The Nude Mouse as an Experimental Lepromatous Leplosy Model (Continued): The Lepromatoid Lesions in Mystacial Vibrissae Located Site of Injection

Yasuko YOGI and Kazunari NAKAMURA

(National Institute for Leprosy Research)

(Received for publication: February 26, 1986)

Key words: Congenitally athymic (nude) mouse, an experimental lepromatous leprosy model, intra-upper lip inoculation, intra-dorsal site inoculation of the fore and hind foot.

We have successfully established an animal model for experimental lepromatous leprosy using the specific pathogen-free (SPF) nude mouse in 1976, and the germ-free nude mouse, receiving footpad inoculation method in 1979⁽²⁾. We have developed a "foot injection" method instead of footpad injection in nude mice, by oneself, in the vinyl isolator. This method involves injection in the dorsal sites of fore and/or hind feet without the plantar aspect (footpad), using a two-step needle. We have also developed a combined method, which involves a second injection in the mystacial vibrissae located site. This combined inoculation method (fore, and hind feet plus lips infection) was useful for obtaining numerous bacilli (10^{10} – 10^{11}) per nude mouse at an early stage (about 9–10 months) after infection as a source of *Mycobacterium leprae*, in 1978⁽¹⁾.

In this report, a long term observation was performed to determine whether or not heavy lepromatoid lesions occur in the mystacial vibrissae located site (swelling) after the inoculation with a "virulent" *M. leprae* strain.

Materials and Methods

The site of injection for experimental mice was the right upper lip at the site of mystacial vibrissae, while for control mice the injection was made at the right dorsal site of the hind foot, using the nude mouse passage of *M. leprae*, namely, which was maintained by successive transfers in normal mice (22nd passage) and then 5th passaged in nude mice. *M. leprae* used was "virulent" because it had caused slight swelling due to *M. leprae* growth in injected hind feet at 6 to 7 months after the foot injection with 10^5 – 10^6 bacilli using BALB/c nude mice. In the present study, 10 each nude mice with background BALB/c of 5–10 weeks old were used. They were maintained in the vinyl isolator under SPF condition and were provided with sterilized autoclavable commercial diet (CE-2, CLEA) and tap water *ad libitum*. In addition, congenitally vibrissaeless nude mice established in our laboratory were also used with mystacial vibrissae injection in the present study as shown in Fig. 1, on the right.

The inoculum size were 5.0×10^5 bacilli for the right intra-upper lip (mystacial vibrissae located part) and the right dorsal site of the hind foot, respectively.

For identification, the acid fast bacilli (AFB) harvested aseptically, were reinoculated into the right hind foot of 6 BALB/c mice in order to confirm the growth pattern (persistent infection) in the mice. The obtained AFB were cultured on 1% Ogawa's medium and a modified Nemoto's egg yolk medium at 33°C and 37°C for 5 to 6 months. Also, the slides with AFB were treated with pyridine, as modified by us for use with smears. This treatment was done as follows; Dry the smears on cover slides in oven for 15-30 minutes or at room temperature for 5-24 hrs. The Ziehl-Neelsen stain for acid fast bacteria. Dehydrate in fresh pyridine for 5-10 minutes at 60°C. Shake, rinse, and wash in water bath for about 30 minutes at 37-40°C.

The tissues were fixed in buffered formaldehyde solution, and stained with Fite-Faraco, and haematoxylin-eosin.

Results

In the event of *M. leprae* injection into the dorsal site, the swelling due to *M. leprae* growth at foot was observed at 6 months in only 2 of 7 nude mice tested, as well as previously reported footpad inoculation method. In the case of intra-upper lip injection, no swelling at injected site was observed. At 10 months after the injection, all nude mice tested were observed with the swelling of injected foot (thickness: less than 2mm), one nude mouse died. In contrast, in the case of the animals injected in the intra-upper lip, only a small amount of swelling of the injected site was observed.

Afterward, at 11-13 months after the intra-upper lip inoculation, the swelling of the upper lip as shown in Fig. 2 was observed in 4 of the 7 nude mice tested. At that time, the bacillary counts were approximately 5.0×10^{11} AFB per gram with swollen, redness and abscess-forming tissues as shown in Fig. 3. In addition, a slight systemic infection had occurred as well as foot injection. At 16-17 months after injection, three of the nude mice showed excellent swelling due to *M. leprae* growth in injected lip. The lepromatoid lesions extended to the neighbouring parts such as the uninoculated left and lower lips, nose, eye lids, ears and rhinarium, skin over diastema, dentine of incisors, skin of muzzle and nasal septum, mandibular nodes and the skin at the site of mandible. As shown in Fig. 4, section of the opposite upper lip at 16 months after injection showed a numerous AFB invaded in muscle layers and in vibrissae follicle. In addition, systemic infection occurred as evidenced by lepromatoid lesions in the extremitas, tail, epididymis, testis, liver, lung, and spleen.

