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Development of delayed hypersensitivity responses in *Mycobacterium lepraemurium* infections in resistant and susceptible strains of mice

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Summary. C57Bl mice are relatively resistant to a moderate subcutaneous infection with *Mycobacterium lepraemurium* while BALB/c mice are much more susceptible. Cutaneous delayed hypersensitivity reactions which develop in the first 3 weeks of infection were compared in these two strains of mice. Both strains gave a peak of delayed hypersensitivity between 6 and 10 days after infection which was followed by a period of low reactivity before the development, in the third week, of a stable persistent delayed hypersensitivity reaction. There was no difference between the strains in the size at 24 h of the delayed hypersensitivity reaction but the reactions differed in their kinetics. The low resistance strain, BALB/c, gave a Jones-Mote-type of response while the high resistance strain gave a response which could be described as a tuberculin-type reaction.

INTRODUCTION

Inbred strains of mice show varying degrees of resistance to a moderate level of infection with *Mycobacterium lepraemurium* (Closs & Haugen, 1974). Closs (1975) has shown that C57Bl mice are able to limit the growth of *M. lepraemurium* following an injection of organisms ($2{\cdot}7 \times 10^6$) into the foot pad, while C3H mice are not. Similarly, in our experiments, BALB/c mice cannot limit the growth of organisms, following foot pad infection, while C57Bl mice can.

Recently, Rook (1978) has described fluctuations in the delayed hypersensitivity response of BALB/c mice in the first 4 weeks following subcutaneous infection with pathogenic and non-pathogenic strains of mycobacteria. He has suggested that mice develop three types of delayed hypersensitivity reactions, one of which is only produced after infection with non-pathogens and that this is associated with resistance.

Poulter & Lefford (1977) have described the production of delayed hypersensitivity by a relatively resistant strain of mice (B6D2F1; C57Bl × DBA/2) infected with *M. lepraemurium* (10^8 organisms into the foot pad) which develops at 2 weeks and peaks at 4–6 weeks after infection.

In this paper, we compare the changes in delayed hypersensitivity reactions which take place during the first 3 weeks of infection with *M. lepraemurium* in a high resistance strain, C57Bl, and in a low resistance strain BALB/c.

MATERIALS AND METHODS

Mice

Ten week old inbred BALB/c and C57Bl female mice were used. They were bred at the National Institute for Medical Research (N.I.M.R.) and at the Institute of

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Basic Medical Sciences, Royal College of Surgeons (R.C.S.).

Mycobacterium lepraemurium

The Douglas strain of *M. lepraemurium* was maintained by serial passage of 10^9 bacilli intravenously in Parkes strain outbred mice.

Preparation of organisms for infection

M. lepraemurium organisms were harvested from the livers of infected Parkes strain mice and purified by the method of Draper (1971). Acid fast bacilli stained by the Ziehl-Neelsen technique were counted by the method described by Hart & Rees (1960).

Infection of mice

10^7 *M. lepraemurium* organisms in 0·05 ml of sterile saline were injected into the right hind foot pad of mice.

Preparation of M. lepraemurium sonicate

M. lepraemurium organisms were prepared by the method of Draper (1971) from the livers and spleens of Parkes strain mice. The organisms were resuspended in distilled water and freeze dried. Suspensions of freeze dried organisms containing 50 mg organisms per ml of phosphate buffered saline pH 7·4 were ultrasonicated for 30 min at 4°. The sonicated material was centrifuged at 35,000 ***g*** for 30 min to remove debris. The protein content of the sonicate was measured by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as the standard. The sonicate was diluted to 200 μg/ml and passed through a 0·22 μm millipore filter for use as skin test antigen.

Skin testing

Mice were injected into the left hind foot pad with 25 μl (containing 5 μg of protein) of *M. lepraemurium* sonicate. The thickness of the foot pad was measured just before and 4, 8, 24 and 48 h and sometimes daily for 6 days after the injection. The foot pads of mice kept at the Royal College of Surgeons were measured using a dial thickness guage (Mitutoyo, Japan) and a screw gauge micrometer (Moore and Wright, Sheffield) was used to measure the foot pads of the mice kept at the N.I.M.R. Increase in foot pad thickness was expressed as a percentage of the thickness before injection.

Assessment of M. lepraemurium growth

Mice were killed by cervical dislocation. Right hind foot pads were excised and homogenized in 2 ml of 0·1% albumin saline and diluted 1:1 in 0·1% albumin in water for counting as above.

Statistical analysis

Means were compared by the Student's *t* test for non-paired data.

