INFECTION AND IMMUNITY, May 1979, p. 492-500
0019-9567/79/05-0492/09$02.00/0

Vol. 24, No. 2

Mitsuda-Type Lepromin Reactions as a Measure of Host Resistance in *Mycobacterium lepraemurium* Infection

JILL CURTIS* AND JOHN L. TURK

Department of Pathology, Royal College of Surgeons, London, WC2, United Kingdom

Received for publication 7 February 1979

The footpad reaction to autoclaved whole *Mycobacterium lepraemurium* organisms (MLM lepromin) in high-resistance (C57BL) and low-resistance (BALB/c) mice was studied. Infected C57BL mice gave a prolonged footpad response persisting for 4 weeks after skin testing with high and low doses of lepromin. This was accompanied by mononuclear cell infiltration. Uninfected C57BL mice gave no response. The majority of infected BALB/c mice gave no increase in footpad thickness. However, a high proportion of infected and control BALB/c mice tested with the high dose showed mononuclear cell infiltration which resembled that in C57BL mice. The low dose caused little infiltration in infected or control BALB/c mice. The course of infection in the two strains was different. Dissemination of organisms from the infected footpad was minimal in C57BL mice 5 months after infection. In BALB/c mice, dissemination to the draining lymph node and to some extent to the liver had occurred by 5 months. The draining lymph node of BALB/c mice showed histological evidence of local antibody formation, which was not found in C57BL mice. On the basis of these findings, it was possible to fit murine leprosy in these two strains into a classification similar to that used for human leprosy.

The lepromin skin test has been in general use in leprosy since 1940. Lepromin is a suspension of autoclaved whole *Mycobacterium leprae* organisms prepared from skin biopsies of highly bacilliferous lepromatous leprosy patients. The skin test is read at 48 h (the Fernandez reaction) or 3 to 4 weeks (the Mitsuda reaction) after lepromin injection. Whereas all lepromatous patients are negative for the Fernandez reaction, some tuberculoid patients also give no reaction at 48 h. However, all tuberculoid patients give a positive Mitsuda reaction and all lepromatous patients are negative. Thus the Mitsuda reaction in leprosy patients is a good indicator of host resistance. Healthy contacts of leprosy patients show a high degree of positivity to lepromin and a significant proportion of people in non-leprosy areas also give a positive lepromin reaction (8, 10). Thus the lepromin test is not specific to leprosy but it is of importance in the classification of leprosy patients.

M. lepraemurium causes a disease in mice analogous to leprosy in man. Different inbred strains of mice vary in their susceptibility to infection with *M. lepraemurium* (3), thus mimicking the spectrum of human leprosy. After subcutaneous infection in the footpad with 10^6 to 10^7 organisms, C57BL mice are able to limit the multiplication of the bacilli while C3H and BALB/c mice are not (1, 2).

It has been shown that $B6D2F_1$ (C57BL/6 × DBA/2) mice infected with 10^8 *M. lepraemurium* organisms in the footpad develop 24-h footpad reactivity to an ultrasonicate of *M. lepraemurium* organisms (7), and Alexander and Curtis (1) have studied delayed footpad reactions to a similar reagent in BALB/c and C57BL mice. The former workers (7) also studied the footpad response of $B6D2F_1$ mice to heat-killed *M. lepraemurium*. Only the 24-h response was measured, and this response was similar in infected and uninfected mice.

A lepromin reagent (MLM lepromin) has been prepared from *M. lepraemurium* organisms by using the protocol recommended for the preparation of lepromin from *M. leprae* (11). This has been used to test the Mitsuda reactions of C57BL (high resistance) and BALB/c (low resistance) mice.

MATERIALS AND METHODS

Mice. Female BALB/c and C57BL mice, bred at the Royal College of Surgeons, were used. Mice were 6 to 12 weeks of age at the start of the experiment.

Preparation of organisms for infection. *M. lepraemurium* (Douglas strain) organisms were maintained by intravenous passage in Parkes strain mice which were kept at the National Institute for Medical Research, Mill Hill, England. Organisms for infection were purified by the method of Draper (4) from the liver of a mouse 5 months after intravenous injection with 10^9 organisms. Acid-fast bacilli (AFB) stained by the Ziehl-Neelsen technique were counted by the method of Hart and Rees (5).

Infection of mice. Mice were infected subcutaneously in the right hind footpad with 10^7 organisms contained in 0.05 ml of sterile saline.

Preparation of MLM lepromin. Livers and spleens were harvested from Parkes strain mice 5 months after an intravenous infection with 10^9 *M. lepraemurium* organisms. The livers and spleens were autoclaved at a pressure of 15 lb/in^2 for 15 min. Organisms were purified by method 2 of Draper (4) from the autoclaved tissues and counted (5). They were resuspended in 0.5% phenol-saline at the appropriate dilutions, aliquoted, autoclaved at 15 lb/in^2 for 15 min, and stored at 4°C. Portions of 0.5% phenol-saline were autoclaved for skin testing control mice.

