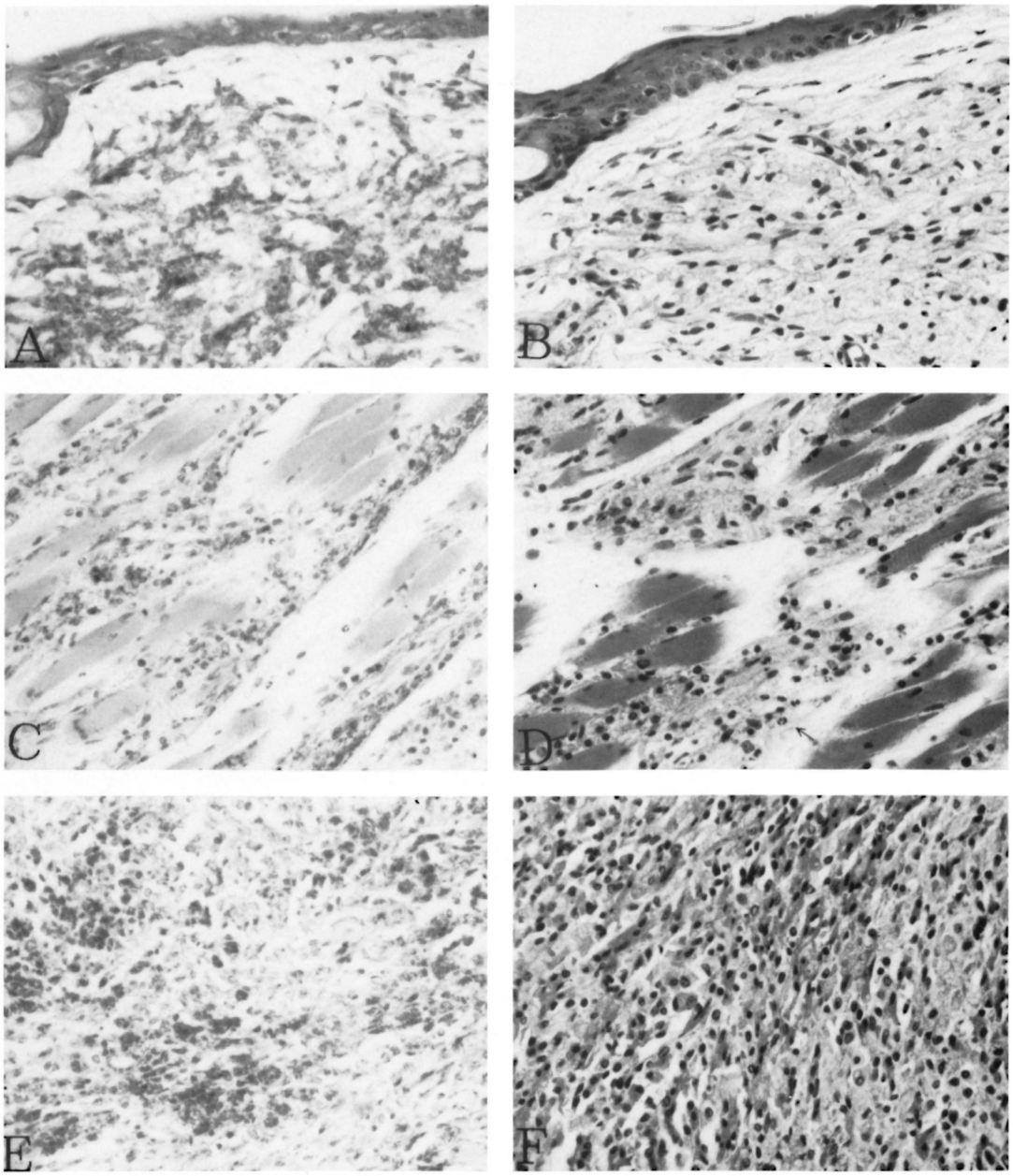

FIG. 9. Histopathological findings for an F344/NJcl-*rnu* rat 12 months after inoculation with *M. leprae*. **A** = Dermis of inoculated hind foot pad (Fite-Faraco ×400); **B** = serial section of **A** (H&E ×400); **C** = intermuscular layer of inoculated hind foot pad (Fite-Faraco ×400); **D** = serial section of **C** showing lymphocytes and a few neutrophils (↖) surrounding *M. leprae* growth (H&E ×400); **E** = subcutaneous tissues of uninoculated fore foot pad with many globi (Fite-Faraco ×400); **F** = serial section of **E** (H&E ×400).