RESULTS

Bacillary counts in the foot pad

Table 1 shows the number of organisms present in the right hind foot pad of BALB/c and C57Bl mice 2 and 3 months after the injection at this site of 10^7 organisms. There were five times as many organisms per foot pad in BALB/c mice as there were in C57Bl mice 2 months after infection and this difference was significant ($P < 0·05$). Three months after infection, BALB/c mice had 100× more organisms per foot pad than had

Table 1. Bacillary counts in the foot pad

Time after infection	No. of organisms in the right hind foot pad. (Mean ± SE) × 10^{-6}		
	C57Bl	BALB/c	P value
2 months	12·0 ± 3·1	63·4 ± 18·6	< 0·05
3 months	3·4 ± 1·3	314·6 ± 64·3	< 0·001
P value	< 0·05	< 0·01	

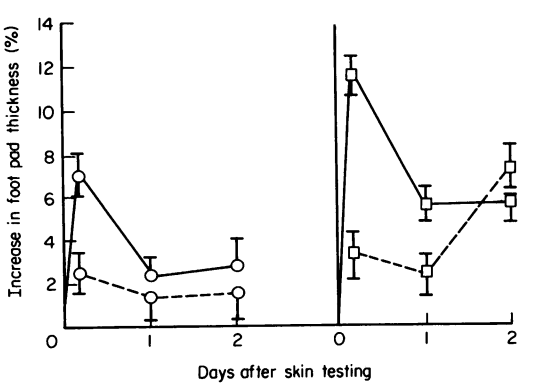

Figure 1. Percentage increase in foot pad thickness in uninfected control mice following skin testing with *M. lepraemurium* antigen (——) in the left hind foot pad and normal saline (----) in the right hind foot pad. Each point represents the mean ± SE of twenty mice, ten from the N.I.M.R. and ten from the R.C.S. ○, C57Bl; □, BALB/c.

C57Bl mice–a highly significant ($P < 0{\cdot}001$) difference. Between 2 and 3 months the number of organisms per foot pad increased significantly ($P < 0{\cdot}01$) in BALB/c mice while there was a small but significant ($P < 0{\cdot}05$) drop in the number of organisms per foot pad in C57Bl mice over this period.

Delayed hypersensitivity reactions

The data presented are the combined results of two experiments carried out simultaneously and independently at the R.C.S. and the N.I.M.R. The same inoculum of infective organisms was used for the two experiments. Groups of five mice (R.C.S.) and four mice (N.I.M.R.) were skin-tested at 2 day intervals for the first 3 weeks of infection. Each mouse was skin tested once only.

Figure 1 shows the percentage increase in foot pad thickness of uninfected control mice injected with 5 μl of skin-test antigen in the left hind foot pad and 5 μl of

