The Experimental Inoculation with Leprosy Bacillus in Various Hybrid Nude Mice (Continued): The Results of CD-1(ICR) Hybrid Nude Mice

KAZUNARI NAKAMURA and YASUKO YOGI

(National Institute for Leprosy Research)

(Received for Publication: July 23, 1988)

Key words: *Mycobacterium leprae*, experimental lepromatous leprosy model, CD-1(ICR) hybrid nude mice

We have successfully established as an animal model for experimental lepromatous leprosy by using NFS/N nude mice originating from a Swiss colony⁽¹⁾ and SHR nude rats originating from a Wistar colony⁽⁴⁾. In addition, non-obese diabetic (NOD) hybrid nude mice originating from Swiss-Webster mice (Jcl: ICR outbred strain), which gave excellent results with the development of a marked lepromatoid lesions as compared with that of "resistant" Crj: CD-1(ICR) or Jcl: ICR nude mice⁽³⁾.

In this report, we compared the susceptibility of CD-1(ICR) and Jcl: AF hybrid nude mice originating from the same Swiss-Webster (ICR outbred) mice with that of "resistant" CD-1(ICR) nude mice.

Materials and Methods

We have established CD-1(ICR) hybrid nude mice (M_1) by mating the CD-1 female mice and NFS/N or N: NIH (s) male nude mice. Jcl: AF hybrid nude mice (IAI inbred female mice originating from Jcl: ICR outbred strain) were obtained from CLEA, Tokyo, Japan. CD-1(ICR) hybrid nude mice (4-7 weeks old, 10 nude mice, respectively) and 10 CD-1(ICR) nude mice 4-7 weeks old were used as control group. The inoculum size was 5.2×10^5 bacilli/foot derived from hind foot passage of Crj: CD-1(ICR) nude mice. Experiment of Jcl: AF hybrid nude mice were performed separately. Ten Jcl: AF nude mice and CD-1(ICR) nude mice (6 male, 7 female) 4-5 weeks old were used. Inoculum size was 7.8×10^5 bacilli/foot derived from passage of Jcl: ICR nude mice. The site of injection was the right hind footpad. Mice were maintained in the vinyl isolator under specific pathogen free (SPF) conditions, and were provided with sterilized autoclavable commercial diet (CE-2, CLEA, JAPAN) and tap water *ad libitum*. For identification, the harvested acid fast bacilli (AFB) were reinoculated into the right hind foot of Jcl: ICR mice in order to confirm the growth pattern (persistent infection with microglobi). The AFB obtained were cultured on 1% Ogawa's medium and a modified Nemoto's egg yolk medium at 33°C and 37°C for 3 months. The smears of AFB were treated with pyridine

Fig. 1 Swelling of the right hind foot, site of inoculation, + swelling foot shows on the left nude mouse, and +++ swelling foot due to *M. leprae* growth shows on the right nude mouse.

Table 1 Inoculated right hind foot swelling due to the growth of *M. leprae* in the hybrid nude mice

Days after inoculation	Genetic background of nude mice											
	CD-1(ICR)				CD-1×NFS/N				CD-1×N: NIH (s)			
242	++	+	+	±	+++	+++	+++	+++	+++	+++	+++	+++
	±	±	±	±	+++	++	++	++	++	++	++	++
434	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++
	++	++	+		+++	++			+++			

Note; Inoculum size..... 5.2×10^5 bacilli/foot

as modified by us. Besides, the tissues obtained from infected nude mice were fixed in buffered formaldehyde solution, and stained with Fite-Faraco, and Heamatoxylin-Eosin.