were present in cells of the mononuclear phagocyte system (Fig. 6). However, in F344/NJcl-*rnu* rats (Fig. 9) lymphocyte proliferation with a few neutrophils was seen both in the inoculated hind foot pads and at every other site of multiplication of *M. leprae*. As shown in Figure 2 and Figure 5, there was a wide difference in the susceptibility of *M. leprae* between the hypertensive nude rats (SHR/NCrj-*rnu*) and the

THE TABLE. *Genetic profiles of the SHR/NCrj-*rnu*, SHR/NCrj and F344/NJcl-*rnu *strains.*

Strain	Number of rats M	F	Chromosome number and Gene locus 1	2	3	5	8	13	14	19	19	19	19	19	19	19	19	19	20	*	*
			Hbb	*Amy1*	*Syp1*	*Mup1*	*Acon1*	*Es6*	*Fh1*	*Gc*	*Es1*	*Es2*	*Es3*	*Es4*	*Es7*	*Es8*	*Es9*	*Es10*	*Es14*	*RT2*	*RT1A*	*Akp1*	*Alp1*
SHR-*rnu/rnu*	1		*a*	*a*	n.t.	*a*	*b*	n.t.	*b*	*a*	*a*	*a*	*b*	*a*	n.t.	n.t.	n.t.	n.t.	*b*	*b*	*k*	*a*	*a*
SHR-*rnu/rnu*	1		*a*	*a*	*a*	*a*	*b*	*a*	*b*	*a*	*a*	*a*	*b*	*a*	*b*	*b*	*a*	*a*	n.t.	*b*	*k*	*a*	*a*
SHR-*rnu/+*		1	*a*	*a*	n.t.	n.t.	*b*	n.t.	*b*	*a*	*a*	*a*	*b*	*a*	n.t.	n.t.	n.t.	n.t.	*b*	*b*	*k*	*a*	*a*
SHR-*rnu/+*	1		*a*	*a*	*a*	*a*	*b*	*a*	*b*	*a*	*a*	*a*	*b*	*a*	*b*	*b*	*a*	*a*	n.t.	*b*	*k*	*a*	*a*
SHR/NCrj		1	*a*	*a*	n.t.	n.t.	*b*	n.t.	*b*	*a*	*a*	*a*	*b*	*a*	n.t.	n.t.	n.t.	n.t.	*b*	*b*	*k*	*a*	*a*
F344-*rnu/rnu*	1		*a*	*a*	*a*	*b*	*b*	*a*	*b*	*a*	*a*	*a*	*a*	*b*	*b*	*b*	*a*	*a*	n.t.	*a*	*l*	*a*	*b*

[a] Genetic testing was carried out by: Hbb, RBC; Amyl, pancreas;' SVP1, seminal vesicle: Mup1, urine: Acon1, kidney; Es6, testis; Fh1, kidney; Gc, plasma; Es1, plasma; Es2, plasma; Es3, small intestine; Es4, kidney; Es7, testis; Es8, testis; Es9, testis; Es10, testis; Es14, plasma; Rt2, RBC; RT1A, RBC; Akp1, kidney; Alp1, kidney.
Abbreviations used: M = male; F = female; RBC = red blood cell; n.t. = Not tested; SHR = SHR/NCrj; F344 = F344/NJcl.
* = Chromosome number not determined.

normotensive nude rats (F344/NJcl-*rnu*). The pathogenesis of hypertension in SHR rats is clearly unknown. Yamori, *et al.* reported that the pathogenesis is multifactorial and more than one system is involved, likely the renal excretory processes, the renin-angiotensin system, changes in cellular processes which regulate vascular growth, and neurogenic effects ([16]). They also reported that two to six genes contribute to blood pressure ([13]). The formation of heavy lepromatoid lesions was enhanced with the corresponding genetic background of nude rats. For example, RT1 and RT2 loci in the SHR/NCrj-*rnu* rat were *k* and *b* while in the F344/NJcl-*rnu* rat they were *l* and *a*. Perhaps these genetic loci and the background to develop hypertension made a difference in the susceptibility to *M. leprae* growth.

