INTERNATIONAL JOURNAL OF LEPROSY Volume 47, Number 3
Printed in the U.S.A.

Partial Inhibition of the Growth of *Mycobacterium lepraemurium* in C3H Mice Immunized with Cell Wall Skeletons[1]

Yoshinori Tanaka[2]

Leprosy is a chronic infectious disease which is related to a complex cell-mediated immunity against *Mycobacterium leprae*. According to various information, including clinical findings, histology and especially skin test reactions to *M. leprae* antigens, leprosy is classified into polar subgroups, tuberculoid leprosy and lepromatous leprosy. Tuberculoid leprosy is a relatively benign form and usually responds well to chemotherapy. A patient with tuberculoid leprosy shows positive lepromin reactivity. On the other hand, lepromatous leprosy is a malignant form and is associated with ineffectual cell-mediated resistance.

When we consider experimental leprosy, we usually find difficulty in searching for an experimental animal because of the quite limited host range of *M. leprae*. It is known that *M. lepraemurium* infection in mice is one of the useful models in experimental leprosy. Through the study of *M. lepraemurium* infection in nude mice and other mouse strains, Kawaguchi ([12, 13]) observed that experimental murine leprosy can be classified into three clinical types, benign, intermediate and malignant, and suggested that mouse strain differences are related to their cell-mediated immunity. The C3H mouse is representative of the malignant type. Therefore, *M. lepraemurium* infection in C3H mice was used in this experiment as a model of lepromatous leprosy.

Immunotherapy has been employed in the treatment of cancer in this decade, and various kinds of agents such as living or killed bacterial cells, bacterial components and drugs have been used. Cell wall fraction or cell wall skeleton (CWS), which had been purified from *Mycobacterium bovis* BCG cells, has been reported to be an effective T cell stimulator ([8, 18]). Cell wall compositions of *in vivo* and *in vitro* grown *M. lepraemurium* cells have been found to be similar to those of *M. bovis* BCG ([4, 7]). Therefore, CWS of *M. lepraemurium* cells (LM-CWS) was used to stimulate T cell function in C3H mice.

C3H mice were either challenged with *M. lepraemurium* immediately after stimulation with LM-CWS or seven weeks after nonspecific stimulation with LM-CWS. Later the status of nonspecific delayed-type hypersensitivity (DTH) was determined. The growth of lepromata and the distribution of murine leprosy bacilli in the various organs were then observed.

MATERIALS AND METHODS

Purification of cell wall skeleton (LM-CWS). *Mycobacterium lepraemurium* Hawaii, grown on one percent Ogawa's egg yolk medium ([17]) for five to six weeks at 37°C, were collected and washed in 0.067 M phosphate buffer (PB) pH 6.8. Cells were delipidated with acetone, ether-ethanol (1:1, v/v), chloroform, and chloroform-methanol (2:1, v/v). Purified cell wall skeletons were prepared from the delipidated cells by using the method of Azuma, *et al.* ([3, 5]). Three and two-tenths g of delipidated cells was suspended in 100 ml of sterilized distilled water and disrupted by sonication at four amperes (Kaijo Denki Co. Ltd., Tokyo, Japan) for 30 minutes in an ice bath (0°C). The disrupted cells were centrifuged several times at 600 × g for 20 minutes to remove the unbroken cells and cell debris, and the cell walls collected by centrifugation of the supernatant at 20,000 × g for one hour. The precipitates were resuspended in 0.067 M PB (pH 7.8) and treated in sequence with trypsin, chymotrypsin and

[1] Original manuscript received for publication on 2 February 1979; revised manuscript received for publication on 7 May 1979.

[2] Y. Tanaka, M.D., Ph.D., Associate Professor, Department of Microbiology, Kurume University School of Medicine, Kurume, Fukuoka, 830 Japan.