The longest survival included only one mouse observed at 25.5 months after upper lip injection. No clinical ulceration due to *M. leprae* growth was observed at any time during this experiment. As shown in Fig. 5, a swelling due to *M. leprae* growth were found to develop in the both upper and lower lips and neighbouring sites such as the nose, eye lids, and ears of each the right and left sites, besides, extremitas and so on. As shown in Fig. 6, section of the opposite vibrissae follicle at 25.5 months after injection contained numerous AFB. In addition, a slight swelling due to *M. leprae* growth was found in extremitas and the epididymis. However, in spite of severe nose lesions at an earlier stage lung lesions could not be accelerated.

These systemic lesions were mostly similar to those of the foot injection at later stage after injection.

The AFB obtained from the swollen, erythematous and abscess-forming tissue have bacteriologically been distinguished from other mycobacteria: The reinoculation test in normal mice confirmed persistent infection at the site of injection only, loss of acid-fastness by pyridine extraction, and the cultivation on modified Nemoto's egg yolk medium and 1% Ogawa's medium at 33°C and 37°C for 5 to 6 months was impossible. Thus, the AFB obtained from the lesions could not be distinguished from *M. leprae*.

Discussion

We have previously described that a slight systemic *M. leprae* infection produced in congenitally athymic (nude) mice (respectively reared under SPF, and conventional condition) using footpad inoculation method⁽¹⁻³⁾. Colston and Hilson independently reported that the longest survival included only two nude mice in the case of conventionally reared, showing the enhanced growth of *M. leprae* at about 9 months after footpad injection⁽⁶⁾, in compared with our report that the similar result observed at 9-12 months after inoculation with reared under conventional condition. Anyhow, nude mice would support strongly enhanced growth of *M. leprae*, if safely maintained longer than that of about 6 months as previously reported by Prabhakaran⁽⁵⁾, using footpad inoculation method.

We have developed a "foot injection" method as well as footpad injection method using nude mice⁽¹⁻³⁾. We reconfirmed that severe lepromatoid lesions of the injected right upper lip, including of neighbouring sites, and systemic lesions occurred as well as hind "foot injection" method or footpad injection as above mentioned. We observed with long-term survival only one nude mouse at 25.5 months after the upper lip injection. In the case of upper lip injection in nude mice, the excellent swelling due to *M. leprae* growth occurred and began to develop swelling in neighbouring sites such as the left and lower lip, nose, ears, eye lips and rhinarium. However, in spite of severe nose lesions, with lung lesions or lesions involving dentine, skin of the mandible site, and tongue could not be accelerated. No clinical ulceration at the root of tail due to *M. leprae* growth was reconfirmed in this study, at any time during both experiments, in contrast with reported by Kohsaka *et al.*⁽⁷⁾ except for injected sites lesions. Hasting *et al.* reported that an excellent result was observed in nude mice inoculated with armadillo derived *M. leprae*⁽⁸⁾.

These results may suggest that differences of a virulent of *M. leprae* or symbiosis with another pathogens as compared with our results, showing no excellent lesions occurred in injected sites in BALB/c nude mice, except for germ-free nude mice⁽²⁾ and thymus grafting⁽³⁾, can also serve as a marker for "virulent" strains of *M. leprae*. This marker can be used to compare different strains of *M. leprae* as well as previously reported that differences in morphology⁽⁴⁾.

Summary

Employing congenitally vibrissaeless nude mice, inoculations of *M. leprae* were made either

into the right upper lip at the site of mystacial vibrissae or the right dorsal site of the hind foot at a dose of 5.0×10^5 , respectively.

We have established severe lepromatoid lesions of the injected upper lip, including neighbouring tissues, and systemic lesions in the vibrissaeless nude mouse with intra-upper lip inoculation method. These systemic lesions occurred after injections into the upper lip as well as after hind foot injections. However, in spite of severe nose lesions at an earlier stage, the intra-upper lip infection, with lung lesions or lesions involving dentine, skin of the mandible site and tongue could not be accelerated. These findings led to the conclusion that the preferable infection sites in the nude mouse, especially in the vinyl isolators, were reconfirmed. That is to say, our simple combined method by which the dorsal sites of the fore and hind feet as well as footpad site, and intra-upper lips were injected with *M. leprae* was useful for increasing the yield of *M. leprae* at an early stage after the infection and also for establishing an experimental lepromatous leprosy model.

Acknowledgments

This study was supported in part by U. S.-Japan Cooperative Medical Science Program, and described in same report, in Japanese, 1979, and also, reported at U. S.-Japan Fifteenth Joint Conference on Leprosy Research, Kagoshima, 1980.