M. leprae is an acid-fast bacterium that has many lipid components in the cell wall ([11]). In human lepromatous leprosy, *M. leprae* have an affinity to blood vessel endothelial cells ([4, 14]). Foamy cells are formed eventually due to fat degeneration in most of the tissues in which *M. leprae* have multiplied. The mechanisms determining how leprosy is manifested after *M. leprae* infection, and how *M. leprae* causes various pathologic changes, such as the necrotic vascular lesions in the hypertensive nude rat, are unknown. Perhaps if the reasons why the hypertensive nude rat shows such high susceptibility were known these other problems could be more easily addressed.

SUMMARY

Since more than a decade ago, we have attempted to develop spontaneously hypertensive rats carrying the nude gene that permits high multiplication of *Mycobacterium leprae*. A congenic strain carrying nude (*rnu*) and hypertensive genes was produced using SHR/NCrj females and F344/NJcl-*rnu* males. Cross-intercross was carried out 12 times to establish the hypertensive nude rat congenic strain. As a result of the genetic monitoring test with NE12F2 generation rats, the genetic profile of the SHR/NCrj-*rnu* rats was the same as that of the SHR/NCrj rats except for the *rnu* gene. We have successfully developed a hypertensive congenic nude rat strain (SHR·F344Hfh11; SHR/NCrj-*rnu*). An in-

crease in the blood pressure in nude rats was found to begin at a slightly delayed age when compared with their hairy litter mates. Both female and male rats showed the highest blood pressure at approximately 20 weeks of age—166 ± 1.4 and 197 ± 11 mm Hg in nude rats and 175 ± 11 and 193 ± 3.2 mm Hg in their hairy litter mates in female and male rats, respectively.

In the present study, comparisons were made on the susceptibility to *M. leprae* in hypertensive SHR/NCrj-*rnu* and normotensive F344/NJcl-*rnu* rats. We have reconfirmed that hypertensive SHR/NCrj-*rnu* rats of the NE12F3 generation were highly susceptible to *M. leprae*. In the SHR/NCrj-*rnu* rats of both sexes excellent massive swelling due to multiplication of *M. leprae* was observed and, also, nodular lesions were produced in uninoculated fore feet and lips while those sites in the F344/NJcl-*rnu* rats showed only a slight swelling of the inoculated feet with mild nodular lesions. Although mild lymphocyte proliferation was seen only in the *M. leprae*-inoculated site with numerous bacilli and partial necrosis in the SHR/NCrj-*rnu* rats, at noninoculated sites, multiplication of *M. leprae* was only observed in the cells of the mononuclear phagocyte system. However, in F344/NJcl-*rnu* rats, lymphocyte proliferation with a few neutrophils was seen at the site of inoculated hind foot pads and everywhere at the site of multiplication of *M. leprae*. There was a wide difference in the susceptibility to *M. leprae* between the SHR/NCrj-*rnu* and the F344/NJcl-*rnu* rats.