TABLE 1. *Design of experiments.*

Exp.	Groups	Challenge simultaneously with IM[a]	Challenge after IM[b]	LM-CWS (μg)	LM-CWS boosters
A	I.	+	–	100	Each 2 wk to 17th wk, then each 4 wk to 35th wk.
	II.	+	–	10	Each 2 wk to 17th wk, then each 4 wk to 35th wk.
	III. (controls)	+	–	–	
B	IV.	–	+	100	1× at 5 wk, then each 4 wk.[c]
	V.	–	+	–	

[a] All mice challenged by live *M. lepraemurium* simultaneously with immunization.
[b] All mice challenged by live *M. lepraemurium* after seven weeks, i.e., after a preliminary immunization.
[c] At seven weeks the level of nonspecific DTH was measured in both groups IV and V by means of picryl chloride. The mice were then challenged and group IV immunized with LM-CWS injections. Group V mice received PB on each occasion when group V was boosted.

pronase at 20°C for 24 hours, followed by washing in turn with saline, water, acetone, ether, and chloroform-methanol (2:1). The LM-CWS (450 mg) consists of a mycolic acid-arabinogalactan-mucopeptide complex and is analogous to the cell wall of the same strain grown *in vivo* and of other mycobacteria ([4, 7, 11, 14]).

Bacterial suspension for challenge. A C3H mouse, which had been inoculated subcutaneously with *M. lepraemurium* Hawaii six months previously, was killed with chloroform and washed in cresol and then in running water. A leproma was excised from the mouse, cut into small pieces, and ground in a mortar. The emulsion was diluted 1,000 times with saline (ml/g). The bacterial suspension used for this experiment was prepared from the supernatant after letting the diluted solution stand for several hours in a refrigerator. It contained about 1–5 × 10^7 cells per ml of suspension.

Animals. Inbred mice of the C3H strain were used at 6–7 weeks of age. Four to six mice of either sex were housed in a stainless steel cage with sterile litter under conditions of controlled humidity at 23°C.

Preparation of oil-attached LM-CWS. LM-CWS was suspended in sterile liquid paraffin, followed by the addition of 0.2% Tween 80 solution, and mixed well by sonication ([6]). The final concentration of LM-CWS was either 1 mg or 100 μg per ml. One tenth ml of one of these emulsions was injected subcutaneously into the C3H mice.

Injection of LM-CWS and challenge of the mice. Experiments were designed as shown in Table 1. In the first experiment (Experiment A), each of 24 C3H mice were challenged subcutaneously in the breast with 0.2 ml of *M. lepraemurium* suspension and divided into three groups, I, II, and III. At the same time, eight mice of groups I and II were injected subcutaneously into their backs with 100 μg or 10 μg of oil-attached LM-CWS per mouse, respectively. Mice of group III were not injected with LM-CWS (control group). Booster injections of the same dose of LM-CWS into groups I and II were performed every two weeks until the 17th week; after that LM-CWS was injected every four weeks to the end of this experiment (at 35 weeks).

In another experiment (Experiment B), C3H mice were subcutaneously injected in their backs with 100 μg each of oil-attached LM-CWS and five weeks later reinjected with the same dose of LM-CWS (group IV). Seven weeks after the first injection of LM-CWS, the mice were checked for nonspecific DTH to picryl chloride (see below) and then challenged subcutaneously in the breast with 0.2 ml of *M. lepraemurium* suspension. Booster injections into the group IV mice were performed every four weeks. Non-stimulated control mice (group V) were injected in their backs with 0.1 ml of PB seven and two weeks before challenge.

Measurement of the size of lepromata and histological examination. The length and breadth of the murine lepromata were measured by a slide caliper, and the size of the

lepromata were expressed as the product of length and breadth.

In Experiment A, four mice from each of the three groups were sacrificed at 17 and 35 weeks, and the lepromata and various organs were carefully removed and weighed. Microscopic examination was carried out by impression preparations. Lymph nodes, spleen, liver and lung were freshly cut apart with a pair of scissors, and the cut surfaces were stamped on glass slides, which were stained by Ziehl-Neelson's method. Results were expressed by symbol and numerical expressions. In Experiment B, autopsies and microscopic examinations were carried out at 21 and 40 weeks.