References

- 1) Nakamura, K. and Yogi, Y.: The development of experimental lepromatous leprosy (continued); Investigations of serial passage, inoculation method and various inoculation routes using the nude mice, Jap. J. Lep. **47**, 166 (1978), (in Japanese).
- 2) Nakamura, K. and Yogi, Y.: Experimental lepromatous leprosy in germ-free (gnotobiotic) nude mice, Jap. J. Lep. **48**, 199 (1979), (in Japanese).
- 3) Nakamura, K. and Yogi, Y.: The nude mouse as an experimental lepromatous leprosy model; The enhancing effect of thymus cells in infected nude mice, Proc. XI Intern. Lep. Congress (1978), In Leprosy, pp. 44-49, Excerpta Medica, Amsterdam (1980).
- 4) Nakamura, K. and Maeda, M.: A proposal of the new sub-type of *M. leprae*, La Lepro, **37**, 233-234 (1968), (in English).
- 5) Prabhakaran, K., *et al*: Hairless mice, human leprosy and thymic derived lymphocytes, Experimentia **31**, 784-785 (1975).
- 6) Colston, M. J. and Hilson, G. R.: Growth of *Mycobacterium leprae* and *M. marinum* in congenitally athymic (nude) mice, Nature **262**, 399-401 (1976).
- 7) Kohsaka, K., *et al*: Lepromatoid lesions developed in nude mouse inoculated with *Mycobacterium leprae*, La Lepro **45**, 177-187 (1976), (in English).
- 8) Hastings, R. C., *et al*: Multiplication of acid-fast bacilli in nude mice inoculated with armadillo derived *M. leprae*, U. S.-Japan Fifteenth Joint Conference on Leprosy Research, Kagoshima, 10-21 (1980), Int. J. Lep. **48**, 490 (1980).

実験的らい腫らいモデルとしての先天性無胸腺 (ヌード) マウス (続報):

上唇内接種法により形成されるらい腫らい病変について

與 儀 ヤ ス 子 中 村 一 成

キーワード: 先天性無胸腺 (ヌード) マウス, 実験的らい腫らいモデル, 上唇部内接種, 前・後肢足背内接種

先天性無胸腺 (ヌード) マウスの上唇内にらい菌を接種すると, 右後肢足内接種法とほぼ同様の全身化像を呈し, またらい菌増殖の好発部位である鼻, 眼瞼, 耳等にも早期にらい病巣を形成した。本報では, *Mystacial vibrissaeless* のヌードマウス (BALB/c) を作出し得たので, このヌードマウスを供試して, 多くのらい菌株中, 後肢足内接種法で明らかな足腫脹を呈するらい菌株を用い, 5.0×10^5 個宛, それぞれ右上唇部内あるいは右後肢足背内に接種して長期観察を実施した。右後肢足背内接種法では, 7 匹中 2 匹にらい菌接種後 6 カ月目より軽度の腫脹がみられ, 10 カ月前後ですべて腫脹した。上唇内に接種したヌードマウスでは, 10 カ月前後から軽度の腫脹を呈し, 菌接種後 16~17 カ月ですべてに肉眼的腫脹を認めた。また, 一匹のみ 25.5 カ月まで観察し得た。

これらの観察期間中, らい菌増殖による肉眼的腫脹が接種部にまず観察され, 病理組織学的にもらい腫らい病巣であった。また, 早期に上・下唇部, 鼻にらい菌がみられるので肺臓内は勿論, 歯髄, 舌等に重篤ならい病巣が形成されるものと期待したが, 病巣の進展像は軽度であった。長期間観察し得たヌードマウスは, 足背内接種群とほぼ同様な全身性らい腫らい病変像であった。したがって, 上唇内接種法は後肢足背・前肢足背内接種法, さらに足底・足掌内接種法と同様, 定量的に, かつ容易にらい菌を接種することができ, 実験的らい腫らいの研究に有用な接種部位であること, また, らい菌接種後早期に多量のらい菌を得るには, これらの組合せ接種は有用であることを再確認した。

Fig. 1 Vibrissaeless nude and nu/+ mice as shown on the right

Fig. 2 Swelling of injected upper lip and neighbouring sites due to *M. leprae* growth at 12 months after inoculation.

Fig. 3 Section of injected upper lip lesions contained numerous globi and lepra cells, Fite-Faraco stain.

Fig. 4 Section of the opposite upper lip at 16 months after injection, showing numerous AFB in muscle layers and in vibrissae follicle. Fite-Faraco stain.

Fig. 5 Swelling of both upper and lower lips and neighbouring sites such as the eye lids, ears, fore feet and so on at 25.5 months after inoculation.

Fig. 6 Section of the opposite vibrissae follicle at 25.5 months after injection, having a numbers of AFB, Fite-Faraco stain