Lepromin dose. Standard human lepromin contains 1.6×10^8 organisms per ml (11) and the skin test dose is 0.1 ml. MLM lepromin was prepared at two concentrations, 6.4×10^8 and 1.6×10^8 organisms per ml, and 0.025 ml was injected into each mouse. Thus, some mice received the same number of organisms as is given to humans (1.6×10^7), and other mice received one-quarter of this number.

Skin testing. Mice were skin tested by subcutaneous injection of 0.025 ml of MLM lepromin in the left hind footpad. Control mice were injected with 0.025 ml of 0.5% phenol saline in the right hind footpad in addition to MLM lepromin in the left hind footpad. Footpad thickness was measured with a dial thickness gauge (Mitutoyo, Japan) immediately before injection of MLM lepromin or saline and 4, 6, 8, 12, 24, and 48 h after and then daily for 4 weeks. The increase in footpad thickness was expressed as a percentage of the preinjection thickness.

Experimental design. Groups of C57BL and BALB/c mice were skin tested at 2, 4, 6, 8, and 16 weeks after infection. The increases in footpad thickness of mice skin tested at 2, 4, 6, and 8 weeks were measured for 4 weeks, and then the mice were killed and the skin-tested footpad was processed for histology. The infected foot was collected for organism counts. Two mice of each strain skin tested at 16 weeks after infection were killed at 6, 12, 24, and 48 h and 8, 15, 22, and 30 days after injection of MLM lepromin, and the footpads were processed as above.

One group of control mice was age-matched to mice at 4 to 6 weeks after infection, and one group was age-matched to mice 16 weeks after infection. The former group was skin tested for 4 weeks and then the skin test site was processed for histology. Two mice of each strain in the latter group were killed at intervals throughout the 4 weeks of skin testing and their footpads were examined histologically.

Histology. Excised feet were fixed in formol acetic alcohol for 24 h, followed by formic acid for 24 to 48 h, and then processed in the usual way. Sections were stained with hematoxylin-eosin and by the Ziehl-Neelsen technique. Livers were fixed in Bouin fixative, and lymph nodes and spleens were fixed in Carnoy's fixative. Liver, lymph node, and spleen sections were stained with hematoxylin-eosin and by the Ziehl-Neelsen technique, and spleens and lymph node sections were also stained with pyronin and methyl green.

Counts of organisms in the infected footpad. Right hind footpads were excised and homogenized in 2 ml of 0.1% albumin saline. The suspension was diluted 1:1 in 0.1% albumin saline and counted (5).

Statistical tests. Means were compared by Student's t test for unpaired data. Where possible, the numbers of responders were compared by the heterogeneity χ^2 test. However, where any of the expected numbers (on the null hypothesis of homogeneity) were less than 5, Fisher's exact test was used.

RESULTS

Increase in footpad thickness. Control mice gave an immediate peak of footpad reactivity between 6 and 8 h after MLM lepromin injection. An immediate peak of reactivity was also elicited by the injection of phenol-saline and in control BALB/c mice, this reaction was not significantly different in size from the immediate peak given in response to MLM lepromin. In C57BL control mice, the immediate response to phenol-saline was smaller ($P < 0.05$ to $P < 0.001$) than the immediate response to MLM lepromin. This immediate response to MLM lepromin was given by all infected mice and was often significantly higher in C57BL mice than in BALB/c mice.

The 24-h reaction to MLM lepromin of control animals (Fig. 1 and 2) was not significantly different from their reaction to phenol-saline and there was no further reaction in control animals throughout the 4 weeks of skin testing.

In general, the 24-h reaction to MLM lepromin in infected C57BL mice was higher ($P < 0.05$) than the 24-h reaction in the appropriate control animals, and at 16 weeks the difference was highly significant ($P < 0.001$). In infected BALB/c mice tested with the higher dose of MLM lepromin, the 24-h reaction was never significantly higher than the 24-h reaction of control animals. At 4 and 6 weeks after infection, BALB/c mice tested with the lower dose of MLM lepromin gave a higher 24-h response than controls ($P < 0.05$).

After the insignificant 24-h reaction, BALB/c mice skin tested 2 weeks after infection showed no further reaction (Figs. 3C and D). In contrast, the skin-tested footpads of C57BL mice remained swollen for 4 weeks after the test (Fig. 3A and B). The increase in footpad thickness was small, averaging about 8%, but at both doses of MLM, lepromin was higher than the change in footpad thickness of the appropriate control mice; P values ranged from <0.05 to <0.01. C57BL mice also gave a prolonged footpad reaction when skin-tested at 4 weeks (Fig. 4A and B), 6 weeks (Fig. 5A and B), 8 weeks (Fig. 6A and B), and 16 weeks (Fig. 7A) after infection, and the reaction was higher ($P < 0.05$ to $P < 0.001$) than the reaction in the appropriate controls at all times.