DTH to picryl chloride. DTH in the mice was measured by the method of Asherson and Ptak ([2, 20]). About 0.1 ml of 7% picryl chloride in ethanol was applied to the skin of the clipped abdomen. Seven days after this sensitization, both ears were challenged with one per cent picryl chloride in olive oil. The thickness of the ear was measured with a dial thickness gauge (Peacock Dial Thickness Gauge, Type G, Ozaki Seisakusho Co. Ltd., Tokyo) before and 24 hours after challenge with the antigen. The results were expressed as follows:

$$\text{Ear swelling (\%)} = \frac{1}{2} \times \left(\frac{R - R_0}{R_0} + \frac{L - L_0}{L_0} \right) \times 100$$

where R_0 and R are thicknesses of the right ear before and 24 hours after challenge, respectively, and L_0 and L are those of the left ear.

Footpad swelling and organ weight data were expressed as the mean and standard deviation within the groups, and statistical significance was determined using the Student's t test.

RESULTS

The progression of murine leprosy and DTH to picryl chloride in mice injected simultaneously with LM-CWS and bacilli. Murine lepromata were clearly recognized 10 weeks after challenge with *M. lepraemurium* (Fig. 1). At 10 weeks the sizes of the lepromata were similar in the three groups. At 13 and 17 weeks, the development of lepromata was supressed in groups I and II and unimpaired in group III.

At 17 weeks four mice from each group were sacrificed for autopsy. From that time on, four remaining mice from each group were successively treated by injecting oil-attached LM-CWS every four weeks. No significant difference in reactivity among the three groups was attributed to an overdose of LM-CWS. At 22 and 28 weeks, the mice in group III had developed the usual soft lepromata expected in unimmunized mice ([1, 12, 13]). During this time period, the hard lepromata in groups I and II were significantly smaller than those in group III. On the other hand, by 35 weeks, development of lepromata in mice in groups I and II was not inhibited. Thus the development of lepromata in these groups was only delayed.

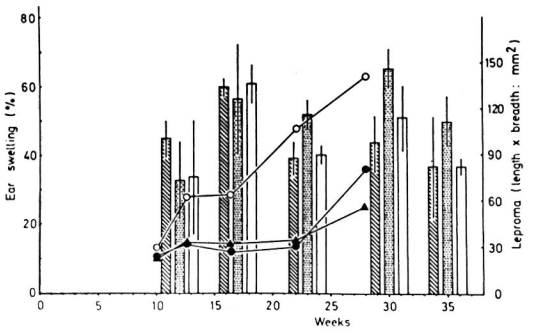

FIG. 1. Relationship between DTH to picryl chloride and the size of lepromata in mice injected simultaneously with *M. lepraemurium* and LM-CWS. Each animal was challenged with 2–5 × 10^6 bacilli and treated as described in Methods. Ear swelling of mice in groups I (▨), II (⊡), and III (□) are indicated in bars. The average size of the lepromata in groups I (●), II (▲), and III (○) is shown as the mean value of the product of the length and breadth.

DTH to picryl chloride is shown in Fig. 1. At 12 weeks the mean reactivity of mice in group I was slightly higher than those of mice in groups II and III. However, no statistically significant differences were found among the three groups at 12 or 17 weeks.

At 23, 30, and 35 weeks, the ear swelling response of group I was similar to that of group III. The reactivity of group II was slightly greater than those of the other two groups (Fig. 1).

Autopsy of mice at 17 weeks. At 17 weeks four mice from each group were killed with chloroform, and the lepromata were carefully removed and weighed (Table 2). The mean weights of the lepromata from the four mice in group I was 0.10% of body

TABLE 2. *Weights of lepromata and organs in Experiment A.*

	Groups	Lepromata	Spleen	Liver
At 17 wk	I (100 μg)	0.10 ± −[a]	0.96 ± 0.07	6.27 ± 0.16
	II (10 μg)	0.36 ± 0.11	0.67 ± 0.07	5.48 ± 0.69
	III (0 μg)	0.40 ± 0.31	0.52 ± 0.09	5.33 ± 0.74
At 35 wk	I (100 μg)	0.55 ± 0.34	1.06 ± 0.11	6.33 ± 0.25
	II (10 μg)	1.29 ± 1.03	1.52 ± 0.42	6.84 ± 1.50
	III (0 μg)	2.05 ± 1.13	0.87 ± 0.43	5.60 ± 0.73

[a] Each value expressed as % of body weight. Data expressed as $\bar{x}$ ± S.D.; N = 4 in each group except group III at 35 weeks where N = 3.

weight and less than that of the other two groups. The weights of the lepromata of groups II and III were almost the same. In appearance, the lepromata in groups I and II were of the same extent (Fig. 1), but there was a great difference in the weights of the excised lepromata between groups I and II, showing that the lepromata in group I were extended but flat and that their volume was very small.

