Dermal Characteristics of the Hereditary Hairless-athymic Mouse and the Multiplication at Early Stage of *Mycobacterium leprae* Inoculated into the Right Hind Foot

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Key words: Hairless-athymic mouse, nude mouse, hairless mouse, *Mycobacterium leprae*.

High susceptibility of the nude mice to *Mycobacterium leprae* as due to the absence of the thymus, namely, the hereditary incomplete immunity which was controlled by the nude gene⁽¹⁻³⁾. When *M. leprae* were inoculated subcutaneously into the foot pad of the hairless mice, moreover, the multiplication of *M. leprae* was found to be slightly better than that in their littermate haired mice at a late stage after inoculation⁽⁴⁾.

Both the nude mouse and the hairless mouse are the mice of dermal mutation of hairlessness, and the skin of the hairless mouse was under the control of a recessive hereditary gene (*hr*) which belongs to the linkage group III⁽⁵⁾, while, the skin of the nude mouse was under the control of a recessive hereditary gene (*nu*) which belongs to the linkage group VII⁽⁵⁾.

In the present study, a hairless mouse and a nude mouse were mated with an intention of knowing the influence of respective characteristics caused by the gene, which develops the skin abnormality of hairlessness, on the growth of *M. leprae* favoring the multiplication in the skin. *M. leprae* was inoculated subcutaneously into the right hind foot of the offspring of these matings.

The hairless-athymic mouse was a F₂ double mutant mouse which possesses the hairless gene and the nude gene at a homozygote, and was a mouse with a new dermal abnormality of developing wrinkles never observed with "*hr*" or "*nu*" gene alone.

Materials and Methods

Animals: BALB/c nude mice were obtained from CLEA, Tokyo and at about the same time, the hairless mice derived from ICR strain were cordially provided by Dr. K. Ezaki of CLEA. They were maintained in our laboratory by mating with the mice of several different strains. In the present study, those mated with C57BL/6 and CF#1 were used. The hereditary hairless-athymic mice and the nude mice were maintained in a vinyl isolator under specific pathogen-free conditions, and were provided with a sterilized autoclavable commercial diet (CE-2, CLEA, Japan Inc.) and tap water *ad libitum*. The other mice were housed in a conventional animal room with sterilized autoclavable pelets, water and beddings.

Mating: Female hairless mice (*hr/hr* +/+) of a 7 weeks old were mated with the male nude mice (+/+ *nu/nu*) of the same age to produce F₁ offsprings. Then, brother sister mat-

ings were done to produce F_2 offsprings.

Confirmation of the hairless gene and the nude gene of the hereditary hairless-athymic mouse: The male hereditary hairless-athymic mice ($hr/hr\ nu/nu$) obtained as F_2 were mated with the female mice of original strains ($+/+ +/+$), and brother sister matings were done with the F_1 offsprings thus obtained. Four kinds of F_2 off springs were produced for the confirmation that the hereditary hairless-athymic mice were the individuals to possess homozygous hairless gene and nude gene.

Inoculation: F_1 and F_2 mice of hairless and nude matings of 4-7 weeks old and the control mice having the background of C57BL/6 and CF#1 also of 4-7 weeks old were used for the subcutaneous inoculation of *M. leprae* derived from the nude mouse into the right hind foot in a dose of 1.0×10^6 bacilli.

Harvest: Following the inoculation of *M. leprae*, the animals were sacrificed at varying periods of 2, 3, 5, 6, and 9 months after inoculation in order to harvest the bacilli.

Identification: The acid fast bacilli (AFB) harvested aseptically were reinoculated into the right hind foot of C57BL/6 and CF#1 mice for confirmation of the growth pattern (persistent infection) in the mice. The obtained AFB were cultured on a 1 % Ogawa's medium and a modified Nemoto's egg yolk medium at 33°C and 37°C for 6 months. Also, the AFB smears were treated with pyridine as modified by us. The tissues were fixed in buffered formaldehyde solution, and stained with Fite-Faraco, and haematoxylin-eosin.

