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Advances in Experimental Leprosy

Kenji Kohsaka

Department of Leprology, Research Institute for Microbial Diseases, Osaka University

Thank you Dr. Choi, although I feel that I do not deserve them, I appreciate your kind word in introducing me.

1873 Hansen (Discoverer) *Mycobacterium leprae*
Attempt on ainmal transmission
Experimental animals
Domestic animals
Wild animals
Pets, Insects, Fish

1960 Shepard
Mouse foot pad method
20°C, *5* × *10*³ (10^3 - 10^4)
↓
10^6 (6 - 8 months)

1965 Hilson
Rat foot pad inoculation

1966 Rees, et al.
Thymectomized-irradiated mouse
$5 \times 10^3 \rightarrow 10^8$ (5%, at risk)

1971 Fieldsteel, et al.
Thymectomized-irradiated rat

1971 Kirchheimer and Storrs
Armadillo: nine-banded armadillo
(*Dasypus novemcinctus*, Linn.)
Experimentally lepromatous leprosy

1973 Lew, et al.
Korean chipmunks
(*Tamias asiatics sibiricus*, Gmelin)

1976 Kohsaka, et al. Colston, et al.
Nude mouse (BALB/c-*nu/nu*)
Experimentally lepromatous leprosy

1977 Kohsaka, et al.
Establishment of experimental leprosy in nude mouse

I am very pleased and honored to be here today. Mr. President, ladies and gentlemen, thank you very much.

I am very grateful that you have invited me to come to your Conference and speak to you about Advances in Experimental Leprosy.

The discovery of leprosy bacillus, *Mycobacterium leprae* was made 9 years earlier than that of tuberculosis, but experimental medicine on leprosy fell far behind the studies of other bacilli, due to mainly unsuccessful attempts at cultivating *M. leprae* as well as the transmitting it to experimental animals.

At the begining of my lecture, looking back over the history of the past.

Since 1873, *Mycobacterium leprae* was discovered by Hansen, as a causative agent of leprosy, many investigators in the world have made efforts to grow *M. leprae* on culture media and animals. However, the cultivation *in vitro* is not succeeded yet. Continual failure to grow the leprosy bacillus *in vitro* means that there is a requirement for highly susceptible experimental animal model for leprosy research. Efforts to develop an animal model of lepromatous leprosy have been done a wide variety of possible experiments not only with laboratory animals but using live-stock, domestic and wild animals, pets, insects and fish by means of various ideas and technics.

In 1960, the first successful transmission of *M. leprae* to animals was made by shepard, who obtained limited multiplication of the organism in the foot pads of mice and the demonstration has been one of the major contributions to both clinical and experimental leprosy research. However, the usefulness of the mouse foot pad model is limited

by the fact that the infection is self-limiting and localized. Growth of the *M. leprae* in normal mouse foot pad is limited and maximum yield of the bacilli from infected foot pad is about 10^6. If amall number of organisms, usually 5,000 bacilli, are inoculated into the foot pads of normal mice, bacillary numbers increase exponentially until a ceiling of approximately 10^6 organisms per foot pad is reached about 6—8 months after inoculation. After this ceiling has been reached the total mumber of organisms change little, but bacillary killing occurs. Thus, although the mouse foot pad model has been of great value, it does not represent a model of lepromatous leprosy.

A similar infection was obtained in the foot pad of the white rat by Hilson.

The ability to produce an enhanced and disseminated *M. leprae* infection in mice and rat by the nonspecific depression of T-cell function has been demonstrated by several workers.

In 1966, Rees reported that thymectomized and irradiated mice produce a significant increase in bacillary yield when inoculated with *M. leprae* into the foot pad, and develop a systemic spread of the infection following both foot pad and intravenous inoculations, 5%, at risk.

This slide shows a foot pad infection presented by Rees.

Neonatally thymectomized rats have also been shown to develop enhanced and generalized *M. leprae* infection when inoculated in the foot pad or intraveneously by Fieldsteel and others.

