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A lack of correlation between antigen-specific cellular reactions and resistance to *Mycobacterium lepraemurium* infection in mice

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Summary. Following infection subcutaneously in the footpad with 10^7 *Mycobacterium lepraemurium* organisms C57BL mice were able to limit multiplication of organisms at the infection site for the 6 months studied and to limit organism spread to the draining lymph node. Large numbers of organisms were present in the footpad and draining lymph node of BALB/c mice at 6 months. In spite of this difference in local immunity the changes in cellular reactivity to specific antigen as assessed by the delayed footpad response and the *in vitro* proliferative response of draining lymph node cells were similar in the two strains over the time studied.

INTRODUCTION

The relationship of delayed hypersensitivity to cell-mediated resistance in chronic infections is of widespread interest. Cell-mediated responses such as delayed skin-test reactivity to specific antigen, antigen-stimulated lymphocyte transformation *in vitro* and the production of leucocyte migration inhibition factor by lymphocytes stimulated by specific antigen, have been studied in human and experimental tuberculosis, leprosy, syphilis and leishmaniasis. In general, high resistance is associated with positive responses and low resistance with negative responses. However, this correlation is not absolute. The lymphocyte transformation responses to *Mycobacterium leprae* organisms of leprosy patients fall into a spectrum corresponding to the clinical and histopathological spectrum of the disease (Myrvang, Godal, Ridley, Froländ & Song, 1973). However, it has been shown that the responses of borderline leprosy patients correlate with hypersensitivity, patients undergoing hypersensitivity reactions having higher responses than stable patients higher in the immunological spectrum (Bjune, Barnetson, Ridley & Kronvall, 1976). A lack of correlation between the intensity of skin reactions to PPD and resistance to tuberculosis has also been noted in man (Hart, Sutherland & Thomas, 1967; McMurray & Echeverri, 1978) and experimental animals (Tuberculosis Program USPHS, 1955; Youmans, 1975). *M. lepraemurium* causes a chronic disease in mice which shows a spectrum similar to that of leprosy in man. C57BL mice have high resistance to moderate levels of subcutaneous infection and BALB/c and C3H mice have low resistance (Closs, 1975a; Alexander & Curtis, 1979). Curtis & Turk (1979) have classified C57BL mice as having BT and BALB/c mice as BL-type of murine leprosy. This classification was made on the basis of skin-test responsiveness to autoclaved whole organisms, histological evidence of local antibody production and ability to limit multiplication at, and spread of organisms from the infective focus.

In this paper we describe the *in vitro* antigen-stimulated lymphocyte proliferative responses and the

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delayed footpad responses of C57BL and BALB/c mice during the first 6 months following subcutaneous infection with *M. lepraemurium*. No correlation could be found between the size and duration of these responses and resistance to infection.

MATERIALS AND METHODS

Mice

Female BALB/c and male and female C57BL mice bred at The Royal College of Surgeons were used in these experiments. Mice were 10–14 weeks of age at the time of infection.

Infection of mice

Organisms for infection were prepared as described elsewhere (Alexander & Curtis, 1979). Mice were injected subcutaneously into the right hind footpad with 10^7 *M. lepraemurium* organisms in 0·05 ml of sterile phosphate-buffered saline pH7·4 (PBS).

Preparation of sonicated organisms

An ultra sonicate of *M. lepraemurium* organisms was prepared as previously described (Alexander & Curtis, 1979), and passed through a 0·22 μm millipore filter before use.

Footpad testing

The ultrasonicate of *M. lepraemurium* organisms was diluted in PBS to a protein content of 200 μg/ml. Mice were injected in the left hind footpad with 25 μl of this preparation, i.e. 5 μg protein/mouse. The thickness of the footpad was measured just before the injection and 4,6,8,12,24 and 48 hr later and on days 3,4,5 and 6 after the injection. The footpad thickness was measured with a dial thickness gauge (Mitutoyo, Japan), and the increase in footpad thickness was expressed as a percentage of the thickness before injection.

