The Experimental Inoculation with *Mycobacterium leprae* in the Congenitally Asplenic Mouse

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**Key words**: Congenitally asplenic mouse, Dh/+ mouse, experimental leprosy, *Mycobacterium leprae*

We have previously observed that neonatal or adult splenectomy has no significant effect on the multiplication of *Mycobacterium leprae* in mice. Additionally, creating abnormal reaction to hind feet with a mixed infection with *M. leprae* and *Corynebacterium kuscheri* or by mixing oil (adjuvant such as Drakeol No. 6, liquid paraffin, or vegetable oil) with *M. leprae* caused either no growth or reduced multiplication of *M. leprae* in mice, which was different from the case of *M. lepraemurium* (6-10).

Congenitally asplenic mice which are referred to the “Dh” gene for dominant hemimelia, were described in 1959 by Searle(1). The homozygous mouse (Dh/Dh) did not survive beyond 4 days of life due to severe visceral anomalies, and the heterozygous mouse (Dh/+) besides lacking spleen was found to have abnormal hind limbs(1).

In this report, descriptions are made as to whether or not a congenitally asplenic mouse is a suitable model for the study of leprosy.

**Materials and Methods**

Animals: Three congenitally asplenic mice (heterozygous female mice-Dh/+ ) and 2 homozygous normal male littermate mice (+/+ ) of B6C3DH strain were cordially obtained from Dr. T. Suzuki, First Department of Pathology, Niigata University School of Medicine. The 3 female mice were observed to have advanced deformities of both hind limbs and 2 of them had already been pregnant. Upon arrival, they were placed in a vinyl isolator under specific pathogen-free (SPF) conditions for breeding by sib-mating. In the present study, 17 Dh/+ mice having highly advanced deformities of both hind limbs of 6-10 weeks old were used with 20 +/+ mice and 8 each of the following strains C3H/He, C57BL/6, BALB/c and ICR mice all of which were 6 weeks old and were used as controls. The Dh/+ mice and +/+ mice were maintained in a vinyl isolator under SPF conditions, and were provided with sterilized autoclavable commercial diet (CE-2, CLEA JAPAN INC.) and tap water *ad libitum*. The other control animals were housed in a conventional animal room.

Inoculation: *M. leprae* used was maintained by successive transfers in normal mice over a prolonged period of time (22nd passage) and then 6th passaged in nude mice. The inoculum
size was $4.0 \times 10^4$ bacilli for the right hind foot and $1.2 \times 10^4$ bacilli for the right fore foot.

Harvests: Following the inoculation of *M. leprae*, the animals were sacrificed at varying periods of 3, 6, 10, and 12 months after inoculation in order to harvest the bacilli.

Identification: The acid fast bacilli (AFB) harvested aseptically from the *Dh/+* mice and *+/+* mice 10 months after inoculation, were reinoculated into the right hind foot of BALB/c and ICR mice in order to confirm the growth pattern (persistent infection) in the mice. The bacilli were also cultured on 1% ogawa's medium and a modified Nemoto's egg yolk medium (formula as follows; asparagine 1.0g, KH$_2$PO$_4$ 1.0g, glycerine 6.0ml, Aq. dest 100.0ml, autoclave this mixture at 120°C for 20 minutes, and then added to 2% malachite green solution 6.0ml, egg yolk 200.0ml, sterilization is carried out at 90°C for 1.5 hours) at 33°C and 37°C for 3 to 5 months. In addition, the slides with AFB were treated with pyridine, as modified by us for use with smears. This treatment was done as follows; 1) Dry the smears on cover slides in oven for 15-30 minutes or at room temperature for 5-24 hrs. 2) The Ziehl-Neelsen stain for acid fast bacteria. 3) Dehydrate in fresh pyridine for 5-10 minutes at 60°C. 4) Shake, rinse, and wash in water bath for about 30 minutes at 37-40°C. After treatment, as one of the characteristics used to identify *M. leprae*, the smears of *M. leprae* and in vitro (cultured on medium) *M. lepraemurium* lost acid fastness, however, the smears of in vivo *M. lepraemurium* did not.