Although both the thymectomized-irradiated mouse and neonatally thymectomized rat play important roles in leprosy research, their usefulness is limited by the fact that they retain a significant degree of immunocompetence.

And, in 1971 a new model of experimentally lepromatous leprosy of armadillo was represented by Kirchheimer and Storrs. This is one of the most important findings for the experimental study of leprosy that the nine-banded armadillo develops a lepromatous leprosy-like disease when inoculated with *M. leprae*. In fact the systemic spread of the infection in armadillos is even more extensive than in man, with bacilli in lepromas and in internal organs being more viable and far more numerous than in man.

However, armadillo is large and difficult to hadle, and is not available in sufficient numbers in certain areas, because of their apparent inability to breed in captivity. In addition, armadillo captured from the wild may have a disease which is indistinguishable from leprosy, or may be harboring cultivable mycobacteria.

In 1973, susceptibility of Korean chipmunks was reported by prof. Lew in your country.

And in 1976, the fact that congenitally athymic nude mice are highly susceptible to infection with *M. leprae* was independently reported by Kohsaka etal. and Colston et al.

It is known that the mouse mutant, nude mouse is born without a thymus and have the immunological defects, and also the nude mouse has been shown to be highly susceptible to a number of infectious agents.

The central role of the T-cell system in control of the *M. leprae* infection in both the mouse and man suggests that the nude mouse might prove to be a suitable model for studies of lepromatous leprosy, in other words, congenitally athymic nude mouse is expected to develop the lesion with more significant multiplication of *M. leprae* compared with normal mouse because of the impairment of thymus-dependent immunity in the animal. Therefore, I attempted to find out a new model of experimentally lepromatous leprosy with laboratory animal and since 1974 I have been studying on animal transmission of *M. leprae* with the nude mouse.

But, when nude mice are reared under conventional conditions they are likely to contract the wasting disease and other microbial infection and die before long. However, they will be able to survive much longer if they are kept under specific pathogen free or sterilized conditions. Then, feed, drinking water and any other material brought into the plastic isolator must be sterilized completely in advance.

At first, I'd like to speak our first experiment.

MATERIALS AND METHODS

Table 1. Growth of *M. leprae* in Nude and Control Mice following Foot pad Inoculation

KK Strain mice (control)			Nude mice (BALB/c-*nu/nu*)		
No of mouse	Months surived	Bacillary count/ foot pad	No. of mouse	Months survived	Bacillary count/foot pad and other findings
1	1 (d)	—	1	2 (d)	—
2	2 (d)	—	2	3 (d)	—
3	8 (s)	4.6×10^5	3	8 (s)	3.3×10^8 acid-fast bacilli in smear of inguinal lymph node, and a few bacilli in liver
4	10 (d)	3.6×10^5	4	8 (s)	4.6×10^5
5	12 (s)	4.2×10^4	5	13 (d)	2.6×10^6 many AFB in inguinal node, and a few bacilli in spleen and liver
6	12 (s)	1.0×10^6	6	17 (s)	Lepromatoid lesions
7	17 (s)	2.8×10^5	7	19 (s)	developed in foot pads at
8	17 (s)	2.0×10^4	8	22 (s)	inoculation site and other sites

Inoculum: 1.0×10^4 0.03 ml inoculated into right hind foot pad.
d, Died; s, Sacrificed.

Animal: Five-weeks old 8 nude mice were used in the experiment. The nude mice *(nu/nu)* of genetic background BALB/c (BALB/c-*nu*) bred under specific pathogen free (SPF) condition, and they were maintained in SPF plastic-isolators to prevent the wasting disease and other microbial infection. Food and water were given after complete sterilization.

Inoculum: Bacillary suspension of *M. leprae* was prepared from a leproma of relapsed case of lepromatous patient. The bacillary suspension was made with Hanks' solution and 1.0×10^4/0.03 ml of *M. leprae* were inoculated into right hind foot pads of 8 nude mice, and as a control, 8 mice of kk strain were infected by the same way.