Lymphocyte transformation test

Cells from the draining (right) popliteal lymph node were used for the antigen-specific lymphocyte transformation test. Single cell suspensions were prepared in RPMI 1640, 'with added glutamine', containing 10% heat-inactivated foetal calf serum and 100 u/ml of penicillin and 100 μg/ml of streptomycin sulphate. Cells were cultured in round-bottomed micro-titre plates (Nunc). Each well contained 200 μl of cell suspension containing 10^6 viable cells (by Nigrosin exclusion) and 25 μl of medium (control culture) or the appropriate dilutions of sonicate in RPMI. Cultures were incubated at 37 ° in a humid atmosphere of 10% CO_2 in air for 3, 4, 5 or 6 days. Seventeen hours before harvesting, 25 μl of RPMI 1640 containing 1 μCi of tritiated thymidine (specific activity 2 Ci/mmol) were added to each well. Wells were harvested onto glass fibre discs in a multi-cell harvester (MASH II) and washed with distilled water. The discs were dried and counted in a liquid scintillation counter. Results were expressed as the arithmetic mean c.p.m. of between 4 and 8 replicate cultures.

Assessment of multiplication of M. lepraemurium *in the footpad*

Mice were killed by cervical dislocation, the right hind footpads were excised and homogenized in 2 ml. of 0·1% bovine serum albumin in water. The homogenate was diluted in albumin water and the organism were counted by the method of Hart & Rees (1960). Since *M. lepraemurium* does not grow *in vitro*, all organism counts refer to the number of intact organisms recovered, not to the number of viable organisms recovered.

Antibody measurement

Antibody to the antigens of *M. lepraemurium* was measured by the ELISA technique (Voller, Bidwell & Bartlett, 1976). The ultrasonicate was used as the antigen. Rabbit anti-mouse IgG labelled with alkaline phosphatase was a gift from Dr D. Adu of Guy's Hospital, London. The known positive serum was a pool of serum from Parkes strain mice killed 5 months after intravenous infection with 10^9 *M. lepraemurium* organisms. The technique was standardized for anti-mycobacterial antibody measurement, as described elsewhere (Adu, Curtis & Turk, manuscript in preparation).

Histology

Livers were fixed in Bouins fixative and spleens and lymph nodes in Carnoys fixative. Feet were fixed in formal acetic alcohol for 24 hr and in 10% formic acid for a further 48 hr. All sections were stained with haematoxylin and eosin and by the Ziehl-Neelson technique. Spleens and lymph nodes were also stained with methyl green and pyronin.

Statistical analysis

Mean increases in footpad thickness were compared by Students *t* test for non-paired data.

Experimental design

In a preliminary experiment it was found that the right popliteal lymph node draining the site of infection of mice in the first 3 weeks of infection was very small and that groups of up to twenty-five mice were necessary to obtain sufficient cells for culture. Because of the large numbers of mice needed, the experiment was performed in two parts; the responses of mice between 4 and 25 weeks of infection were measured and in a separate series of experiments the responses of mice from 8 days to 6 weeks. Groups of ten mice were used for measuring the footpad response and the lymph node cells of these mice were used for the antigen-specific lymphocyte transformation test later in the experiment. For the antigen- specific *in vitro* lymphocytic response of uninfected animals, groups of thirty-three to forty mice were used and both right and left popliteal lymph nodes were collected. At the end of each series of experiments, i.e. at 25 weeks and 6 weeks, ten mice were killed, their livers, spleens and right popliteal lymph nodes were fixed for histological examination, and their serum was collected for antibody measurement.

RESULTS

Delayed footpad responses

Footpad reactions to the soluble antigens of *M. lepraemurium* were measured at 8 days, 2, 3, 4, 6, 8, 12, 16, 21 and 25 weeks after infection. Mice were tested at 8 days because in previous experiments (Alexander & Curtis, 1979), a peak of delayed footpad reactivity was found at this time.

Infected mice of both strains gave a peak of response at 4–6 hours after skin testing (Fig. 1c and d), which was also given by uninfected (control) mice (Fig. 1a and b). The injection of 25 μl of PBS into the contralateral footpads of control mice induced detectable swelling at 4 and 6 h (4·3±1·0% C57BL,