Results

The F_1 offsprings produced by mating of the hairless female mice and the nude male mice were all haired as both of the recessive genes were heterozygous ($hr/+ \ nu/+$). Arbitrary brother sister matings of the F_1 mice, moreover, produced 4 kinds of F_2 offsprings as shown in Fig. 1. Namely, the appearance of genotype was controlled by the arrangement of the two recessive genes such as the nude mice ($hr/+ \ nu/nu$), hairless mice ($hr/hr \ nu/+ \ +$), haired mice ($hr/+ \ + \ nu/+ \ +$), and lastly, the mice with temporary unusual wrinkles having the homozygous recessive nude gene and the hairless gene simultaneously ($hr/hr \ nu/nu$). Namely, the mice which were hereditary hairless-athymic with temporary wrinkles were deficient of the thymus, and have characteristic dermal changes which vary with aging. Upon histological investigation of the skin of hereditary hairless-athymic mice which showed varying changes by aging, their hypotrichosis type change was found to belong to the cystica, and moreover, the formation of cysts changed following the advance in aging. Macroscopic discrimination of the hereditary hairless-athymic mice from the nude mice was difficult for about 10 days after birth. Abnormal dermal wrinkles of the hereditary hairless-athymic mice, however, began to appear from around 14 days after birth, namely, the period same as the appearance of depilation with the hairless mice. The wrinkles increased following the advance in days after birth, and the skin became like that of rhino mice⁽⁵⁾ by the time about 40 days after birth as shown in Figs. 2 and 3. Histologically, the skin at this time showed numerous cysts in both the surface layer and deep layer, especially, cyst formation in the surface layer causing the appearance of wrinkles as shown in Fig. 5. On the other hand, the wrinkles disappeared gradually

Fig. 1 Four kinds of F_2 off springs littermates were produced by mating hairless mice and nude mice. (hr/hr nu/nu), (hr^{+/+} nu/nu), (hr/hr nu^{+/+}) and (hr^{+/+} nu^{+/+}) from the left.

Fig. 2 Hairless-athymic mouse of 35 days after birth.

Fig. 3 Hairless-athymic mouse of 90 days after birth.

Fig. 4 Hairless-athymic mouse of 210 days after birth.

Fig. 5 Dermal histology of a hairless-athymic mouse 35 days after birth. H & E stain.

Fig. 6 Dermal histology of a hairless-athymic mouse 210 days after birth. H & E stain.

following the advance in thickening of the skin. By the time about 6 months after birth, the skin of hereditary hairless-athymic mice showed an appearance of the skin of hairless mice, and the cyst formation moves from the skin surface into deeper layers as shown in Fig. 6. By this time, as shown in Fig. 4 both the hereditary hairless-athymic mice and the hairless

Table 1 Bacillary counts of *M. leprae* in hind foot of hairless-athymic mice at early stage after the inoculation

C57BL/6;

Mouse (back- ground and/or genera- tion)	Genotype	Phenotype	Months of after the inoculation				
			2	3	5	6	9
C57BL/6 hr/hr F ₂ +/+ × +/+ F ₁ hr/hr Control	hr/hr nu/nu	H-A	5.8×10 ⁵	2.0×10 ⁷ 6.0×10 ⁷	3.0×10 ⁷ 1.8×10 ⁸	3.1×10 ⁸ 5.0×10 ⁹	8.5×10 ⁸ 7.9×10 ⁹
	hr ⁺ /+ nu/nu	nude	7.5×10 ⁵	3.5×10 ⁷	7.3×10 ⁸	2.1×10 ⁸	6.5×10 ⁹ 1.3×10 ⁹
	hr/hr nu ⁺ /+	hairless	•	6.5×10 ⁶	•	1.2×10 ⁷ 4.5×10 ⁶	5.5×10 ⁵ 7.0×10 ⁶
	hr ⁺ /+ nu ⁺ /+	haired	6.5×10 ⁵	4.5×10 ⁶	2.5×10 ⁶	3.2×10 ⁷ 9.1×10 ⁵	3.8×10 ⁶ 8.5×10 ⁵
	hr/+ nu/+	haired	•	1.6×10 ⁶	3.9×10 ⁶ 8.1×10 ⁶	9.3×10 ⁵ 4.2×10 ⁷	6.8×10 ⁶ 4.1×10 ⁶
	+/+ nu/nu	nude	3.1×10 ⁶	•	2.0×10 ⁸	5.5×10 ⁸ 3.3×10 ⁸	7.8×10 ⁸ 6.7×10 ⁹
	hr/hr +/+	hairless	1.9×10 ⁵	•	•	3.1×10 ⁷ 7.3×10 ⁵	4.7×10 ⁶ 9.2×10 ⁵
	+/+ +/+	haired	•	3.0×10 ⁶	2.8×10 ⁶ 7.5×10 ⁵	5.5×10 ⁷ 6.2×10 ⁵	2.5×10 ⁶ 3.9×10 ⁵

Note; Inoculum size...1.0×10⁶/mouse

• ...not tested

mice became very much alike each other macroscopically as well as histologically.