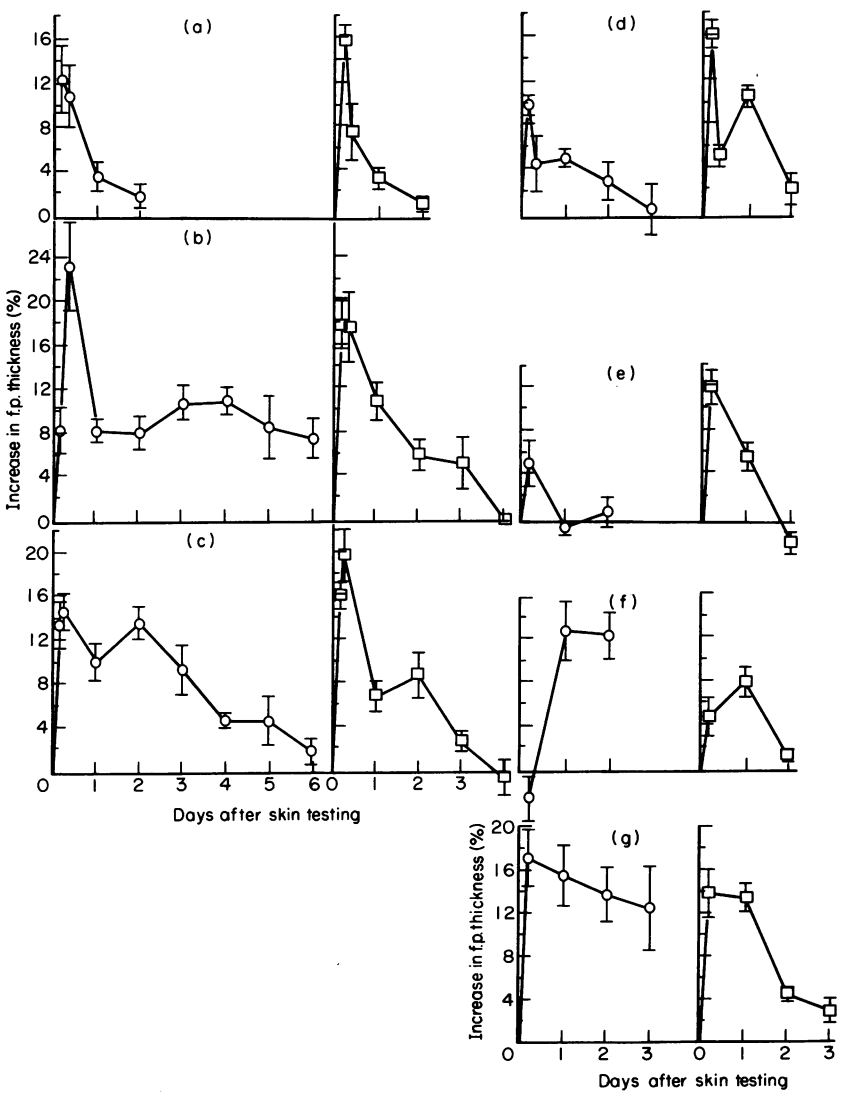

Figure 2. Percentage increase in foot pad thickness, Mean ± SE. Mice skin tested (a) 4 days, (b) 6 days, (c) 8 days, (d) 10 days, (e) 12 days, (f) 16 days and (g) 23 days after infection. o, C57Bl; □, BALB/c.

normal saline into the right hind foot pad. There was a 7% increase in foot pad thickness 4 h after the injection of skin-test antigen in C57Bl mice and a 2–3% increase in thickness at 24 and 48 h. The 4 h response was higher (11%) in BALB/c mice and these mice showed a 5–6% increase in foot pad thickness at 24 and 48 h. The increase at 48 h stimulated by antigen in BALB/c mice was not significantly different from the increase at this time in the saline-injected foot pad.

Four days after infection, all the mice gave an early reaction peaking at 4–8 h after skin testing, but there was no evidence of a delayed response (Fig 2a). The two strains of mice showed very different patterns of reactivity when skin tested 6 days after infection (Fig. 2b). In C57Bl mice, a very high early reaction was followed by a prolonged delayed reaction which was highest 3–4 days after skin testing. There was a similar large early reaction in BALB/c mice which diminished rapidly and the foot pads had returned to their pre-injection thickness by 3 days in some mice and in all mice by 4 days after skin testing. At 8 days of infection (Fig 2c) the pattern of reactivity was similar in the two strains of mice which both showed a peak of delayed hypersensitivity (DH) 48 h after skin testing. This peak was higher, but not significantly so, in C57Bl mice and there was some prolongation of the response up to 6 days in this strain. Again, the foot pads had returned to normal thickness in all BALB/c mice by 4 days. C57Bl mice gave very little delayed response when skin tested on day 10 of infection and no delayed response on day 12 (Fig. 2d and e). In contrast, BALB/c mice gave a delayed response which peaked at 24 h and was very low at 48 h on day 10 and possibly a similar but lower response on day 12 (Fig. 2d and e).

By day 16 of infection both strains of mice were giving a delayed response which was highest 24 h after skin testing (Fig. 2f). There was a difference in the kinetics of the response in the two strains which was also present at 23 days (Fig. 2g) and, in another experiment, at 2 months (Fig. 3a) and 3 months (Fig. 3b) after infection. In C57Bl mice the delayed response was maximum at 24 h and persisted at a high level for up to 4 days. In BALB/c mice the delayed reaction also peaked at 24 h but then fell to a low level at 48 h. At 16 days, 23 days, 2 months and 3 months after infection, there was no significant difference between the 24 h responses of the two strains. By 3 months of infection, the skin test responses of C57Bl mice were very variable and it appeared that some of these mice were losing their reactivity possibly due to their having controlled the infection (Table 1).

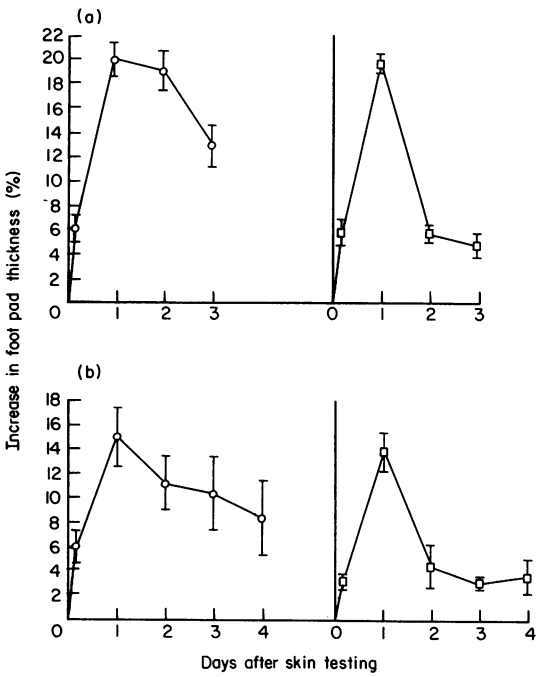

Figure 3. Percentage increase in foot pad thickness, mean ± SE. Mice tested (a) 2 months, (b) 3 months after infection. o, C57Bl; □, BALB/c.