Because it is well known that cell walls of mycobacteria stimulate lymphoid tissues, the spleen and liver were removed from each of the mice and weighed. The spleen weights of group I mice were 0.96% of body weight and greater than those of the other two groups ($p < 0.01$). The liver weights were 6.27% of body weight in group I animals, and this was not significantly greater than in the other two groups. There were no differences in spleen and liver weights between the mice in groups II and III.

The right or left axillary lymph node, which is the regional lymph node for the primary lesion, inguinal lymph node, spleen, liver, and lung were evaluated for bacilli by impression preparations (Table 3). Acid-fast bacilli were found essentially only in the axillary lymph nodes of group I mice at 17 weeks. However, in mice in groups II and III, acid-fast bacilli were found in more or less all of the organs examined.

These results show that in group I mice injection of oil-attached LM-CWS stimulated lymphoid tissues and that the growth and dissemination of murine leprosy bacilli were partially inhibited, and the bacilli remained in local lesions on the breast up to 17 weeks after inoculation.

Autopsy of mice at 35 weeks. The lepromata were quite enlarged, and their weights in groups I, II, and III were 0.55, 1.29, and 2.05% of body weights, respectively. Spleen weights in group II mice averaged 1.52% of body weights and were slightly greater than those of groups I and III. The

TABLE 3. *Acid-fast bacilli in impression preparations of the various organs (Experiment A). Data expressed as* $\bar{x}$ ± *S.D. of numerical gradings.*[b]

		Organs				
Weeks	Groups[a]	Axillary Lymph Node	Inguinal Lymph Node	Spleen	Liver	Lung
17	I (100 μg)	1.6 ± 1.1	0.0	0.0	0.3 ± 0.3	0.0
	II (10 μg)	2.5 ± 1.0	1.8 ± 1.0	0.8 ± 0.3	1.5 ± 1.0	1.0 ± 0.0
	III (0 μg)	3.0 ± 0.0	1.0 ± 0.7	1.1 ± 0.6	1.6 ± 0.8	1.4 ± 0.8
35	I (100 μg)	2.5 ± 1.0	0.5 ± 0.4	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.3
	II (10 μg)	2.8 ± 0.5	1.6 ± 1.1	0.9 ± 0.8	1.0 ± 0.7	1.1 ± 0.6
	III (0 μg)	3.0 ± 0.0	1.7 ± 0.6	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0

[a] N = 4 in each group except group III at 35 weeks where N = 3.

[b] Number of acid-fast bacilli:
− (0) no acid-fast bacilli found in the microscopic fields examined.
± (0.5) a very few bacilli found in several microscopic fields.
+ (1.0) a few bacilli found in each microscopic field.
++ (2.0) 10 to 100 bacilli found in each microscopic field.
+++ (3.0) many bacilli and globi.

TABLE 4. *Weights of lepromata and organs in Experiment B.*

	Groups	Lepromata	Spleen	Liver
At 21 wk	IV (100 μg)	0.64 ± 0.21[a]	0.82 ± 0.09	6.13 ± 0.38
	V (0 μg)	2.49 ± 0.89	0.71 ± 0.19	5.57 ± 0.17
At 40 wk	IV (100 μg)	4.11 ± 1.81	0.88 ± 0.16	7.01 ± 0.87
	V (0 μg)	6.70 ± 4.37	2.96 ± 0.67	10.34 ± 3.41

[a] Each value was expressed as % of body weight. Data expressed as $\bar{x}$ ± S.D.; N = 4 at 21 weeks and N = 3 at 40 weeks.

apparent increase of this spleen weight might be related to the fact that the DTH of group II mice was greater than that of the other two groups after 22 weeks ($p < 0.05$). However, since many acid-fast bacilli were observed in the spleens by impression preparations, the apparent increase in spleen weight might be due to microlepromata. Actually, one of the mice in group II was found to have innumerable macroscopic lepromata in both the spleen and the liver with associated increases in the weights of these organs. There were no significant differences in liver weights among the three groups.

