In 1960, Shepard reported a model of experimental leprosy of mouse by means of foot-pad inoculation technic. This experimental leprosy has been widely utilized in the field of leprosy research, but growth of *Mycobacterium leprae* in normal mouse foot-pad is limited and maximum harvest of *M. leprae* from infected normal mouse foot-pad is about $10^6$. Several studies were reported concerning immunosuppressed animals in which *M. leprae* can grow more significantly than in normal mouse. In 1971, a new model of experimental lepromatous leprosy of armadillos was represented by Kirchheimer and Storrs.

We attempted to find out another model of experimental lepromatous leprosy with laboratory animal, and since 1974 we have been studying on animal inoculation of *M. leprae* with nude mouse.

As authors reported previously, a lepromatoid lesion developed in nude mouse inoculated with *M. leprae* according to Shepard method, and acid-fast bacilli obtained from the lesion were identified as *M. leprae*.

It is important to confirm the success of secondary passage of *M. leprae* proliferated in lesion of first infected mouse into the other nude mice for establishing a new animal model of experimental leprosy. The next important problem is the reproducibility of animal transmission of *M. leprae* derived from different patients. We, therefore, carried out the studies on secondary passage of *M. leprae* which proliferated in lesion of first infected nude mouse to the other nude mice, and studies on animal transmission with *M. leprae* derived from 8 different patients by use of nude mice. The results of these experiments will be shown here.

**Materials and Methods**

The nude of genetic background (BALB/c-nu/nu) and BALB/c-nu/+ bred in our laboratory under SPF conditions were used in the experiments. They were maintained in plastic isolators to prevent the wasting disease and other microbial infection during a period of the experiment.

Bacillary suspensions of *M. leprae* were prepared from the lesions of foot pads of 3 first infected nude mice. *M. leprae* derived from different source of 8 patients biopsied in 1976 were also used for inoculation, and 0.03 ml of the suspensions were inoculated into hind foot pads of mice.

Body temperature of nude mice at various parts was examined with electronic thermometer.
and was compared with normal littermates.

Results

1) The results of secondary passage of *M. leprae* proliferated in the first infected nude mice.

Three mice developed lepromatoid lesion in the first infection were killed at 17th, 19th and 22nd month after infection. They were named as No. 6, No. 7 and No. 8, and $1.8 \times 10^6$, $1.0 \times 10^6$ and $2.2 \times 10^6$ of *M. leprae* were inoculated into the foot pad of nude mice respectively for second infection. Large inoculum size was used to examine if the infection of large number of *M. leprae* could shorten the time for developing lepromatoid lesion at the site of inoculation. At the 10 months after the infection, swelling of foot pad was found at the inoculation sites of all infected animals macroscopically, and lepromatoid lesion was sighted at the foot pads by histopathological examination. In the group of No. 6, two mice were killed at 7 and 10 months after infection, and the number of acid-fast bacilli in the foot pads was counted. The number of organisms of respective mouse was $6.8 \times 10^6$ and $3.6 \times 10^8$. $5.4 \times 10^5$ of the bacilli harvested at 10th month were used for third inoculation, and slight swelling of foot pad occurred in all infected mice at 7th month after inoculation, and the swelling was gradually increased.

The results of secondary passage and other findings were summarized as follows. All mice of 3 groups showed swelling of foot pads, and lepromatoid lesions in the inoculation sites were noted histopathologically. Bacteriological tests on the acid-fast organisms obtained from the lesions were carried out. The bacilli were cultivated on Ogawa’s egg meaium and agar medium, but there was not growth of any organism on the media. Dopa oxidase activity of the bacilli was also examined, and it was noted that the bacilli showed a marked activity of D-dopa oxidase. These results support the fact that the acid-fast bacilli are *M. leprae*.