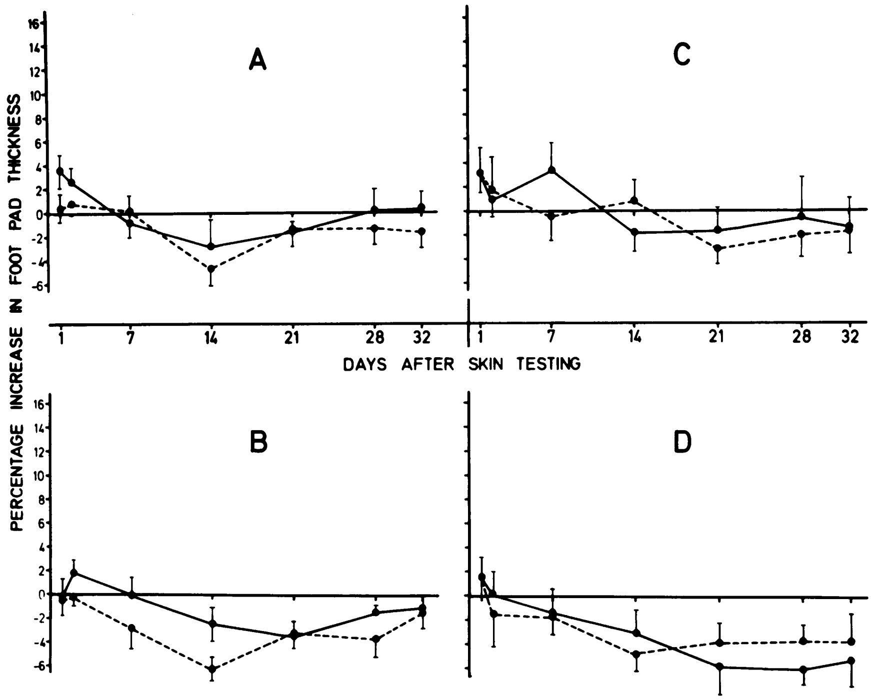

FIG. 1. *Footpad increases between 24 h and 4 weeks in C57BL and BALB/c control mice age-matched to mice in weeks 4 through 6 of infection. (A) C57BL mice skin tested with 1.6 × 10^7 organisms per mouse. (B) C57BL mice skin tested with 0.4 × 10^7 organisms per mouse. (C) BALB/c mice skin tested with 1.6 × 10^7 organisms per mouse. (D) BALB/c mice skin tested with 0.4 × 10^7 organisms per mouse. Each point is the mean ± standard error of (A) 10 readings, (B) 10 readings, (C) 8 readings, and (D) 7 readings. Symbols: ——, Response to MLM lepromin; -----, Response to 0.5% phenol-saline.*

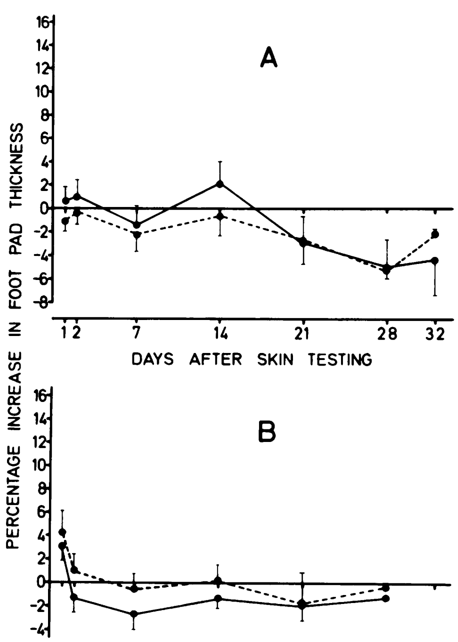

FIG. 2. *Footpad increases between 24 h and 4 weeks in C57BL and BALB/c control mice age-matched to mice in week 16 of infection. (A) C57BL mice skin tested with 1.6 × 10^7 organisms per mouse. (B) BALB/c mice skin tested with 1.6 × 10^7 organisms per mouse. Each point is the mean ± standard error of 2 to 20 readings. Standard errors of means of three or fewer readings were not calculated. Symbols: ——, Response to MLM lepromin; -----, Response to 0.5% phenol-saline.*

Individual BALB/c mice skin-tested at 4, 6, 8, and 16 weeks after infection showed some prolonged footpad reactivity. The mean increase in footpad thickness, during weeks 3 and 4 after skin testing, of BALB/c mice tested 4 weeks after infection (Fig. 4C and D), was higher ($P < 0.05$ to $P < 0.01$) than the mean reactivity of control animals. Mean increases in footpad thickness of BALB/c mice skin tested at 6 weeks (Fig. 5C and D), 8 weeks (Fig. 6C and D), and 16 weeks (Fig. 7B) were not significantly different from those of controls.

For each animal tested, the mean increase in footpad thickness between 8 days and 4 weeks was calculated. Table 1 shows the number of mice in each group with a mean footpad increase of greater than 5% (responders).