Results

As shown in Fig. 1, one plus swelling due to *M. leprae* growth indicate on the left nude mouse hind foot, four plus swelling shows on the right nude mouse hind foot. Also, the swelling shows due to *M. leprae* growth in injected hind foot of CD-1(ICR) hybrid nude mice and CD-1(ICR) nude mice are given in Table 1 at 242 days and 434 days after injection. At 434 days after injection, there were marked nodular lesions in the infected foot, developing to the lower leg and toes of CD-1(ICR)×NFS/N and CD-1(ICR)×N: NIH(s) hybrid nude mice (M_1) indicating no significant differences against susceptibility to *M. leprae* in either mouse group. In contrast, CD-1(ICR) nude mice showed

Table 2 Inoculated right hind foot swelling due to the growth of *M. leprae* in the AF hybrid nude mice

Days after inoculation	Genetic background of nude mice							
	CD-1(ICR)				AF (IAI×NFS/N)			
300	++	++	++	+	##	##	##	##
	+	+	+	+	++	++	++	+
	+	±	±					
433	##	##	##	++	###	###	###	###
	++	++	++	+	##	++	++	

Note; Inoculum size..... 7.8×10^5 bacilli/foot

lesions with a slight swelling at the infected paw.

As shown in Table 2, the swelling due to *M. leprae* growth of Jcl: AF hybrid nude mice gave excellent results as compared with CD-1(ICR) nude mice at 300 days and 433 days after inoculation, as well as CD-1(ICR) hybrid nude mice, above mentioned. Thus, CD-1(ICR) hybrid nude mice and Jcl: AF hybrid nude mice were highly susceptibility to *M. leprae* as compared with CD-1(ICR) nude mice originating in spite of the same Swiss-Webster mice (ICR outbred strain). The AFB isolated from the swollen tissues were distinguished from other mycobacteria, namely, the reinoculation test by using Jcl: ICR normal mice indicated with persistent resistant infection only at the site of injection; loss of acid-fastness by pyridine extraction; the cultivation on a modified Nemoto's egg yolk medium as well as on 1% Ogawa's medium at 33°C and 37°C for 3 months which showed no growth. In addition, sections of the inoculated feet of CD-1(ICR) and Jcl: AF hybrid nude mice showed the presence of severe lepromatoid lesions, when stained with Fite-Faraco and Haematoxylin-Eosin as compared with CD-1(ICR) nude mice. Therefore, we have established the CD-1(ICR) hybrid nude mice and Jcl: AF hybrid nude mice at the M₁ circle as a new model for experimental lepromatous leprosy, resembling the cases of ICR-Lasat and their athymic counterparts, and NOD hybrid nude mice as compared with that of "resistant" CD-1(ICR) nude mice.

Discussion

We showed as an animal model for experimental lepromatous leprosy, such as NOD hybrid nude mice originated from Swiss-Webster mice (Jcl: ICR outbred strain)⁽⁵⁾ and NFS/N and NIH(s) nude mice originated from Swiss mice^(2,4). In addition, CD-1(ICR) hybrid nude mice; CD-1(ICR)×NFS/N, CD-1(ICR)×N: NIH(s) and IAI inbred strain originated from Jcl: ICR mouse by mating the cross-intercross system (M₁ circle) with CD-1 outbred strain or IAI inbred strain female and NFS/N or N: NIH(s) male nude mice, they were produced highly susceptibility to *M. leprae*. Rice and O'Brien reported that the genetic variance of laboratory outbred Swiss mice⁽⁶⁾. However, in spite of the fact these mice originated from a Swiss or Swiss-Webster colony, gave excellent results with the development of marked

lepromatoid lesions, resembling NFS/N and N : NIH(s) nude mice originating a Swiss strain. Thus, the formation of lepromatoid lesions in the Swiss and Swiss-Webster hybrid nude mice (M_1) may be produced by the genetic background except for CD-1(ICR) nude mice. The same may be true for SHR hybrid nude rats originating from the Wistar colony which differ from "resistant" WKY, WM, and LOU nude rats in experimental lepromatous leprosy.

Summary

Hybrid nude mice by mating with CD-1(ICR) outbred and IAI inbred strain originating Jcl : ICR strain female and NFS/N inbred or N : NIH (s) outbred nude male mice, they were given excellent results with the development of marked lepromatoid lesions in comparison with that of "resistant" CD-1(ICR) nude mice. In summary, we have established CD-1(ICR)×NFS/N, CD-1(ICR)×NIH(s) and IAI×NFS/N hybrid nude mice at the M_1 circle (generation) as a new model for the experimental lepromatous leprosy. The development of marked lepromatoid lesions formation in nude mice may be produced by the genetic background of the Swiss colony except for CD-1(ICR) nude mice.