This slide shows the results of control in the left column and that of nude mice in the right column in the table. As shown in the Table 1, two nude mice among 8 and 2 out of 8 kk strain mice were dead at the early stage of the experiment, then 6 mice are available for study. At 8th and 13th month after the infection, it was observed that gradual increase of acid-fast bacilli in infected foot pad, lymph-nodes and internal organs, so the possibility of the generalized infection by *M. leprae* was suggested. At 17th month, swelling of right hind foot pad of all 3 mice survived was noted macroscopically, and these 3 mice survived more than 17 months, eventually developed lepromatoid lesion at the inoculated site and low temperature parts of body such as eyelid, earlobe, tail and nose. These 3 mice were sacrificed at 17th, 19th, and 22nd month after the inoculation respectively, and histopathological and bacteriological examination were carried out.

Table 2. Results of Identification Tests

1) Failure to grow in vitro (Ogawa's egg medium)
2) No granuloma formation in ddO strain mice
3) Loss of acid-fastness by pyridine extraction
4) Positive D-dopa oxidase activity
5) Lepromin reaction in patents:
   Five lepromatous −
   Four tuberculoid +

This slide shows a swelling of right hind foot pad as compare to uninoculated left side. Two nude mice developed ulceration at the root of tail other than swelling of foot pad.

Two mice showed swelling of both eyelids.

Numerous acid-fast bacilli were seen in the smear obtained from the lesion.

This slide shows a section of infected foot pad stained by Fite-Faraco's technic. Histopathologically, lepromatous lesion consist of foamy cells and histiocytic cells with remarkable proliferation of acid-fast bacilli was observed in the section of infected foot pads.

Shows the same subcutaneous lesion of foot pad.

Marked invasion of acid-fast bacilli into peripheral nerves with remarkable multiplication

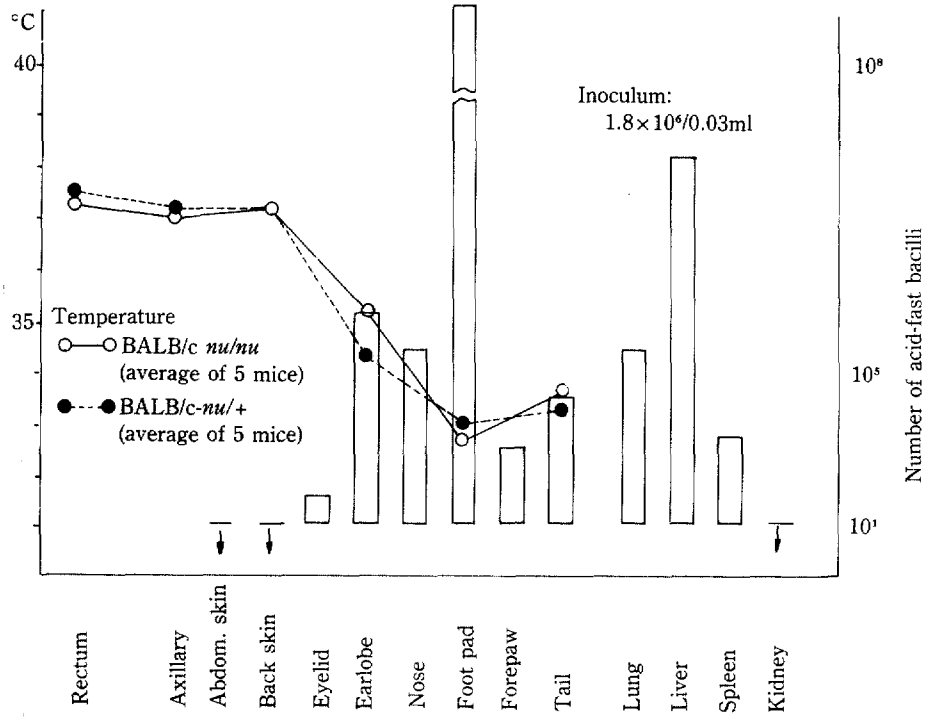
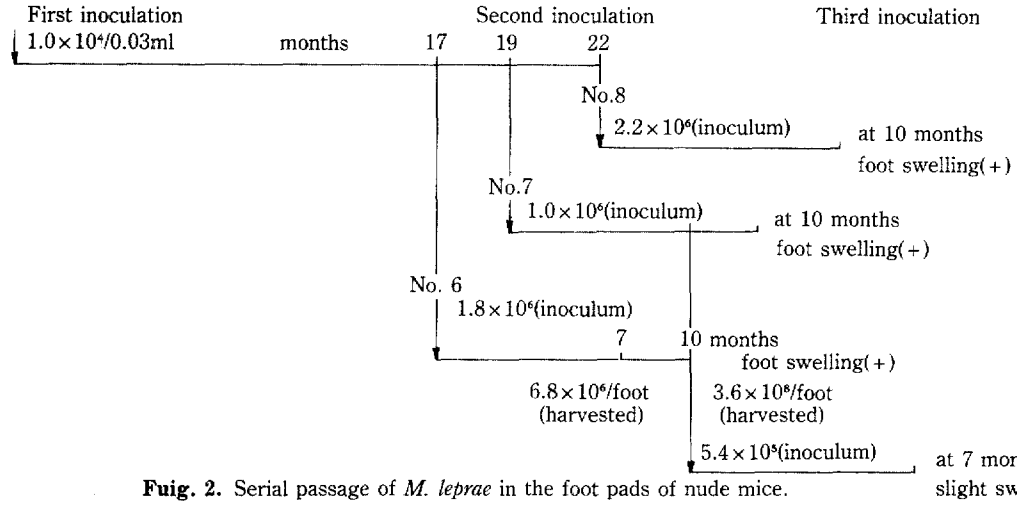