At the same time, the relationship between the body temperature of nude mice and distribution of the bacilli was examined, because in previous experiment the lepromatoid lesions and many bacilli were noted in lower body temperature between nude mice and normal mice under circumstance of 25°C in platic-isolator at 24°C of animal room. Hind foot pad showed the lowest temperature and there were the most amount of organisms as a matter of course, because of the foot pad was site of infection. *M. leprae* were seen in the skin of low body temperature parts such as eyelid, earlobe, nose and tail other than foot pad, but no organism was detected in the skin of higher temperature parts of abdominal wall and back at 10 months after infection. In the internal organs, *M. leprae* were seen in lung, liver ($4.6 \times 10^6$) and spleen, but not kidney. $2.0 \times 10^5$ of bacilli were counted in homogenate of liver 0.1 g. Although the weight of liver was only about 12 times heavier than spleen, 100 times of the bacilli were found in liver as compared with spleen. Therefore, the distribution of bacilli in liver was significantly higher than in spleen as far as examined mouse is concern. Further experiments on this study should be carried out repeatedly.
2) The results of animal transmission with *M. lepraе* derived from 8 different patients.

Up to now, *M. lepraе* derived from 8 different patients were successfully transmitted into the foot pads of nude mice. Swelling of foot pads of all animals except one group was noted, and the maximum yield of *M. lepraе* reached $1.1 \times 10^{10}$ per foot pad at only 8 month after inoculation. The maximum swelling of foot pad was 5 mm thickness. Histopathologically, lepromatoid lesion was seen in a infected foot pad of mice. The mice which were infected with the material from a case of relapsed patient, did not show swelling of foot pad until 12 month after infection macroscopically, however, $2.0 \times 10^8$ organisms were counted in a foot pad (inoculum; $3.4 \times 10^5$). The acid-fast bacilli obtained from the foot pads were tested cultivation *in vitro* and dopa oxidase activity. No growth of any organism was observed and D-dopa oxidase activity of the bacilli was positive.

Fig. 1 Swelling of right hind foot pad of mouse at 8 months after infection (1st infection)

Fig. 2 Ditto Swelling of right hind foot pad of mouse at 9 months after infection (2nd passage)
Discussion and Conclusion

According to Colston and Hilson\(^1\), if the problems of survival can be overcome, the nude mouse could prove to be of considerable value as a model for the study of lepromatous leprosy. As authors reported already, nude mice could survive for about two years when they were maintained under SPF condition in plastic-isolators, and they developed lepromatoid lesions at inoculation site and low temperature parts of the body by the infection of \textit{M. leprae}. Generalzed infection of \textit{M. leprae} was also seen in the nude mice.

In this paper, the success of secondary passage with \textit{M. leprae} which proliferated in the lesion of first infected nude mice to the other nude mice was confirmed by the experiment, and reproducibility of animal transmission of \textit{M. leprae} derived from 8 different patients by the use of nude mice was also proved. It was suggested that the period of the development of lepromatoid lesion can be shortened by infection with large inoculum size. Therefore, it is believed that the results of our experiments revealed the establishment of a new model of experimental lepromatous leprosy with laboratory animal by use of the nude mice\(^6\).

This study received financial support from the World Health Organization, The U. S.-Japan Cooperative Medical Science Program, the Ohyama Health Foundation, the Sasakawa Memorial Health Foundation and the Osaka Dermatological Institute for which grateful acknowledgment is made.

References

ヌードマウスによる実験らい

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Shepard が開発した方法にもとづき、ヌードマウスを
用いて著者らがこれを一層発展させた実験動物らいが、
らい研究における真の動物モデルとして確立され、広く
研究に用いる実験系になるためには、初代発症マウス
の病巣内増殖菌が、次代接種マウスにおいても、初代と
同様に増殖して病変を作せる必要はなければならない。
そして由来の異なる患者材料でも同一疾病が再現される必
要があり、従ってこれらを検討したのが本研究である。

ヌードマウス内増殖菌は、次代接種ヌードマウスにお
いても顕著な著的増菌と病変の進展を示し、病巣は病
理組織学的に瘻の病像を呈した。すなわち、病巣内
増殖菌はヌードマウスにおいて確実可能であることが確
認された。次いで実験の再現性については、最初にマウ
スを発症させた既報の材料以外に、現在までに由来の
異なる8名の患者材料によってヌードマウスらいの発
症をきたし、再現性が立証された。これらの結果から、
ヌードマウスの実験らいが確立され、らい研究の動物モ
デルとして利用できることが示された。従って、今後の
広範な応用が期待される。また10⁴の菌量接種では発症
までに1カ年半以上も長期間要したが、10⁵〜10⁶の
菌量接種によりかなり短縮されて、早いものは8カ月
後に接種足趾の腫脹を伴って発症し、1足当り1.1×10¹⁰
の菌が算出された。