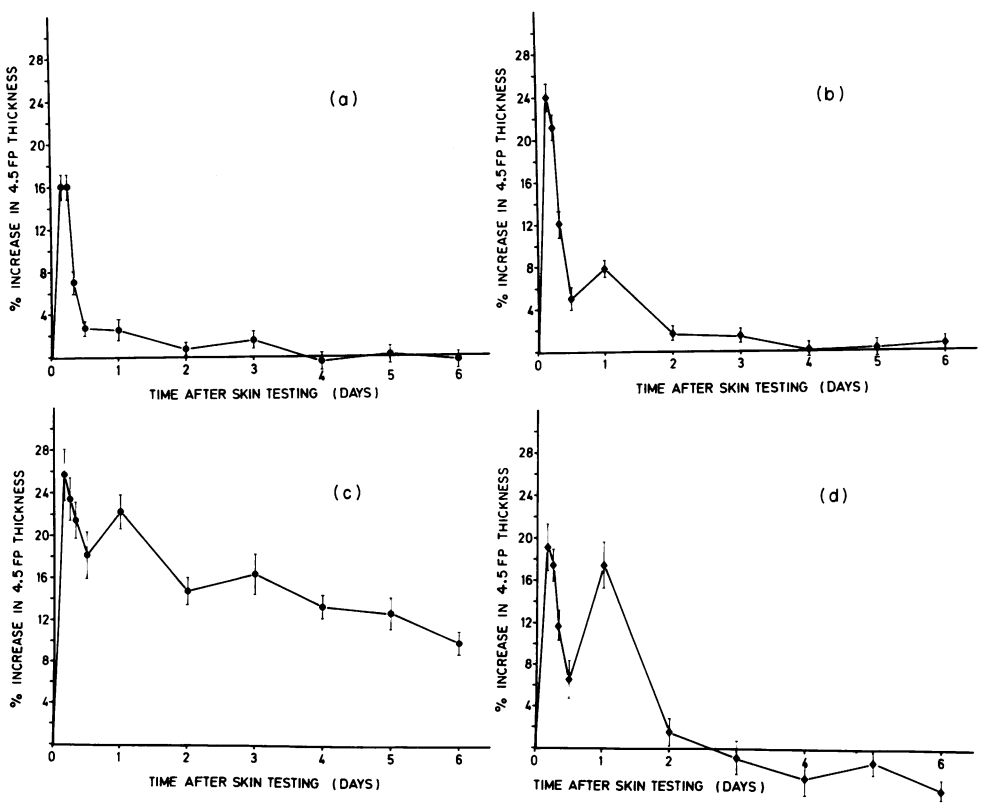

Figure 1. Footpad responses of mice to an ultrasonicate of *M. lepraemurium* organisms. (a) Uninfected C57BL mice $n = 30$; (b) uninfected BALB/c mice $n = 40$; (c) 6 week infected C57BL mice, $n = 10$; (d) 6 week infected BALB/c mice $n = 10$.

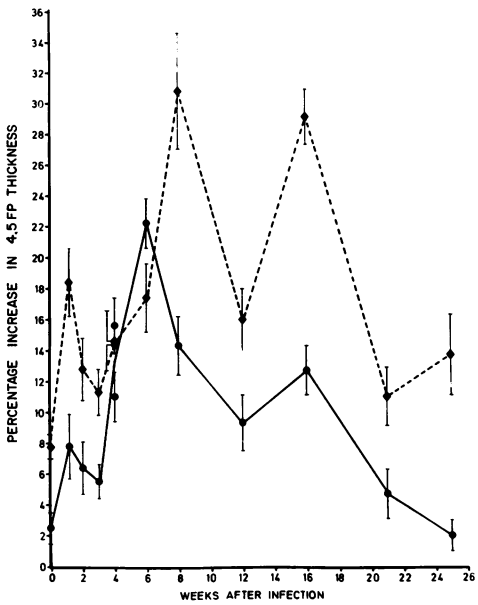

Figure 2. Twenty-four hour footpad responses to an ultrasonicate of *M. lepraemurium* organisms throughout infection. ---- BALB/c mice, ●——● C57BL mice. Each point is the mean ± SE of ten readings. The responses at 4 weeks were measured in both sets of experiments (see Experimental Design) and both results are shown.

11·4 ± 1·2% BALB/c at 4 hr which, however, was significantly ($P < 0.001$) lower than the responses of the same mice to antigen. The 4–6 hr peak declined to a minimum at 12 hr and was followed by a delayed reaction at 24 hr. The kinetics of the delayed response were different in the two strains and an example of the kinetics is shown in Fig. 1c and d for mice footpad tested 6 weeks after infection. Groups of ten uninfected mice were tested at intervals throughout the experiment. There appeared to be no age-related component of the 24 hr footpad response of uninfected mice, so the data from all uninfected mice were pooled, Fig. 1a and b.