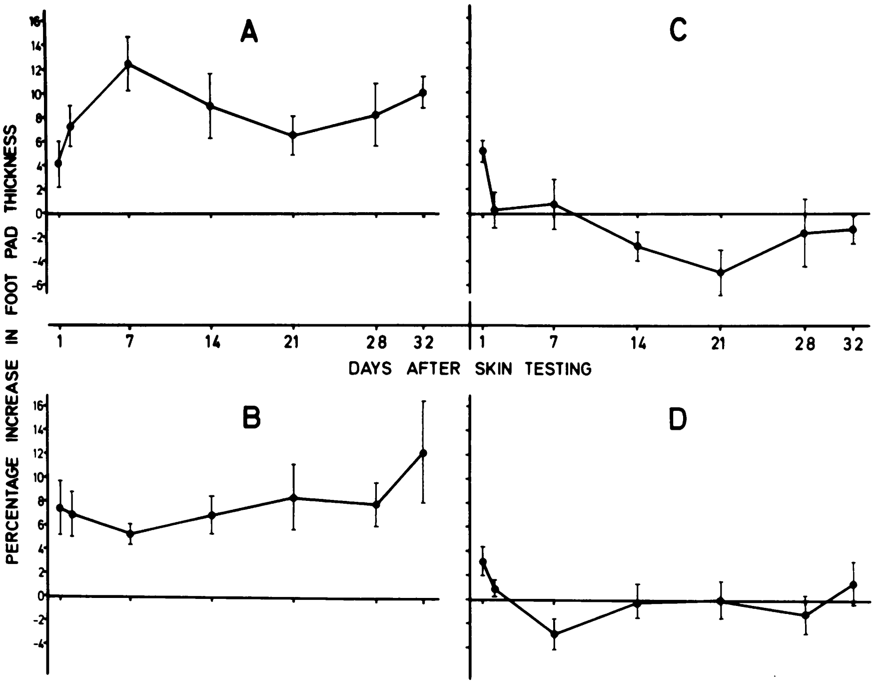

FIG. 3. *Footpad increases between 24 h and 4 weeks in C57BL and BALB/c mice skin tested at week 2 after infection. (A), (B), (C), and (D) as in Fig. 1. Each point is the mean ± standard error of five readings.*

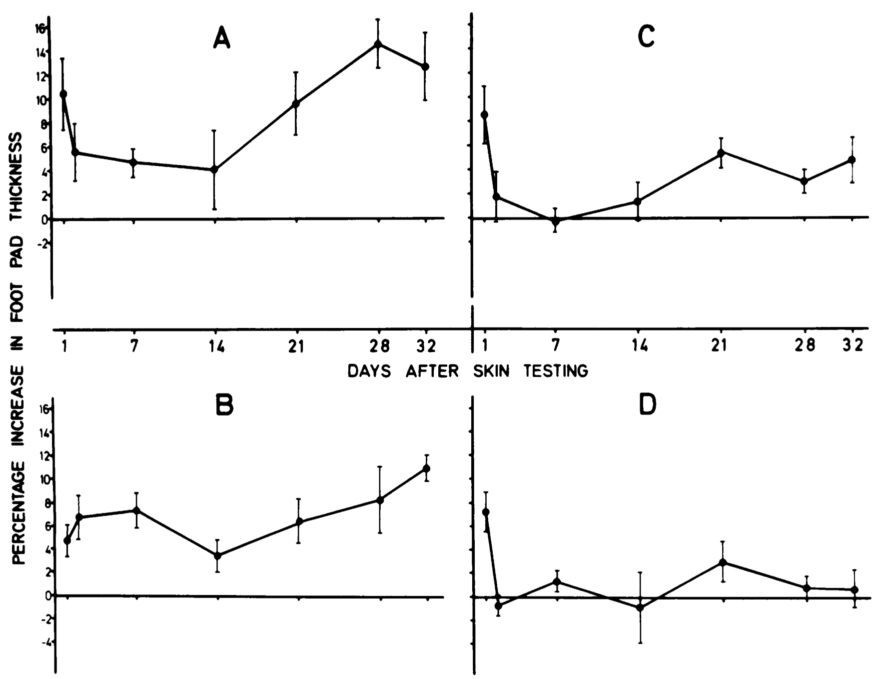

FIG. 4. *Footpad increases between 24 h and 4 weeks in C57BL and BALB/c mice skin tested at week 4 after infection. (A), (B), (C), and (D) as in Fig. 1. Each point is the mean ± standard error of five readings.*