Fig. 1. Organ temperature and distribution of *M. leprae* in various organs of nude mice inoculated 10 months previously with *M. leprae.*



Fuig. 2. Serial passage of *M. leprae* in the foot pads of nude mice.

of the bacilli were also seen. You can find a nerve bundle in lower part of the section.

Shows the same section with hematoxylin-eosin stain.

Shows high magnification. Invasion and proliferation of acid-fast bacilli were seen in the cross section of peripheral parts of right peroneal nerve.

Furthermore, numerous acid-fast bacilli were seen in tibial nerve in the thigh.

In order to ascertain whether the acid-fast bacilli growing in the lepromatous lesion in the nude mice was identical with that isolated from the leprosy patient, several identification tests were carried out. This slide summarizes the results of the tests, that is

1) Failure to grow *in vitro*
2) No granuloma formation in normal mice
3) Loss of acid-fastness by pyridine extraction
4) Positive D-dopa oxidase activity
5) Lepromin test to patients

From the results, the acid-fast bacilli proliferated in the lesion were identified as *Mycobacterium leprae*, and it was confirmed that the lepromatous lesion and disseminated infection developed in the nude mice were caused by proliferation of inoculated *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 model of experimental leprosy, and the next important problem is the reproducibility of animal transimission of *M. leprae* derived from different patients. Therefore, we attempted the studies on secondary passage of *M. leprae* which proliferated in lesion of first infected nude mouse to the other nude mice, and on animal transmission with leprosy bacilli derived from 8 different patients by use of nude mice.

This slide shows the result of serial passage of *M. leprae* proliferated in the first infected nude mice.

Three mice developed lepromatous lesion in the first infection were sacrificed at 17th, 19th and 22nd month after inoculation and they were named as No. 6, 7 and 8. All infected mice of 3 groups showed swelling of foot pad with bacillary proliferation, and lepromatous lesions in the inoculation site were noted histopathologically. Bacteriological tests on the acid-fast organisms obtained from the lesions support the fact that the acid-fast organisms are *M. leprae*.

In 2nd passaged nude mouse, the infection was accompanied by swelling of right hind foot pad at 11 months after inoculation, and 1.5×10^{11} of *M. leprae* were harvested from the foot pad.