BALB/c mice gave a strong 24 hr response 8 days after infection and then the response declined to a level similar to that of control mice at 3 weeks (Fig. 2). After 3 weeks, the 24 hr responses of BALB/c mice were higher ($P < 0.01$–$P < 0.001$) than those of uninfected mice throughout except at 21 weeks. The 24 hr response of C57BL mice was higher ($P < 0.01$) than that of control mice at 8 days, and between 4 and 16 weeks ($P < 0.001$). However, the 48 hr response of C57BL mice was higher ($P < 0.05$ – $P < 0.001$) than that of control mice throughout, except at 21 weeks and the response between 3 and 6 days of skin testing was also usually greater than that of controls. Except at 4 and 6 weeks the 24 hr response of BALB/c mice was higher ($P < 0.05$ – $P < 0.001$) than that of C57BL mice, and this was also so in the uninfected mice (Fig. 2).

Histological examination of the skin-test site of mice infected for 6 weeks showed that there was infiltration with polymorphs between 4 and 24 hr which was maximal at 12 hr. Mononuclear cell infiltration appeared at 12 hr and was maximal at 48 hr in both strains. Between 3 and 6 days, there was a decrease in the cellular infiltrate in the feet of BALB/c mice, but in C57BL mice the mononuclear cell infiltrate persisted. At 4–6 hr and at 24 hr the sections suggested the presence of an oedematous response which decreased in BALB/c mice at 48 hr, but persisted in the C57BL mice for the 6 days of testing.

Lymphocyte transformation test

In a small preliminary experiment it was found that the optimum dose of antigen and the optimum day for harvesting cultures could vary at different times of the infection. It was therefore decided to use a range of antigen doses and to harvest the cultures on days 3, 4, 5 and 6. Too few popliteal lymph node cells were obtained from uninfected controls and mice at the beginning of the infection to harvest on each day. Results from the later part of the infection indicated that the highest counts were obtained when the cells were harvested on day 3, and so cultures were always harvested on day 3 followed by day 4, day 5 and day 6, if sufficient cells were available.

The lymph node cells of BALB/c mice at 8 days and 2 weeks after infection gave the highest counts from cultures harvested on day 4 or day 5. At subsequent times, the highest counts were obtained on the third day of culture. Throughout the infection the lymph node cells of C57BL mice gave the highest counts on day 3 of culture. A preliminary experiment had also established that an antigen dose range of 2·5, 5, 10, 25 and 50 μg/ml was optimal.

Responses of cells from the right popliteal lymph node

Sufficient cells were obtained from the popliteal lymph nodes of uninfected BALB/c mice to harvest the cultures on days 3 and 4, but only sufficient cells to harvest on day 3 were obtained from the popliteal lymph nodes of uninfected C57BL mice (Table 1). Two groups of uninfected mice of each strain were

Table 1. The responses of popliteal lymph node cells from uninfected BALB/c and C57BL mice

Antigen concentration (μg/ml)	Day of Harvest 3 BALB/c	4	3 C57Bl
(i)			
0	*3345	3338	1643
2·5	7477	6749	3089
5	7747	7094	5002
10	9473	7439	6236
25	12,761	9362	7565
50	14,691	9516	6266
(ii)			
0	3766	1825	2335
2·5	4895	2538	NT†
5	7218	3146	NT†
10	7318	3125	NT†
25	10 320	4684	6312
50	11.301	4595	6128

*Each figure is the arithmetic mean c.p.m. of 4–8 replicate cultures.
†NT not tested due to lack of cells.

tested and the results given by the two groups were similar.

The counts incorporated by unstimulated cultures and by antigen-stimulated cultures in experiments performed throughout 6 months of infection are presented in Fig. 3. The highest counts were incorporated by cells stimulated with antigen doses of 25 or 50 μg/ml and the highest count obtained is shown. Data from cells harvested on the 3rd day of culture are given.

Cellular responses in the infected footpad

The size of the infected footpad was measured throughout the infection and compared with the uninfected contralateral footpad (Fig. 4). In C57BL mice, there was a rapid increase in the size of the infected footpad during the 4th week of infection, and the footpad remained swollen until 6 weeks, after which the swelling subsided. The infected footpad of BALB/c mice increased throughout the experiment. By 21 weeks, the whole foot and ankle were grossly swollen and there was evidence of necrosis.