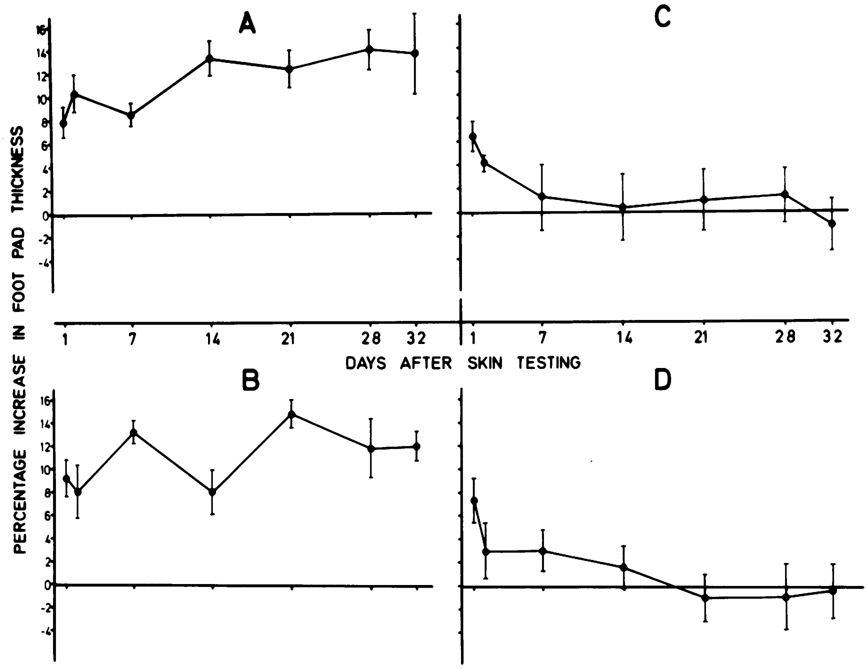

FIG. 5. *Footpad increases between 24 h and 4 weeks in C57BL and BALB/c mice skin tested at week 6 after infection. (A), (B), (C), and (D) as in Fig. 1. Each point is the mean ± standard error of five readings.*

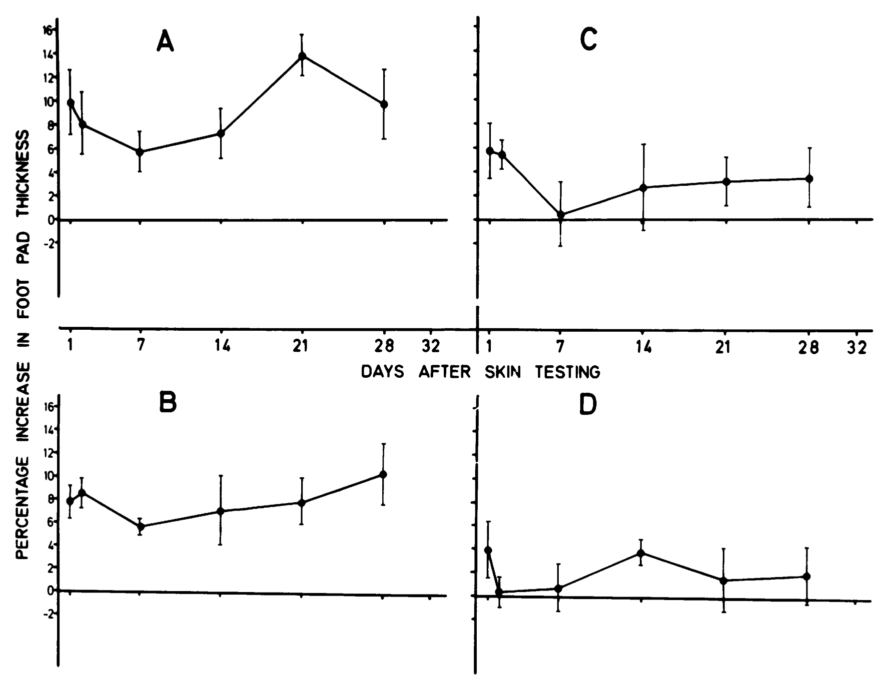

FIG. 6. *Footpad increases between 24 h and 4 weeks in C57BL and BALB/c mice skin tested at week 8 after infection. (A), (B), (C), and (D) as in Fig. 1. Each point is the mean ± standard error of five readings.*

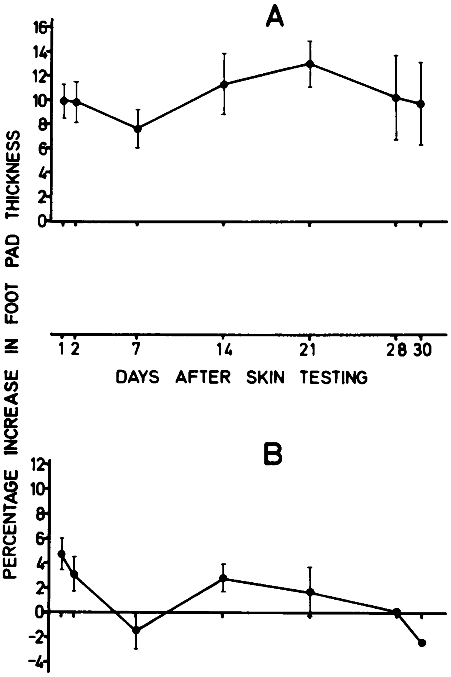

FIG. 7. *Footpad increases between 24 h and 4 weeks in C57BL and BALB/c mice skin tested at week 16 of infection. (A) and (B) as in Fig. 2. Each point is the mean ± standard error of 3 to 20 readings. Standard errors of means of three readings were not calculated.*

Infected and control BALB/c mice contained a similar proportion of responders. There was a significantly higher proportion of responders in infected C57BL mice than in the C57BL controls.