At the same time, the relationship between the body temperature of nude mouse and distribution of the bacilli was examined, because in previous first experiment the lepromatous lesions and many bacilli were noted in lower body temperature parts. 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 inoculation.

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, and the bacilli were also seen in the internal organs except in kindney.

This table shows the results of the reproducibility of transmission of *M. leprae*. Leprosy bacilli derived from 8 different patients were successfully transmitted into the foot pads of nude mice, and the maximum yield of the bacilli reached 1.1×10^{10} per foot pad at only 8 months after inoculation. The maximum swelling of foot pad was 5 mm thickness.

Histopathologically, lepromatous lesion was seen in a infected foot pad of mice, and the harvested bacilli showed the properties of *M. leprae*.

From the above results, the success of secondary passage with *M. leprae* which proliferated in the lesion of 3 first infected nude mice to the other nude mice was confirmed by the experiment, and reproducibility of animal transmission of *M. leprae* derived from 8 different patients by the use of mice was also proved.

Therefore, it was believed that the results of our experiments revealed the establishment of new model of experimental leprosy with laboratory

animal by use of nude mice.

As mentioned above, a new model of experimental leprosy with nude mouse was established, and the further investigations in the fields of leprosy research such as chemotherapy and immunology have been expected a great deal. We therefore, attempted the application of the new model for chemotherapeutic and immunotherapeutic studies of leprosy.

At first, in order to the nude mouse will be useful for the chemotherapy study, it is very important to clarify the effect of antileprosy drugs which has been used clinically against *M. leprae* in nude mouse, so we carried out following experiments.

This table shows the effect of rifampicin in experimental leprosy with nude mice. 5.2×10^6 *M. leprae* were inoculated into foot pads of mice, and the mice were devided into 3 groups. One group was treated with 0.5 mg of rifampicin once at 1 month after inoculation, the second group received twelve doses of 0.2 mg of rifampicin during a 2-week period, commencing one month after inoculation, and the third group were untreated. The dose of 0.5 and 0.2 mg are equivalent to 1,500 mg and 600 mg to man respectively.

In the untreated control group, 7-month harvests yielded 2.3×10^6 bacilli per foot pad, and by 12 months foot pad swelling was observed, indicating bacillary growth. However, no bacilli could be detected in either group of nude mice which had been treated with rifampicin, indicating a tremendously rapid bactericidal effect of rifampicin against *M. leprae*.

From the above preliminary results, we conducted the next experiment as this slide. Using material taken before treatement of the donor patient, 7.6×10^5 of *M. leprae* were inoculated into foot pad and the number of bacilli reached up to 5.6×10^{10} after 20 months. Remarkable swelling of infected foot pad was observed macroscopically and lepromatous lesions were seen at the site of inoculation. However, there was no growth of acid-fast bacilli in the 3 groups infected with the materials taken after rifampicin treatment of the patient for 2 days, 5 days and 1 month. Especially, even in the group infected with *M. leprae* recovered after only 2 days treatment, and no acid-fast organisms were seen in tissue homogenates of the foot pads after 20 months of infection, and no tendency of multiplication of the organisms was observed by histopathological examination. So, the results of the experiments also indicated that rifampicin shows tremendous initial killing effect on *M. leprae*, and the bacilli lost their infectivity to nude mice after only 2 days administration with 450 mg per day of the drug to man.

The slide shows the use of isonicotinic acid hydrazid, INH, minocycline and clindamycin for chemoprophylaxis of experimental leprosy, but the administration of INH for 6 months, minocycline for 3 months and also clindamycin for 1 month was not effective enough to depress the growth of *M. leprae* in nude mice.

Diamino-diphenyl sulfone, DDS or dapson has been widely used for treatment of leprosy as a primary drug, and it has been sown to have a bacteriostiatic or partially bactericidal effect to *M. leprae*.