The axillary lymph nodes from all three groups of mice became enlarged and contained many acid-fast bacilli microscopically. Even in the inguinal lymph nodes, spleens, livers, and lungs of mice in groups I and II, acid-fast bacilli were found regularly (Table 3).

These results differ from the results at 17 weeks. It is obvious that at 35 weeks the increases in weights of lepromata and the growth and dissemination of acid-fast bacilli into the various organs are seen not only in group III (control animals) but also in groups I and II (animals treated with LM-CWS).

Observations in mice challenged with *M. lepraemurium* seven weeks after injection of LM-CWS (Experiment B). As shown in Fig. 2, nonspecific DTH was activated in group IV mice injected with oil-attached LM-CWS seven and two weeks before challenge with *M. lepraemurium* (at 0 time). At 8, 13, and 21 weeks, DTH to picryl chloride was significantly greater in mice in group IV than that in group V mice. Thereafter, no difference in DTH to picryl chloride was observed between the two groups of mice.

At 9 and 13 weeks there were no differences in the sizes of the lepromata between the two groups. After 17 weeks the development of lepromata was suppressed and delayed in group IV animals receiving LM-CWS similar to the observations made in the LM-CWS treated mice described in Experiment A.

Three to four mice from each of the two groups were sacrificed at 21 and 40 weeks. Results are shown in Tables 4 and 5 and are almost the same as those in Experiment A (Tables 1 and 2). At 21 weeks the weights of the lepromata in group V were much greater than those in group IV ($p < 0.05$). However, there were no significant differences in the weights of the spleens and livers between the two groups at 21 weeks. Multiplication of acid-fast bacilli and their dissemination into various organs were partially inhibited in group IV mice. At 40 weeks, the lepromata were quite large. There was enlargement of the spleen and

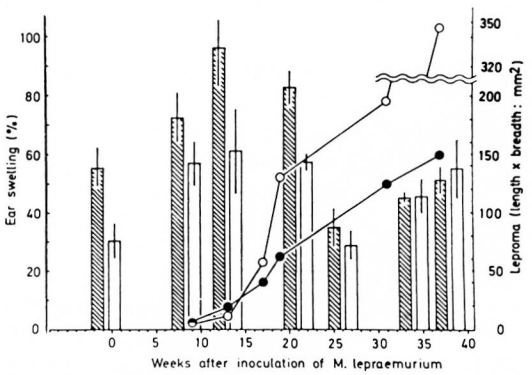

FIG. 2. Relationship between DTH to picryl chloride and the size of lepromata in mice challenged with *M. lepraemurium* seven weeks after injection of LM-CWS. Each animal was stimulated nonspecifically by injecting LM-CWS before challenge with 2–5 × 10^6 bacilli as described in Methods. Ear swelling of mice in groups IV (▨) and V (□) is shown by column. The average size of lepromata in groups IV (●) and V (○) is shown as the mean value of the product of the length and breadth.

TABLE 5. *Acid-fast bacilli observed in the impression preparation of the various organs (Experiment B). Data expressed as* $\bar{x} \pm S.D.$ *of numerical gradings as in Table 3.*

Weeks	Groups[a]	Organs				
		Axillary Lymph Node	Inguinal Lymph Node	Spleen	Liver	Lung
21	IV (100 μg)	0.9 ± 0.3	0.4 ± 0.3	0.1 ± 0.3	0.3 ± 0.3	0.5 ± 0.0
	V (0 μg)	2.8 ± 0.5	0.5 ± 0.4	1.0 ± 0.7	1.0 ± 0.7	0.9 ± 0.8
40	IV (100 μg)	3.0 ± 0.0	2.7 ± 0.6	2.0 ± 1.0	1.8 ± 1.3	1.7 ± 0.6
	V (0 μg)	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0

[a] N = 4 at 21 weeks and N = 3 at 40 weeks.

liver in group V animals, and these organs were found to have innumerable small lepromata macroscopically. Acid-fast bacilli were observed microscopically in all the organs examined.