Histological appearance of skin test site. Histological examination of skin test sites of both strains 4 weeks after lepromin testing revealed mononuclear cell infiltration consisting of a mixture of varying proportions of lymphocytes and histiocytes. The distribution of these cells varied from subepidermal sites to areas lying between muscle bundles. The infiltrate had mainly a perivascular distribution (Fig. 8). Frequently the infiltrate showed some organization, and occasionally there was some central necrosis and edema. There was no qualitative difference in the type of infiltrate found in C57BL and BALB/c mice or in the infiltrate in the reactions induced at different times after infection.

The degree of infiltration was scored on an arbitrary scale of −, ±, +, ++, and +++. The numbers of mice in each group showing footpad infiltration of greater than + are shown in Table 1. A high proportion of BALB/c mice given the higher dose of MLM lepromin had extensive cellular infiltration of the footpad, but the proportions of infected and control mice with infiltration were not significantly different ($P = 0.308$). No control BALB/c mice given the lower dose of MLM lepromin showed infiltration of the footpad but 5 out of 19 infected BALB/c mice did. However, this difference was not significant ($P = 0.053$). In the group of infected BALB/c skin tested with the higher dose of MLM lepromin, a higher proportion had extensive footpad infiltration than showed footpad swelling ($P < 0.001$).

A higher proportion of infected C57BL mice than of control C57BL mice showed cellular infiltration in the footpad after skin testing with either dose of MLM lepromin (higher dose of lepromin $P < 0.001$; lower dose of lepromin $P = 0.007$). The proportion of infected C57BL mice injected with the low dose of MLM lepromin giving a footpad swelling of greater than 5% was higher ($P < 0.05$) than the proportion of these mice with extensive infiltration of the footpad.

The development of the cellular infiltrate in the footpad after the injection of 1.6×10^7 autoclaved organisms was studied in mice 16 weeks after infection and in age-matched controls.

The immediate response at 6 h in both infected and control mice of both strains was accompanied by a polymorphonuclear cell infiltrate. AFB were seen inside these cells. The polymorph infiltrate persisted in control animals for up to 48 h. Some mononuclear cells were present at 48 h in control animals, and small numbers of these cells were still present in some sections at 8 and 15 days. At 4 weeks, infiltration was minimal in all C57BL control sections, but the two sections from control BALB/c mice showed ++ infiltration. AFB persisted in control footpads for up to 22 days.

In infected C57BL mice the infiltrate at 24 h consisted mainly of mononuclear cells, and this infiltrate persisted throughout the 4 weeks of skin testing. Some mononuclear cells were present at 24 h in sections from infected BALB/c mice, but the majority of the cells were polymorphs. The latter cells were replaced by mononuclear cells by 8 days and these persisted throughout the 4 weeks of testing. AFB were difficult to detect in sections collected 4 weeks after skin testing in infected mice of either strain.

Numbers of organisms in infected footpads and degree of dissemination of organisms from the footpad. The number of AFB in the infected footpads was counted in the mice used for skin testing (Table 2).

At each time, the mean number of organisms present in the footpad of BALB/c mice was more ($P < 0.001$) than the mean number of

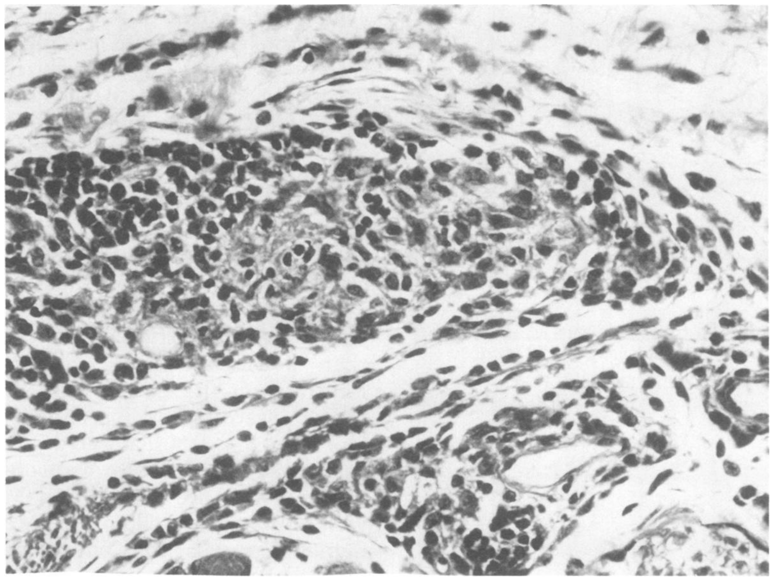

FIG. 8. *Appearance of the test site in the footpad 4 weeks after the injection of 1.6 × 10*^{7} *autoclaved whole organisms into a C57BL mouse 8 weeks after infection. Hematoxylin and eosin, ×400.*

TABLE 1. *Number of mice giving a mean increase in footpad thickness of greater than 5% and number of mice with a cellular infiltrate in footpad of greater than +*