The multiplication at early stage of *M. leprae* inoculated into the right hind foot of the hereditary hairless-athymic mice and the other F₂ littermate mice as well as the control mice observed for 9 months were as shown in Tables 1 and 2. Contrary to our expectation, no appreciable growth promoting effect on *M. leprae* characteristic to the hereditary hairless-athymic mice which were the double mutant of "hr" and "nu" genes was observable in the findings at an early stage of the observation. The findings being mostly the same as those of F₂ littermate nude mice (hr⁺/+ nu/nu) and control nude mice (+/+ nu/nu), the growth promoting effect with *M. leprae* observed in the present study was considered to be derived from the deficiency of the thymus which is the characteristics of the "nu" gene control.

The AFB obtained from the hereditary hairless-athymic mice and the nude mice were distinguished from other mycobacteria on the basis of the following findings: reinoculation into normal mice produced no swelling nor any other macroscopic lesions, the multiplication was confined to the site of inoculation, the cultivation on a 1 % Ogawa's medium and a modified Nemoto's egg yolk medium at 33°C and 37°C for 6 months was negative, and the pyridine

Table 2 Bacillary counts of *M. leprae* in hind foot of hairless-athymic mice at early stage after the inoculation

CF #1;

Mouse (back- ground and/or genera- tion)	Genotype	Phenotype	Months of after the inoculation				
			2	3	5	6	9
F 2	hr/hr nu/nu	H-A	2.3×10 ⁵		1.9×10 ⁸	4.3×10 ⁸ 8.6×10 ⁸	3.2×10 ⁹ 6.6×10 ⁸
	hr ⁺ /+ nu/nu	nude	.	2.4×10 ⁷	5.1×10 ⁸	7.2×10 ⁸ 3.3×10 ⁷	3.8×10 ⁹ 3.4×10 ⁸
	hr/hr nu ⁺ /+	hairless	.	3.7×10 ⁶	7.7×10 ⁶	8.2×10 ⁶ 1.2×10 ⁷	9.3×10 ⁶ 2.1×10 ⁷
	hr ⁺ /+ nu ⁺ /+	haired	.	.	2.9×10 ⁵ 3.8×10 ⁶	4.1×10 ⁷ 3.5×10 ⁶	6.7×10 ⁶ 8.5×10 ⁶
F 1	hr/+ nu/+	haired	.	4.5×10 ⁶	1.8×10 ⁷ 5.3×10 ⁵	7.7×10 ⁶ 8.1×10 ⁶	3.2×10 ⁷ 6.9×10 ⁶
Control	+/+ nu/nu	nude	3.7×10 ⁵	5.0×10 ⁶	2.3×10 ⁸ 8.9×10 ⁷	5.4×10 ⁸ 2.9×10 ⁹	3.3×10 ⁸ 9.4×10 ⁷
	hr/hr +/+	hairless	.	.	1.7×10 ⁷ 8.6×10 ⁶	6.7×10 ⁶	1.8×10 ⁷ 3.9×10 ⁶
	+/+ +/+	haired	.	3.6×10 ⁵	3.1×10 ⁷ 8.1×10 ⁵	5.4×10 ⁶ 4.8×10 ⁷	6.1×10 ⁶ 3.9×10 ⁶

Note ; Inoculum size...1.0×10⁶/mouse
· ...not tested

extraction test showed that the acid fastness of the bacilli disappeared immediately. Thus, in view of these findings, the AFB obtained from the experimental mice were identified as *M. leprae*.

Discussion

Nude mice are known to support the growth of a large number of *M. leprae*⁽¹⁻³⁾. On the other hand, Pattyn⁽⁶⁾ reported on the multiplication of *M. leprae* in the foot pad of hairless mice in 1969. We⁽⁴⁾ have also reported previously that the hairless mice support the multiplication of *M. leprae* slightly better than the their littermate haired mice at a late stage after inoculation. Heiniger⁽⁷⁾ reported that hairless mice (HRS/J) contained *Retroviridae*, oncovirus (endogenous murine C-type RNA), therefore, the onset of leukemia in hairless mice was earlier and incidence was somewhat greater than in the littermate haired mice. Meier et al.⁽⁸⁾ reported that approximately 70 % of the hairless mice developed lymphoid leukemia after 8 months of age or myeloid leukemia after 18 months of age.