DISCUSSION

The relationship between progression of *M. lepraemurium* infection and DTH has been investigated by several authors ([9, 15, 16, 19]). They inoculated murine leprosy bacilli into the mouse footpad and detected DTH to *M. lepraemurium* antigens. In the mice infected with *M. lepraemurium*, DTH to antigens of *M. lepraemurium* is a specific and desirable reaction. However, apparent loss of DTH occurs after 8 weeks of infection in association with high levels of systemic antigen ([19]). Therefore, in this study picryl chloride, a different antigen from *M. lepraemurium*, was used as the antigen for DTH. DTH to picryl chloride was detected after over 20 weeks of infection (Fig. 2).

Oil-attached CWS from mycobacteria stimulates T cell functions nonspecifically ([18]), and it can be expected that DTH against *M. lepraemurium* and picryl chloride are activated when LM-CWS is injected with both these antigens. When the non-infected normal C3H mice were injected with 10 or 100 μg of oil-attached LM-CWS, DTH to picryl chloride was significantly developed at 4 weeks, and an increased level of DTH persisted over 20 weeks as compared with the non-stimulated group (data not shown). In Experiment B, DTH to picryl chloride clearly developed after over 20 weeks of infection. On the other hand, in Experiment A, there were no significant differences in DTH at 12 and 17 weeks among the three groups. These findings indicate that DTH was more easily detected when the antigen challenge was administered after the animals were stimulated with LM-CWS.

In Experiment A, LM-CWS presumably exerted a general immunostimulation, and very few acid-fast bacilli were observed in the inguinal lymph node, spleen, liver, and lung (Table 3). However, DTH to picryl chloride was not detected clearly at 12 and 17 weeks. Because only a small amount of picryl chloride was in the circulation, it is not sufficient to explain the facts on the theory of Schlossman, *et al.* ([21]), who reported that the presence of high concentrations of specific antigen in the circulation can result in suppression of DTH. Two possible reasons may be considered: 1) the occurence of suppressor T cells as recognized in tumor-bearing hosts ([10, 22]), and 2) toxic substances which are produced by *M. lepraemurium* and which inhibit the expression of DTH. These problems should be investigated in the future.

After 21 weeks of infection in both Experiments A and B, no differences in DTH were observed between the groups, and from that time on lepromata grew larger and larger (Figs. 1 and 2). Poulter, *et al.* ([19]) reported that a decay of DTH reactivity was associated with a progressive increase in the number of *M. lepraemurium*. It seems that the loss of detectable DTH to picryl chloride after 21 weeks was also associated with an increase in the number of bacilli.

C3H mice injected with oil-attached LM-CWS developed nonspecific DTH up to about 20 weeks of infection. However, the growth of *M. lepraemurium* was inhibited only in part, and both the increase in size of the lepromata and the multiplication of

M. lepraemurium were observed after some delay. Since it is well known that the C3H mouse is a low responder to *M. lepraemurium* antigen, any other mouse strain of higher responder status should also be used for this kind of experiment. After evaluation of LM-CWS in such animals, the appropriateness of LM-CWS for the immunotherapy of leprosy could be considered.

SUMMARY

C3H mice stimulated with *M. lepraemurium* cell wall skeletons (LM-CWS) were challenged with viable *M. lepraemurium*, and delayed-type hypersensitivity (DTH) to picryl chloride was measured. In one type of experiment the mice were challenged at the time when stimulation of cell-mediated resistance by means of LM-CWS was undertaken. The major purpose was to investigate principles pertaining to immunotherapy. In contrast to the loss of a detectable DTH response to picryl chloride, development of murine leprosy was partially suppressed. In the second type of experiment the mice were stimulated with oil-attached LM-CWS seven weeks before challenge with *M. lepraemurium*. Findings were: a) that nonspecific DTH as measured by sensitization and challenge with picryl chloride was activiated before the infection with *M. lepraemurium* and b) that the DTH which developed was associated with partial protection against the growth of lepromata. The murine leprosy which developed in C3H mice stimulated with LM-CWS was progressive after some delay.