Acknowledgements

This study was supported in part by grants from the U. S. -Japan Cooperative Medical Science Program and the Sasakawa Memorial Health Foundation, and the results of CD-1 (ICR) and Jcl : AF hybrid nude mice described in Ann. Rep. for Lep. Res. Tokyo. pp. 31-35 (1987). (In Japanese)

References

- 1) Nakamura, K. and Yogi, Y.: The nude mouse as an experimental lepromatous leprosy model (continued): The NFS/N nude mouse as a new model using the intra-upper lip inoculation method. Int. J. Lepr., **50**, 585, (1982).
- 2) Nakamura, K. and Yogi, Y.: The hereditary asplenic athymic (LASAT) mouse as an experimental lepromatous leprosy model (continued): Role of the spleen in the formation of the lepromatoid lesion. Int. J. Lepr., **50**, 586, (1982).
- 3) Nakamura, K. and Yogi, Y.: The nude rat as an experimental lepromatous leprosy model (continued): The effects of carrageenans splenectomy, and treatment with thymus cells. Int. J. Lepr., **50**, 587-588, (1982).
- 4) Nakamura, K., and Yogi, Y. The athymic rodent as an experimental lepromatous leprosy model (continued): The SHR nude rat as a new model and the effect of host in the formation of the lepromatoid lesions in nude mice. Int. J. Lepr., **52**, 605-606, (1984).
- 5) Nakamura, K. and Yogi, Y.: *M. leprae*-susceptibility of NOD hybrid nude mice. Int. J. Lepr., **55**, 787-788, (1987).
- 6) Rice, M. C. and O'Brien, S. J.: Genetic variance of laboratory outbred Swiss mice. Nature, **283**, 157-161, (1980).

雑種ヌードマウスへのらい菌接種（続報） CD-1 (ICR) 雑種ヌードマウスの成績

中 村 一 成 興 儀 ヤ ス 子

キーワード：らい菌，実験的らい腫らいモデル，CD-1(ICR) 雑種ヌードマウス

ヌードマウスの遺伝的背景の遺伝子が異なるとらい腫らい病巣の形成能も異なることを始めて明らかにし得た。すなわち，スイス系で純系である NFS/N ヌードマウスは outbred の N : NIH(s) ヌードマウスと同様，らい腫らい病巣の形成が高く，感受性能が良好であったのに対し，同様にスイス系である outbred の Jcl : ICR ヌードマウス および Crj : CD-1 ヌードマウスはいずれもらい腫らいの形成能は軽度でらい菌に対し“抵抗性”であった。しかしながら，Jcl : ICR より分離・系統化された NOD マウスに Crj : CD-1(ICR) のヌード遺伝子を導入した雑種ヌードマウスはらい菌に高い感受性能を有することを明らかにし得た。

そこで本報では，NFS/N および N : NIH(s) ヌードマウスの雄を用いてのクロスインタークロス方式により雑種ヌードマウス (M₁) を作出した。一方，クレアで

は Jcl : ICR マウスより分離・系統化した IAI と雄の NFS/N ヌードマウスとの雑種ヌードマウス Jcl : AF-*nu*を開発市販しているので購入し，らい菌に対する感受性能をそれぞれ検討したところ，らい腫らい病巣の形成能が高いことが判明した。この知見は，既報の ICR ラザットマウスを作出する過程で得られた同胞ヌードマウスが，らい菌感受性能がよいこと，さらにヌードラットでも遺伝的背景が同じウイスター系であるのに SHR 雑種ヌードラットはらい菌感受性能が高いのに対し，WKY, WM, あるいは LOU/N 雑種ヌードラットでは感受性能が低いことを解明し得たことを考え合せると興味ある免疫遺伝学的知見と考えられる。従って，今後さらにらい菌感受性能あるいは“抵抗性”の遺伝的解析を続行し，その生体防御の機能を究明してみたい。