DISCUSSION

Recently, Lefford, Patel, Poulter & Mackaness (1977) have reported that C57Bl mice and BALB/c mice show a similar degree of susceptibility to *M. lepraemurium* infection when given 10^8 organisms intravenously, the time to 50% death being 17 and 16 weeks respectively compared with a time to 50% death of 24 weeks in C3H mice and 25 weeks in A/J mice. In our experiments, however, BALB/c and C57Bl mice show a marked difference in their ability to limit the division of organisms at the site of infection following a subcutaneous injection of 10^7 organisms into the foot pad. The number of organisms present in the foot pad 2 and 3 months after infection appears to be a good criterion for assessing the relative resistance of C57Bl and BALB/c mice to a moderate subcutaneous infection with *M. lepraemurium*.

Both strains of mice showed a peak of delayed foot pad reactivity between 6 and 10 days after infection. This peak was followed by a period of low reactivity before the development in the third week of a stable persistent delayed hypersensitivity reaction. This pattern of development of delayed hypersensitivity is similar to the changes in the delayed hypersensitivity

response of BALB/c mice infected with pathogenic strains of mycobacteria described by Rook (1978). This author found that non-pathogenic mycobacteria induced two peaks of delayed reactivity in the first 2 weeks of infection, a small peak at 6 days and a large peak at 10–12 days, followed by a long-lasting delayed hypersensitivity response after 24 days while pathogenic strains induced only the 6 day and late delayed hypersensitivity responses. He suggested that the production of the delayed hypersensitivity response at 10 days was related to resistance and that pathogenic mycobacteria suppress or do not stimulate this response. In our experiments neither the high resistance nor the low resistance strain of mice studied gave this second peak of reactivity at 10–12 days in response to *M. lepraemurium* infection. The fluctuations in delayed response in the first 2 weeks of *M. lepraemurium* infection probably occur while the balance of effector and regulator cells is being established and the system appears to be equilibrated after 2 or 3 weeks.

It appears from the data presented here that after this initial equilibration phase, the two strains of mice show delayed hypersensitivity reactions which differ not in the degree of foot pad swelling at 24 h but in the kinetics of the response. The low resistance BALB/c strain give a delayed reaction which peaks 24 h after skin testing and falls away sharply at 48 h. This type of reaction may be likened to the Jones-Mote reaction, given by guinea-pigs and man, which has similar kinetics. The high resistance strain, C57Bl, gives a delayed reaction which peaks at 24 h and then is prolonged at a high level for 3 or 4 days. This could be described as a tuberculin-type reaction.

Lenzini, Rottoli & Rottoli (1977) have studied the kinetics of the PPD response in tuberculosis patients showing a spectrum of clinical disease. Patients with localized lesions who respond well to treatment (RR patients) develop a tuberculin-type response while the majority of patients with fairly low resistance and a more disseminated disease (UI patients) develop a Jones-Mote type of response. In tuberculosis therefore it appears that there is a marked correlation between the kinetics of the delayed hypersensitivity reaction and the clinical disease spectrum. From our results, it appears that a similar correlation occurs in murine leprosy.

In human leprosy, the best known mycobacterial disease showing a spectrum of resistance, a suitable skin-test antigen has not been available for such studies up to the present time. In this disease, it appears that the degree of delayed hypersensitivity as measured by the 48 h cutaneous delayed hypersensitivity reaction to lepromin (the Fernandez reaction) and its *in vitro* correlate the antigen specific lymphocyte transformation test, is related to tissue-destructive hypersensitivity and not to resistance (Bjune, Barnetson, Ridley & Kronvall, 1976). It is possible, however, that the kinetics of the delayed hypersensitivity response to soluble antigens of *M. leprae* may show a similar correlation with resistance as that which appears to occur in tuberculosis and murine leprosy. The WHO Immunology of Leprosy programme is now producing soluble *M. leprae* antigens from organisms purified from armadillo tissue and it should be possible to carry out kinetic studies of delayed hypersensitivity with this material. Such studies should be of importance in establishing the potential degree of resistance of individuals in populations exposed to leprosy.

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