RESUMEN

Se estimularon ratones C3H con paredes de *M. lepraemurium* y posteriormente se inocularon (desafiaron) con *M. lepraemurium* vivo. En estos ratones se indujo un estado de hipersensibilidad retardada al cloruro de picrilo que posteriormente se midió. En un tipo de experimentos, los ratones se desafiaron al mismo tiempo que se hizo la estimulación de la resistencia celular con las paredes micobacterianas. El propósito fundamental de este tipo de experimentos fue el de investigar aspectos relacionados con inmunoterapia. En contraste con la pérdida de la respuesta tardía hacia el cloruro de picrilo, el desarrollo de la lepra murina sólo se suprimió parcialmente. En el segundo tipo de experimentos, los ratones se estimularon con paredes micobacterianas emulsificadas con aceite, siete semanas antes del desafío con *M. lepraemurium*. Los hallazgos fueron: a) que no se observó depresión inespecífica de la reactividad tipo tardío (ya que ésta se pudo inducir con el cloruro de picrilo) antes de la infección con el *M. lepraemurium* y b) que la respuesta tardía desarrollada estuvo asociada con una protección parcial contra el crecimiento de lepromas. La lepra murina desarrollada por los ratones C3H estimulados con paredes micobacterianas fue progresiva después de cierto retardo.

RÉSUMÉ

Chez des souris de souche C3H, stimulées par des squelettes de paroi cellulaire de *M. lepraemurium* (LM-CWS), on a étudié la réponse à l'administration d'une préparation de *M. lepraemurium* viable. On a également mesuré l'hypersensibilité de type retardé au chlorure picrique. Dans une expérience, on a étudié la réponse des souris au moment de la stimulation de la résistance cellulaire au moyen de LM-CWS. L'objectif principal était d'investiguer les principes qui régissent l'immunothérapie. Par ailleurs, alors qu'il était devenu impossible de détecter une réponse d'hypersensibilité retardée au chlorure picrique, le développement de la lèpre murine était en partie supprimé. Dans le second type d'expérience, les souris étaient stimulées avec l'extrait LM-CWS en préparation huileuse, sept semaines avant l'administration de *M. lepraemurium*. Les observations ont été les suivantes: a) l'hypersensibilité de type retardé non spécifique, telle qu'elle est mesurée par la sensibilisation et par l'administration de chlorure picrique, était activée avant l'infection par *M. lepraemurium*; b) l'hypersensibilité de type retardé qui s'est ainsi développée, était associée avec une protection partielle contre la croissance de léprômes. La lèpre murine qui s'est développée dans les souris de souche C3H stimulée par la préparation LM-CWS, montrait après un certain délai, une progression.

Acknowledgments. Grateful acknowledgment is expressed to Prof. M. Nakamura for his constant interest during the course of this investigation and is also expressed to Prof. I. Azuma for his useful advice and discussion. I thank Dr. K. Chiba for his technical advice.

REFERENCES

1. Aronson, J. D., Parr, E. H. and Saylor, R. M. BCG vaccine. Its preparation and the local reaction to its injection. Am. Rev. Tuberc. **42** (1940) 651–666.
2. Asherson, G. L. and Ptak, W. Contact and delayed hypersensitivity in the mouse. I. Active sensitization and passive transfer. Immunology **15** (1968) 405–416.
3. Azuma, I., Thomas, D. W., Adam, A., Ghuysen, J. M., Bonaly, R., Petit, J. F. and Lederer, E. Occurrence of N-glycolylmuramic acid in bacterial cell walls. A preliminary survey. Biochim. Biophys. Acta **208** (1970) 444–451.
4. Azuma, I., Yamamura, Y., Tanaka, Y., Koh-

SAKA, K., MORI, T. and ITO, T. Cell wall of *Mycobacterium lepraemurium* strain Hawaii. J. Bacteriol. **113** (1973) 515–518.