Table 3. Results of transmission of *M. leprae* derived from 8 different patients in nude mice

Materials	Inocula	Months	Harvests
1) OSAKA-HS	3.6×10^5	10	3.6×10^9
2) AMAMI-AW	3.6×10^6	10	3.0×10^9
3) TAMAZ-SS	1.5×10^6	9	1.7×10^8
4) KURUME-NT	3.6×10^6	8	1.1×10^{10}
5) OHSHIMA-DDS8	4.3×10^7	16	leproma +
6) OSAKA-MH	7.6×10^5	12	3.0×10^9
7) OSAKA-YD	3.4×10^5	12	2.0×10^8
8) KURUME-NK	5.2×10^6	8	leproma +

As shown in this table, 3 experiments were carried out on preventive effect of DDS upon the growth of *M. leprae* in nude mice. Two strains of *M. leprae* were used, both which were originated from untreated patients and subsequently maintained in mouse passage. Exception of first experiment, a small and a large number of *M. leprae* were inoculated into nude mice, and they were devided 2 groups, respectively, one group was untreated control, and the other was treated by administrating 0.01% DDS in food from the next day after inoculation and continued until the termination of experiment. 0.01% DDS in food is approximately 10 times the ordinary dose to human leprosy patient a day.

About the results, in the case of inoculating a small number of bacilli 5×10^3 although the number of bacilli in treated group is lower than in untreated control, the bacillary population had increased during administrating the drug. When leprosy bacilli were injected into the mice foot pads, the fix-ratio of the bacilli at the site of inoculation in earlier stage was approximately 30%. Subsequently the bacillary population in nude mice had increased much more than the inoculating number, so we could not absolutely find the effect of DDS against *M. leprae*.

On the other hand, in the case of inoculating a large number of bacilli 8.0×10^6 into nude mice and treated with DDS, the bacillary population reduced significantly as compared to the untreated control. But, considering the fixation-ratio of bacilli, the level of bacillary population during administrating the drug was similar to the inoculated bacilli, so the number of inoculated bacilli did not change in the nude mice treated with 0.01% DDS. Although we should continue the study on DDS activity against *M. leprae*, from the above experiments, the results supported that DDS activity is bacteriostatic or partially suppressive effect on the growth of *M. leprae* in nude mouse. In addition, DDS-resistant strains of *M. leprae* were also detected during the experiment.

In the present, there are 2 most important problems in clinical aspect, one of them is a resistant *M. leprae* against antileprosy drugs, and the other is a persister in leprosy patients. The data obtained from the experiments suggested that the nude mouse could be of great value as a model for chemotherapy studies of leprosy, such as screening for antileprosy drugs, detecting of drug-rsistant strain, and monitoring of the progress of chemotherapy.

Furthermore, the use of the nude mouse as a highly sensitive means of detecting small numbers of viable organisms against a background of large numbers of dead organisms has been investigated by Colstion, and others.

As the next study, in order to investigate the induction of reversal reaction in *M. leprae*-infected nude mouse by transplanting thymus, we have studied the effect of thymus transplantation on the growth of *M. leprae* in nude mice.

Two experiments were carring out. In the first experiment, 35 nude mice were inoculated with 2.5×10^7 of *M. leprae* into foot pads, and the mice were devided into 2 groups. One group was untreated control, and the other was transplanted thymus obtained from new-born heterozygous mice, monthly

Table 4. Effect of rifampicin treatment on the *M. leprae* infection in nude mice

Group	Inoculum	Foot pad findings
Untreated control (15 mice)	5.2×10^6/ foot pad	2.3×10^6/foot pad (7 months); swelling (12 months)
Rifampicin, 0.5mg 1 dose (10 mice)	5.2×10^6/ foot pad	$< 8 \times 10^4$/foot pad (11 months); no swelling (12 months)
Rifampicin, 0.2mg daily for 2 weeks (10 mice)	5.2×10^6/ foot pad	$< 8 \times 10^4$/foot pad (11 months); no swelling (12 months)

BALB/c-*nu/nu*, female

for 1 year from a month after inoculation. In the second experiment, a half mice of the above control group which were inoculated with *M. leprae* prior 7.5 months were used as recipients of immunotherapy. They were also transplanted thymus after establishing infection as same as the first experiment. Each mouse was treated with 1 thymus from 1 mouse into back subcutaneously or intraperitioneally by using of traker.