Results

In this present study, we used *Dh/+* mice having severe abnormalities only as shown in Fig. 1 and 2. The hind limbs of *Dh/+* mice were observed to have highly advanced deformities of the femur and tibia and their toes were shortened or fused (Fig. 1 and 2), their muscles also showed marked atrophy as shown in Fig. 3. *M. leprae* was inoculated into the highly deformed right hind foot of *Dh/+* mice. The bacillary counts were less than $10^4$ bacilli per foot 3, 6 and 12 months after the inoculation

<table>
<thead>
<tr>
<th>Strain</th>
<th>Months after inoculation</th>
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<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td><em>Dh/+</em> (B6C3DH)</td>
<td>less than $10^4$</td>
</tr>
<tr>
<td></td>
<td>less than $10^4$</td>
</tr>
<tr>
<td><em>+/+</em> (B6C3DH)</td>
<td>$1.2 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>$8.5 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>$5.2 \times 10^4$</td>
</tr>
<tr>
<td>C3H/He</td>
<td>$4.6 \times 10^4$</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>$3.2 \times 10^4$</td>
</tr>
<tr>
<td>BALB/c</td>
<td>$5.0 \times 10^4$</td>
</tr>
<tr>
<td>ICR</td>
<td>$6.2 \times 10^4$</td>
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</tbody>
</table>

Inoculum size; $4.0 \times 10^4$/mouse
Table 2. Bacillary counts of *M. leprae* in mice with the fore foot inoculation

<table>
<thead>
<tr>
<th>Strain</th>
<th>Months after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Dh/+ (B6C3DH)</td>
<td>1.0×10^5</td>
</tr>
<tr>
<td></td>
<td>5.7×10^4</td>
</tr>
<tr>
<td></td>
<td>3.8×10^4</td>
</tr>
<tr>
<td>+/- (B6C3DH)</td>
<td>3.6×10^4</td>
</tr>
<tr>
<td></td>
<td>2.2×10^4</td>
</tr>
<tr>
<td></td>
<td>5.5×10^4</td>
</tr>
<tr>
<td>C3H/He</td>
<td>2.2×10^4</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>5.0×10^4</td>
</tr>
<tr>
<td>ICR</td>
<td>1.0×10^5</td>
</tr>
</tbody>
</table>

Inoculum size: 1.2×10^4/mouse

as shown in Table 1. In contrast, the bacillary counts in the hind foot of the control mice, namely, +/- mice, C3H/He, C57BL/6, BALB/c and ICR mice increased to 10^6 bacilli per foot by approximately 6 months after inoculation as described in the same table.

The bacillary counts of 5-10 months after inoculation with the fore foot of Dh/+ mice observed was almost the same as that in control mice, showing no differences from the *M. leprae* growth in normal mice as shown in Table 2.

In all the experiment mice, the growth of *M. leprae* was confined to the site of the inoculation, except for highly advanced deformities of hind foot.

Results of reinoculation in normal mice were no swelling nor any other macroscopic lesions and the multiplication was confined to the site of inoculation.

Cultivation on 1% Ogawa’s medium and modified Nemoto’s egg yolk medium at 33°C and 37°C for 3 to 5 months was impossible.

The pyridine extraction test showed that the acid fastness of the bacilli disappeared immediately.

Thus, in view of these findings, the AFB obtained from experimental mice were identified as *M. leprae*.

### Discussion

The congenitally asplenic mouse (*Dh/+*) was considered as a luxoid mouse at the time of its discovery, but some of its outcross offspring lacked the hallex and had tibial hemimelia with luxation of the hind legs. Since such severe abnormalities were unknown in luxoid mice, these mice were considered to be a new mutant of dominant hemimelia(2).

A *Dh/+* mouse is interesting mutant mouse demonstrating continued neutrophilia, lymphocytosis and thrombocytosis which are present only for a short period of time in the case of splenectomized mice(3). Lozzio and Wargon reported that they are consistent with the concept of decreased antibody production associated with asplenia and demonstrated the important function
of the spleen during embryogenesis to achieve normal humoral immunity in adult life\(^{(4)}\), and another workers reported that the spleen plays significant effects on the rate of murine T cell maturation\(^{(5)}\).