5. AZUMA, I., RIBI, E., MEYER, T. J. and ZBAR, B. Biologically active components from mycobacterial cell walls. I. Isolation and composition of cell wall skeleton and components P_3. J. Natl. Cancer Inst. **52** (1974) 95–101.
6. AZUMA, I., TANIYAMA, T., HIRAO, F. and YAMAMURA, Y. Antitumor activity of cell-wall skeletons and peptidoglycolipids of mycobacteria and related microorganisms. Gann **65** (1974) 493–505.
7. AZUMA, I., YAMAMURA, Y. and MORI, T. Chemical and immunological studies on *Mycobacterium lepraemurium* strain Hawaii cultured on Ogawa's one per cent egg yolk medium. Jap. J. Microbiol. **19** (1975) 333–336.
8. AZUMA, I., YAMAWAKI, M., OGURA, T., YOSHIMOTO, T., TOKUZEN, R., HIRAO, F. and YAMAMURA, Y. Antitumor activity of BCG cell-wall skeleton and related materials. Gann Monogr. Cancer Res. **21** (1978) 73–86.
9. CLOSS, O. Experimental murine leprosy: Growth of *Mycobacterium lepraemurium* in C3H and C57/BL mice after footpad inoculation. Infect. Immun. **12** (1975) 480–489.
10. FUJIMOTO, S., GREENE, M. I. and SEHON, A. H. Regulation of the immune response to tumor antigens. I. Immunosuppressor cells in tumor-bearing hosts. J. Immunol. **116** (1976) 791–799.
11. GOREN, M. B. Mycobacterial lipids: Selected topics. Bacteriol. Rev. **36** (1972) 33–64.
12. KAWAGUCHI, Y. Classification of mouse leprosy. Jap. J. Exp. Med. **29** (1959) 651–663.
13. KAWAGUCHI, Y., MATSUOKA, M., KAWATSU, K., HOMMA, A. and ABE, C. Susceptibility to murine leprosy bacilli of nude mice. Jap. J. Exp. Med. **46** (1976) 167–180.
14. LEDERER, E. The mycobacterial cell wall. Pure Appl. Chem. **25** (1971) 135–165.
15. LEFFORD, M. J. and MACKANESS, G. B. Suppression of immunity to *Mycobacterium lepraemurium* infection. Infect. Immun. **18** (1977) 363–369.
16. LEFFORD, M. J., PATEL, P. J., POULTER, L. W. and MACKANESS, G. B. Induction of cell-mediated immunity to *Mycobacterium lepraemurium* in susceptible mice. Infect. Immun. **18** (1977) 654–659.
17. OGAWA, T. and MOTOMURA, K. Studies on *Mycobacterium lepraemurium*. First report: Attempts to cultivate *M. lepraemurium*. La Lepro (Tokyo) **38** (1969) 246–254 (in Japanese).
18. OHNO, R., EZAKI, K., KOBAYASHI, M., TAKEYAMA, H., SUZUKI, H., YAMADA, K., AZUMA, I. and YAMAMURA, Y. Immune response of regional lymph node to BCG cell-wall skeleton and other immunopotentiators. Gann Monogr. Cancer Res. **21** (1978) 109–126.
19. POULTER, L. W. and LEFFORD, M. J. Relationship between delayed-type hypersensitivity and the progression of *Mycobacterium lepraemurium* infection. Infect. Immun. **20** (1978) 530–540.
20. PTAK, W. and ASHERSON, G. L. Contact and delayed hypersensitivity in the mouse. II. The role of different cell populations. Immunology **17** (1969) 769–775.
21. SCHLOSSMAN, S. F. and LEVINE, H. A. Desensitization to delayed hypersensitivity reactions: with special reference to the requirement for an immunogenic molecule. J. Immunol. **99** (1967) 111–114.
22. TREVES, A. J., COHEN, I. R. and FELDMAN, M. Suppressor factor secreted by T-lymphocytes from tumor-bearing mice. Eur. J. Immunol. **4** (1974) 722–727.