The results of the both experiments are shown

Table 5. Effect of infectivity to nude mice of *M. leprae* in skin biopsy specimens of leprosy patient with Rifampicin treatment

Group	No. of AFB inoculated/ foot pad	No. of AFB harvested/ foot pad
Untreated control	7.6×10^5	2.3×10^6 (9 months) 3.0×10^9 (12 months) 5.6×10^{10} (20 months)
Treated with 450 mg Rifampicin daily, for 2 days	3.6×10^6	$<8.9\times10^4$ (9 months) $<7.6\times10^4$ (20 months)
Treated with 450 mg Rifampicin daily, for 5 days	3.2×10^7	$<8.9\times10^4$ (9 months) $<8.0\times10^4$ (12 months) $<8.0\times10^4$ (20 months)
Treated with 450 mg Rifampicin daily, for 1 month	2.7×10^5	$<9.0\times10^4$ (9 months) $<8.0\times10^4$ (20 months)

Five mice were used for each group.
M. leprae obtained from untreated lepromatous leprosy patient was inoculated into foot pad of BALB/c-*nu/nu* mouse.
Values preceded by ">" indicate that no AFB were found during counting procedure.

Table 6. Effect of of INAH, Minocycline and Clindamycin to *Mycobacterium leprae* in nude mouse

Experiment No.	Treatment	No. of AFB inoculated/ foot pad	No. of AFB harvested/ foot pad	Time of harvest (months)
1	Untreated control	1.5×16^5	9.2×10^7	7
	INAH, 0.008% in food, daily, for 6 months	1.5×10^6	5.9×10^7	7
2	Untreated control	3.2×10^6	6.0×10^7	8
	Minocycline, 0.03mg daily, for 3 months	3.2×10^6	7.4×10^7	8
3	Untreated control	2.7×10^7	1.2×10^{10}	9
	Clindamycin, 0.2mg daily, for 1 month	2.7×10^7	1.5×10^{10}	9

Five mice were used for each group. INAH: Isonicotinic acid hydrazide.

in this slide.

Donor thymus shows the both of suppressive effect on the growth of *M. leprae* and therapeutic effect on experimental leprosy in nude mice. In he first experiment, the bacilli increased gradually in control group, and 2.6×10^{10} bacilli were harvested

Table 7. Preventive effect of DDS on the *M. leprae* infection of nude mouse

Strain of *M. leprae*	Treatment	No. of AFB inoculated/ foot pad	No. of AFB harvested/ foot pad	Time of harvest (months)
1) OI	Untreated control	5.0×10^{3}	5.0×10^{5}	5
			1.6×10^{6}	6
2nd passage	0.01% DDS in food		7.7×10^{4}	5
			6.0×10^{5}	6
2) OI	Untreated control	5.0×10^{3}	2.1×10^{6}	9
	0.01% DDS in food		8.8×10^{4}	9
(3rd passage)	Untreated control	8.0×10^{6}	7.4×10^{8}	8
	0.01% DDS in food		4.8×10^{6}	8
	0.01% DDS in food		1.4×10^{5}	9
3) KN	Untreated control	5.0×10^{3}	1.3×10^{5}	9
(6th passage)	Untreated control	8.0×10^{6}	2.2×10^{9}	9
	0.01% DDS in food		4.3×10^{6}	9

DDS: diamino-diphenyl sulfone The figure given is an average for the number of 5 mice. Treatment with DDS was started on the next day of the inoculation. *M. leprae* used was nude mouse passaged strain.