In the present study, an attempt was made to demonstrate the influence of the immunobiological characteristic of \(Dh/+\) mice on the growth of \(M. leprae\) following the inoculation into the hind and fore foot. There were various manifestations of the hind limbs abnormalities on \(Dh/+\) mice\(^{(2)}\). \(M. leprae\) was inoculated into the severe abnormalities right hind foot on \(Dh/+\) mice as shown in Fig. 1 and 2, the hind limbs were observed to have highly advanced deformities of the femur and tibia and their digits were short or missing. Because of such advanced deformities, as shown in Fig. 3 their striated muscles showed marked atrophy which probably prevented the growth of \(M. leprae\).

While, the fore limbs of \(Dh/+\) mice showed no deformities. We expected to show some differences in the growth of \(M. leprae\) due to the absence of the spleen, however, the growth pattern observed was almost the same as that in control mice, showing no differences from the \(M. leprae\) growth in normal mice as shown in Table 2. The reason why the multiplication of \(M. leprae\) was not necessarily more than that in the control in the fore foot of \(Dh/+\) mice was due to the immunobiological characteristics of congenitally asplenic mice which had nothing to do with the multiplication of \(M. leprae\).

**Summary**

Using congenitally asplenic mice and their litter mate \(+/+\) mice as the control, as well as 4 different mice strains, inoculations of \(M. leprae\) were made either into the fore foot at a dose of \(1.2 \times 10^4\) or in the hind foot on a dose of \(4.0 \times 10^4\) in order to study the influence of the immunobiological characteristics of \(Dh/+\) mice on the growth of \(M. leprae\).

Multiplication of \(M. leprae\) was not detected in the hind foot of \(Dh/+\) mice showing highly advanced bone deformities with muscular atrophy at any time following inoculation. Growth of about \(10^6\) bacilli per foot was observed in the hind foot of \(+/+\) mice and all the other strains of control mice.

On the other hand, the fore limbs of \(Dh/+\) mice having no deformities, showed the growth of \(M. leprae\) to almost the same extent as that of control mice.

In conclusion, the immunobiological characteristics of congenitally asplenic mice had no effect on the multiplication of \(M. leprae\).

**Acknowledgements**

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**References**


先天性無脾臓マウスへのらい菌感染実験

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キーワード：先天性無脾臓マウス，Dh/+マウス，実験らい菌

先天性無脾臓マウスは遺伝的に脾臓を欠損することに加えて後肢・足の奇形（臓器も含む場合がある）を呈し、人工的脾摘出マウスとは中性好性症、リンパ球および血小板増加症等が一時的でなく永久に出現するなどの相異がみられる興味ある免疫不全変異マウスである。先天性無脾臓マウス（B6C3DH, Dh/+）およびその同腹正常マウス（+/+），さらに対照として4系統のマウスを供試して，それぞれの前脚足，後肢足，それらの前肢足，後肢足，それぞれ1.2×10^4, 4.0×10^4のらい菌を接種した。高度に異常な奇形肢・足と共に骨格筋の萎縮を呈するDh/+マウスの後脚足部では，+/-マウス，対照マウスでは10^6個/足前後の菌数が得られたのに反して，いずれの時期においても，らい菌の増殖は認められなかった。また，Dh/+マウスは正常な前肢足掌を呈している。その前肢足にもらい菌を接種して比較検討を行ったところ，Dh/+マウスは用いた対照マウスとほぼ同様な増殖像を示した。したがって，先天性無脾臓マウスの有する免疫生物学的性状は，らい菌の増殖能に対してさしたる影響はなかった。
Fig. 1 Congenitally asplenic mouse, showing advanced deformities, such as reduction and twisting of both hind limbs, associated with oligodactyly.

Fig. 2 Congenitally asplenic mouse showing advanced deformities of both hind limbs with twisting and fusion of the right toes.

Fig. 3 Section of the hind foot with highly advanced deformities of congenitally asplenic mouse, showing marked muscular atrophy. H & E stain.