Strain and treatment	No. of mice with a mean increase in footpad thickness of >5%/no. of mice tested		No. of mice with cellular infiltration in footpad of >+/no. of mice tested.	
C57BL				
High lepromin				
Control	0/16	$P < 0.001$[a]	2/16	$P < 0.001$[a]
Infected	20/24		17/24	
Low lepromin				
Control	0/10	$P < 0.001$[a]	0/10	$P = 0.007$[b]
Infected	16/18		9/18	
		$P < 0.05$[a]		
BALB/c				
High lepromin				
Controls	2/10	$P = 0.838$[b]	5/10	$P = 0.308$[b]
Infected	3/22		14/21	
		$P < 0.001$[a]		
Low lepromin				
Control	0/7	$P = 0.731$[b]	0/7	$P = 0.053$[b]
Infected	1/19		5/19	

[a] Calculated by the χ^2 heterogeneity test.
[b] Calculated by Fisher's exact test.

organisms per footpad in C57BL mice. Over the period of 6 to 20 weeks, the number of organisms per footpad in BALB/c mice increased significantly ($P < 0.01$), whereas the increase in the organisms in the footpads of C57BL mice was barely significant ($P \leq 0.05$).

The livers, spleens, and draining (right) popliteal lymph nodes of mice killed 16 and 20 weeks after infection were examined histologically for evidence of dissemination of organisms from the infection site in the footpad.

The livers of C57BL mice showed no evidence of infiltration of AFB. Occasional mononuclear cell granulomas containing a few AFB were seen in the livers of BALB/c mice. The spleens of both strains showed no evidence of infiltration.

TABLE 2. *Logarithmic mean number of organisms per footpad in infected C57BL and BALB/c mice*

Time after infection (weeks)	Logarithmic mean no. of organisms/footpad ± SE[a]		
	BALB/c	C57BL	P
6	7.786 ± 0.069 $n = 10$	6.782 ± 0.077 $n = 10$	<0.001
8	7.953 ± 0.046 $n = 5$	6.817 ± 0.122 $n = 5$	<0.001
10	8.118 ± 0.055 $n = 5$	6.854 ± 0.098 $n = 5$	<0.001
12	8.112 ± 0.141 $n = 5$	7.016 ± 0.077 $n = 5$	<0.001
16–20	8.550 ± 0.199 $n = 8$	7.041 ± 0.118 $n = 10$	<0.001

[a] SE, standard error of the mean.

The draining popliteal lymph node of a C57BL mouse killed at 16 weeks after infection contained a few AFB, but the popliteal lymph nodes of another mouse at this time and four mice killed at 20 weeks contained no bacilli. The draining lymph nodes of BALB/c mice killed at 16 and 20 weeks after infection contained mononuclear cells packed with AFB.

Popliteal lymph nodes in C57BL mice contained many mononuclear phagocyte granulomas limited to the paracortical areas of the node. Similar granulomas were seen in the nodes of BALB/c mice, although the paracortical areas of nodes in this strain were not distended to the same extent as in C57BL mice. A striking difference, however, was the presence of large numbers of plasma cells in the medullary cords of BALB/c popliteal lymph nodes, which were not seen in the nodes of C57BL mice. This would indicate a greater degree of local B-cell stimulation in BALB/c as compared with that of C57BL mice.

DISCUSSION

C57BL mice were able to limit the growth of *M. lepraemurium* in the footpad. At 5 months after infection, there appeared to be little dissemination of organisms to the liver, spleen, and draining lymph nodes. BALB/c mice were not able to limit the growth of *M. lepraemurium*. Dissemination of organisms to the draining lymph node and, to some extent, to the liver occurred by 5 months after infection. Histological examination of the draining popliteal lymph nodes revealed evidence of a strong antibody response in BALB/c but not in C57BL mice.

Two doses of MLM lepromin were used for skin testing: 1.6×10^7 organisms per mouse, the dose used in humans, and 0.4×10^7 organisms per mouse. When footpad swelling after 4 weeks of skin testing was assessed, there was little difference between the responses to the two doses of MLM lepromin. The majority of infected C57BL mice responded to either dose and no uninfected C57BL mice gave a footpad response. A small number of infected and control BALB/c mice gave a footpad response to the higher dose of MLM lepromin, and 1 infected BALB/c mouse out of 19 responded to the lower dose. Thus, footpad swelling at 4 weeks in response to MLM lepromin at either dose correlated well with the ability to limit organism growth in infected mice.

Histological examination of the skin test site in C57BL mice revealed that the majority of infected mice with footpad swelling had extensive mononuclear cell infiltration of the footpad 4 weeks after skin testing and only two uninfected mice, both given the higher dose of MLM lepromin, showed footpad infiltration. However, although only a small number of infected BALB/c mice given the higher dose of MLM lepromin gave a macroscopic response, a high proportion of these mice had extensive infiltration of the skin-tested footpad 4 weeks after lepromin injection. Similarly, a proportion of uninfected BALB/c mice given the higher dose of MLM lepromin showed footpad infiltration. Footpads from the majority of BALB/c mice given the lower dose of MLM lepromin showed minimal infiltration and no macroscopic response.