RESUMEN

Desde hace más de una década, hemos intentado desarrollar ratas hipertensas portadoras del gene desnudeo *rnu* que permitan la multiplicación masiva de *Mycobacterium leprae*. Asi desarrollamos una cepa congénica portadora de los genes desnudo (*rnu*) y de hipertensión, usando hembras SHR/NCrj y machos F344/NJcl-*rnu*. Se neccesitaron 12 intercruces para establecer la cepa de ratas desnudas congénicas e hipertensas. El análisis de las ratas de la generación NE12F2 indicó que el perfil genético de las ratas SHR/NCrj-*rnu* fue el mismo que el de las ratas SHR/NCrj, excepto por el gene rnu. Hemos desarrollado, exitosamente, una cepa de ratas desnudas congénicas e hipertensas (SHR-F344Hfh11; SHR/NCrj-*rnu*). Comparadas con las camadas con pelo, las ratas desnudas presentaran un incremento en la presión sanguinea a una edad ligeramente tardia. Tanto las hembras como los machos mostraron la presión sanguinea más alta alrededor de las 20 semanas de edad: 166 ± 1.4 mm Hg (hembras) y 197 ± 11 mm Hg (machos) en las ratas desnudas, y 175 ± 11 mm Hg (hembras) y 193 ± 3.2 mm Hg (machos) en las ratas con pelo. En el presente estudio, se hicieron comparaciones en la susceptibilidad a *M. leprae* en las ratas hipertensas SHR/NCrj-*rnu* y en las ratas normotensas F344/NJcl-*rnu*. Confiramamos que las ratas hipertensas SHR/NCrj-*rnu* de la generación NE12F3 fueron altamente susceptibles a *M. leprae*. En las ratas SHR/NCrj-*rnu* de ambos sexos se observó una multiplicación masiva de *M. leprae* en las patas traseras inoculadas y lesiones nodulares en las patas delanteras y en los labios, mientras que las ratas F344/NJcl-*rnu* sólo mostraron una hinchazon moderada de las patas inoculadas y lesiones nodulares discretas. Aunque en las ratas SHR/NCrj-*rnu* sólo se observó ligera proliferación de linfocitos con numerosos bacilos y necrosis parcial en los sitios inoculados con *M. leprae*, en los sitios no inoculados la multiplicación de *M. leprae* sólo se observó en las células del sistema fagocitico mononuclear. Sin embargo, en las ratas F344/NJcl-*rnu*, la proliferación de linfocitos, con escasos neutrófilos, se observó tanto en el sitio de inoculación como en todos aquellos sitios de multiplicación de *M. leprae*. Hubo una gran diferencia en la susceptibilidad a *M. leprae* entre las ratas SHR/NCrj-*rnu* y las ratas F344/NJcl-*rnu*.

RÉSUMÉ

Il y a plus de 10 ans, nous avons essayé de développer des rats spontanément hypertendus porteurs du gène nu qui permet une forte multiplication de *Mycobacterium leprae*. Une souche congénique porteuse des gènes nu (*rnu*) et hypertensifs fut produite en croisant des femelles SHR/NCrj et des mâles F344/Njcl-*rnu*. Des croisements successifs furent entrepris 12 fois pour établir la souche consanguine congénique de rats nu hypertendus. D'après les résultats des tests génétiques à partir des rats de génération NE12F2, le profil génétique des rats SHR/NCrj-*rnu* était le même que celui des rats SHR/NCrj, excepté pour le gène *rnu*. Nous avons donc développé avec succès une souche congénique de rats nus hypertendus (SHR•F344Hfh11;SHR/NCrj-*rnu*). L' augmentation de la pression artérielle chez ces rats nus débutait légèrement après leur homologues porteurs de poils. Les rats tant femelles que mâles présentaient la plus haute pression artérielle autour de 20 semaines: 166 ± 1,4 et 197 ± 11 mm Hg chez les rats nus et 175 ± 11 et 193 ± 3,2 mm Hg chez leur homologues porteurs de poils, respectivement.

Dans cette étude, il fut entrepris une comparaison de la susceptibilité des rats hypertendus SHR/NCrj-*rnu* et des rats normotendus F344/NJcl-*rnu* à l'infection par *M. leprae*. Nous avons reconfirmés que les rats hypertendus SHR/NCrj-*rnu* de la génération NE12F3 étaient hautement susceptibles à *M. leprae*. Chez les rats SHR/NCrj-*rnu* des deux sexes, des lésions massives dues à des multiplications de *M. leprae* furent

observées aux sites d'inoculation. De plus, des lésions nodulaires étaient présentes sur les pattes avant non-inoculées et les lèvres tandis que ces sites, chez les rats F344/NJcl-*rnu*, ne montraient que des lésions légères au niveau des pattes inoculées avec des lésions nodulaires légères. Bien que des infiltrations lymphocytaires légères ne furent observées que dans des sites d'inoculation comportant de nombreux bacilles et de la nécrose partielle chez les rats SHR/NCrj-*rnu*, dans les sites non-inoculés, la multiplication de *M. leprae* ne fut observée que dans les cellules du système des phagocytes mononucléés. Cependant, chez les rats F344/NJcl-*rnu*, des infiltrations lymphocytaires avec quelques neutrophiles ont été vues dans les sites d'inoculation des pattes arrières et à chaque site de multiplication de *M. leprae*. En conclusion, une différence importante de la susceptiblité à entre les rats SHR/NCrj-*rnu* et les rats F344/NJcl-*rnu* a été observée.

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