In the present study, the hairless mice and the nude mice were mated with an intention of

knowing the influence of respective characteristics caused by the gene on the growth of *M. leprae* which favors to multiply in the skin.

Appearance of the gene type in the offsprings produced by mating of the hairless mice and the nude mice was controlled by the arrangement of the two recessive genes.

The F₂ offspring mice with unusual wrinkles were produced by mating the hairless mice and the nude mice. That is to say, the mice were hereditary hairless-athymic mice as they possess the two homozygous recessive nude gene and hairless gene simultaneously. The mice were deficient of the thymus and had dermal characteristics which showed changes with aging. The type of hypotrichosis was classified as cystica. The formation of cysts changed with aging, and the dermal appearance of hereditary hairless-athymic mice changed from nude mice like to rhino mice like, and then to hairless mice like as shown in Figs. 2, 3 and 4.

The multiplication of *M. leprae* inoculated into the right hind foot of the hereditary hairless-athymic mice at an early stage was mostly the same as the respective findings of F₂ littermate nude mice and control nude mice. Namely, the growth promoting effect observed in the mice was considered to be derived from the deficiency of the thymus, the characteristics of hereditary hairless-athymic mice which were the double mutant of "hr" and "nu" genes had nothing to do with the multiplication of *M. leprae* at an early stage. But in consideration of the fact that the detection of numerous *M. leprae* at a later stage from the skin of the nude mice which received the foot pad inoculation (eye, ear, nose, lip, loin, femur and tail), and that the favorable results observed at a later stage on the hairless mice which received the inoculation of *M. leprae* into the foot pad, we had a great expectation to know the results at a later stage of *M. leprae* inoculation on hereditary hairless-athymic mice which were deficient of the thymus and possessed the hereditary genes of both dermal abnormality and immunological abnormality with the possibility of expanding systemic dermal sites favorite to the proliferation of *M. leprae*. A separate report will be published on this subject in the near future.

Summary

The F₂ mice with unusual wrinkles were produced by mating hairless mice and nude mice. The mice were hereditary hairless-athymic mice as they possess the two homozygous recessive nude gene and hairless gene simultaneously. These mice were deficient of the thymus, and the dermal appearance of hereditary hairless-athymic mice changed from nude mice like to rhino mice like, and then to hairless mice like. The type of hypotrichosis was classified as cystica. The early growth promoting effects observed on the *M. leprae* inoculated into the right hind foot of hereditary hairless-athymic mice were mostly the same as the respective findings of littermate F₂ nude mice and control nude mice.

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先天性 Hairless-athymic マウスの皮膚性状 およびその右後肢足へのらい菌接種 早期の成績について

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キーワード : Hairless-athymic マウス, ノードマウス, ヘアレスマウス, らい菌

ヘアレスマウスとノードマウスの交雑第2代において皮膚に異常なしわを呈するマウスが得られた。このマウスは、ヘアレス遺伝子座とノード遺伝子座をホモ接合体に持つ先天性 Hairless-athymic マウスであることが判明した。このマウスは胸腺を欠如することと、Aging とともにその皮膚性状がノードマウス様—ライノマウス

様—ヘアレスマウス様と変貌していく外観に大きな特徴があり、その皮膚の Hypotrichosis type は Cystica であった。

先天性 Hairless-athymic マウスの右後肢足へらい菌を接種し得られた早期の成績は、同腹ノードマウスおよび対照ノードマウスとほぼ同様な増殖像を示した。

編集者宛の手紙

The Leonard Wood Memorial (American Leprosy Foundation)
is grieved by the passing of Mrs. Monina G. Madarang
on January 8, 1988.

She was Administrative Director of the Cebu, Philippines facility as well as an excellent laboratory technician. Mrs. Madarang received a BS in Pharmacy from the University of San Carlos, Cebu City, Philippines, a MBA from the University of Philippines, Cebu city, Philippines. She also studied at Mercy Hospital of Medical Technology, San Diego, California and Marquette University, Milwaukee, Wisconsin.

In her 24 years of employment with the Memorial she held a variety of duties. Her wisdom and knowledge of Leonard Wood Memorial procedures in dealing with International leaders made many lasting friendships and created workable cooperation and collaboration in leprosy projects. She received specialized training in many phases of leprosy work and applied the new skills in furthering our research.

Truly committed to the leprosy cause, often giving far more than duty demanded, her caring, dedication and loyalty is truly a loss to the Memorial.