Microscopic assessment of the skin test lesion after injection of 0.4×10^7 autoclaved *M. lepraemurium* organisms correlated with resistance, but the higher dose of MLM lepromin induced cellular infiltration in a high proportion of low-resistance BALB/c mice, both infected and controls. Histologically, the infiltration in the two strains appeared to be similar, consisting of lymphocytes and histiocytes, and AFB were usually cleared by 4 weeks after skin testing.

The 24-h lesion was very similar in appearance to the Fernandez reaction in humans, which is described as containing a large number of polymorphs (8) as well as mononuclear cells. The later reaction at 4 weeks differed from the Mitsuda reaction in humans in that there was no significant organization and that the cells of the mononuclear phagocyte series did not show a typical epithelioid cell appearance. However, it is doubtful whether mouse mononuclear phagocytes ever take on a truly epithelioid cell form.

Uninfected mice of either strain showed little footpad swelling in response to either dose of MLM lepromin. This contrasts with studies on non-contacts of leprosy where up to 90% of people from non-leprosy areas give positive Mitsuda reactions in response to human lepromin (8, 10). This suggests that the lepromin test in humans is nonspecific and that sensitization by other mycobacteria might lead to lepromin positivity. Laboratory mice kept in reasonably controlled conditions do not appear to become sensitized to environmental mycobacteria.

On the basis of the following criteria, it is possible to classify the mouse leprosy type of C57BL and BALB/c mice after infection with 10^7 *M. lepraemurium* organisms in the footpad: (i) ability to limit organism growth at the infective focus: C57BL, +++; BALB/c, −; (ii) ability to prevent dissemination of organisms from site of infection: C57BL, +++; BALB/c, +; (iii) mitsuda reaction to 0.4×10^7 autoclaved whole organisms: macroscopic C57BL, +++; macroscopic BALB/c, ±; microscopic C57BL, ++; microscopic BALB/c, ±; (iv) local antibody response (as evidenced by the histological appearance of the draining L.N.): C57BL, −; BALB/c, +++.

Thus, C57BL mice could be classified according to the Ridley-Jopling scale (9) as borderline tuberculoid BT and BALB/c mice could be classified as borderline lepromatous BL.

These two strains of mice show equal susceptibility to intravenous infection with large numbers of organisms (6; Curtis and Alexander, unpublished data). Thus any classification of mouse leprosy has to be clearly defined as to the route of infection and the number of organisms given.

ACKNOWLEDGMENTS

This work was supported by a grant from The Wellcome Foundation.

We thank J. Manders, F. J. Schindler, H. Adu, and C. Smith for their assistance and M. Ridley for her advice on interpretation of the histology of lepromin reactions.

LITERATURE CITED

1. **Alexander, J., and J. Curtis.** 1979. Development of delayed hypersensitivity responses in *Mycobacterium lepraemurium* infections in resistant and susceptible strains of mice. Immunology **36**:563–567.
2. **Closs, O.** 1975. Experimental murine leprosy: growth of *Mycobacterium lepraemurium* in C3H and C57/BL mice after footpad inoculation. Infect. Immun. **12**:480–489.
3. **Closs, O., and O. A. Haugen.** 1974. Experimental murine leprosy 2. Further evidence for varying susceptibility of outbred mice and and evaluation of response of 5 inbred mouse strains to infection with *Mycobacterium lepraemurium.* Acta Pathol. Microbiol. Scand. Sect A **82**:459–474.
4. **Draper, P.** 1971. The walls of *Mycobacterium lepraemurium:* chemistry and ultrastructure. J. Gen. Microbiol. **69**:313–324.
5. **Hart, P. D., and R. J. W. Rees.** 1960. Effect of macrocyclon in acute and chronic pulmonary tuberculosis infection in mice as shown by viable and total bacterial counts. Br. J. Exp. Pathol. **41**:414–421.
6. **Lefford, M. J., P. J. Patel, L. W. Poulter, and G. B. Mackaness.** 1977. Induction of cell-mediated immunity to *Mycobacterium lepraemurium* in susceptible mice. Infect. Immun. **18**:654–659.
7. **Poulter, L. W., and M. J. Lefford.** 1977. Development of delayed-type hypersensitivity during *Mycobacterium lepraemurium* infection in mice. Infect. Immun. **17**: 439–446.
8. **Rees, R. J. W.** 1964. The significance of the lepromin reaction in man. Progr. Allergy **8**:224–258.
9. **Ridley, D. S., and W. H. Jopling.** 1966. Classification of leprosy according to immunity. A five-group system. Int. J. Leprosy **34**:255–273.
10. **Shepard, C. C., and E. W. Saitz.** 1967. Lepromin and tuberculin reactivity in adults not exposed to leprosy. J. Immunol. **99**:637–642.
11. **World Health Organization.** 1970. Expert committee of leprosy, fourth report. WHO Tech. Rep. Ser. no. 459.