Table 8. Effect of thymus transplanting on the growth of *M. leprae* in nude mouse

1) Suppressive

Group	Inoculum	Months after inoculation 7.5 M	10 M	13 M	15 M
Untreated control (20 mice)	2.5×10^{7}	1.5×10^{9}	/	6.2×10^{9}	2.6×1^{10}
Thymus (subcutan.) (10 mice)	2.5×10^{7}	1.7×10^{8}	2.6×10^{7}	3.7×10^{7}	9.6×10^{7} (n=2)
Thymus (intraperit) (5 mice)	2.5×10^{7}	/	/ (n=2)	4.5×10^{7}	3.0×10^{7}

2) Therapeutic

Group	Initiat. "0" (7.5 M)	5 M (13 M)	7 M (Months of treatment) (15 M) (Months after inoculation)
Untreated control (8 mice)	1.5×10^{9}	6.2×10^{9}	2.6×10^{10} (n=2)
Thymus(intraperit.) (8 mice)	1.5×10^{9}	1.1×10^{9}	5.5×10^{8} (n=2)

at 15 months after inoculation. On the contrary, nearly same amount of bacilli of inoculum 10^7 were recovered from the both treated groups subcutaneously and intraperitioneally. This result indicated that the thymus transplanting was effected suppressively on the growth of *M. leprae* in nude mouse.

On the other hand, 16 infected nude mice among the control group in the above first experiment were used in the second therapeutic experiment at 7.5 months after inoculation, in that time, the number of organisms in growth reached up 1.5×10^9 in the foot pad. The mice were devided into 2 groups, one group was treated by transplanting thymus and the other was untreated control.

In the untreated control group, the organisms gradually increased up to 2.0×10^{10} at 15 months after the inoculation, but in the treated group the bacillary population reduced significantly compared with the untreated control and the inoculum after 6 times of the transplanting of thymus for 7 months.

The results suggested that the thymus transplanting is effective for immunotherapy of experimental leprosy in nude mouse.

This picture shows the macroscopical finding of the infected foot pads of nude mice in the first experiment. From the left to right, as you can see the remarkable swelling of the both hind foot pads in untreated control mouse and treated mice by transplanting of thymus subcutaneously and intraperitioneally at 15 months after infection.

This slide shows the findings of the second therapeutic experiment. As same as previous slide, from the left, the picture indicates untreated control and treated mice with thymus transplanting intraperitoneally.

Histopathologically, the untreated infected foot pad showed a typical leproma at 15 months after infection.

This is high magnification. Macrophages with numerous acid-fast bacilli were seen.

However, animals transplanted with thymus showed significant changes in the sections. In the first suppressive experiment, growth of the bacilli were strongly suppressed as shown in this slide.

In the second therapeutic experiment, some spots of epithelioid transformation of macrophage were observed in the infected foot pads, and the bacilli decreased significantly. This section shows Hematoxylin-eosin stain.

So, the findings revealed the induction of reversal reaction in experimentally infected leprosy by thymus transplanting.

I can summarize the advances in experimental leprosy with the following statements. We expected to find out another model of experimentally lepromatous leprosy with laboratory animal, and since 1974 I attempted on animal transmission of leprosy bacillus with congenitally athymic nude mouse. As described above, a new model of experimental leprosy in nude mouse is histopathologically similar to that in lepromatous leprosy patients, and several experiments on chemotherapy indicated that the response of infected nude mouse to the antileprosy agents DDS, rifampicin, and others is similar to that of lepromatous patients. The growth of *M. leprae* is suppressive by thymus transplanting, and the results suggested the induction of reversal reaction in experimentally infected leprosy.

The results and the further utilization of this experimental technique will strongly promote immunologic and chemotherapeutic studies of leprosy, especially by making evaluation of therapeutic results possible by experiments. The nude mouse provides the amounts of leprosy bacilli which supply the materials for bacteriologic, metabolic and cultivation studies. In addition, it is indicating that the nude mouse is very sensitive as a means of detecting small numbers of viable organisms against a background of large numbers of dead organisms.

Therefore, although nude mouse has been used in Thailand and Nepal in cooperating with us now, it is hoped that the nude mouse technic will be adopted at many institutes or laboratories, and the studies of leprosy will be promoted by experimental leprosy of nude mouse.

In closing, I sincerely hope that this academic meeting of your society will reep fruitful results. Thank you very much again, all of you.

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