Histological examination of the infected foot revealed that for the first 3 weeks after infection in both strains, the foot contained small pockets of macrophages packed with bright red acid-fast bacilli

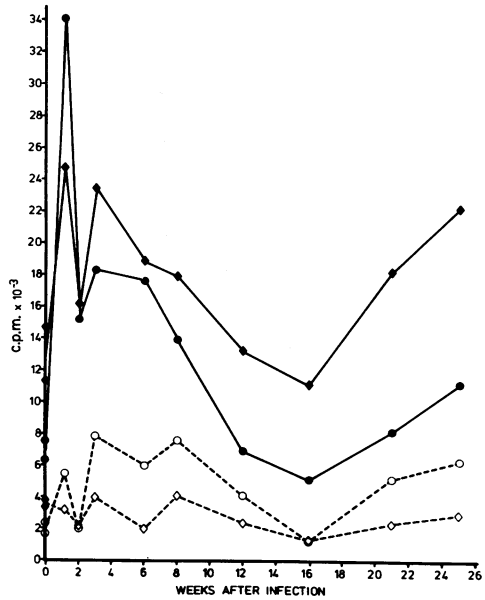

Figure 3. Lymphocyte transformation responses of cells from the right popliteal lymph node to an ultrasonicate of *M. lepraemurium* organisms: responses (c.p.m. × 10^{-3}) of cells harvested on the third day of culture. Each point is the arithmetic mean count of four–eight replicate cultures. The highest counts obtained with a dose range of 5–50 μg/ml of antigen are given. ——— Response of BALB/c cells stimulated with antigen; - - - - response of BLAB/c cells without antigen stimulation; ●———● response of C57BL cells stimulated with antigen; o- - -o response of C57BL cells without antigen stimulation.

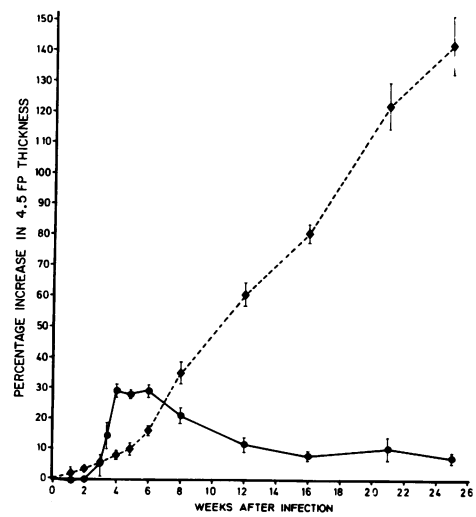

Figure 4. Increase in size of the infected foot throughout infection. - - - - BALB/c; ●———● C57 BL. Each point is the mean ± SE of the readings of at least ten mice.

(AFB). By 28 days after infection, the feet of both strains were infiltrated with mononuclear cells, both lymphocytes and macrophages, the infiltration being slightly more in the feet of C57BL mice. At 6 weeks there was more mononuclear cell infiltration in the feet of C57BL mice than in BALB/c mice, and very few bacilli were detectable in the former strain.

Bacilli were never cleared from the feet of C57BL mice. At 8, 12 and 16 weeks the bacilli appeared to be granular and in some fields there were only a few acid fast dots. The mixed lymphocyte macrophage infiltrate in the foot persisted throughout the period studied. The infiltration in the feet of BALB/c mice increased progressively throughout the infection and consisted, after 12 weeks, largely of macrophages packed with AFB. At 8 and 12 weeks the bacilli were palely stained, which possibly indicates that they were being destroyed, but at 16, 21 and 25 weeks the bacilli appeared as bright red rods. At 21 and 25 weeks some areas of the foot contained large numbers of polymorphs interspersed between loosely packed macrophages.

Infected footpads were harvested at intervals throughout the experiment and the number of bacilli present were counted (Fig. 5). From 6 weeks onwards

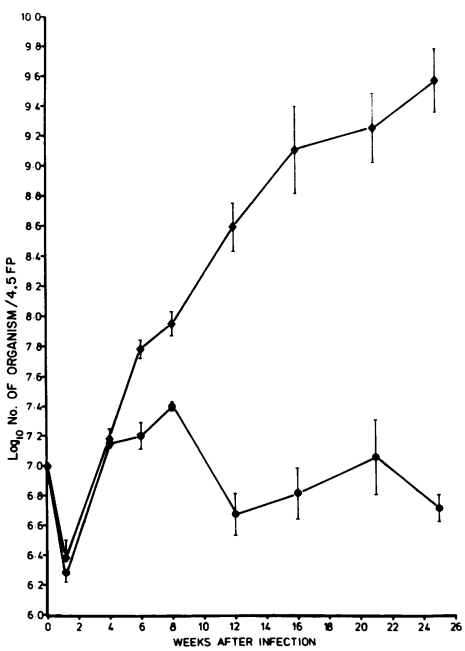

Figure 5. Number ($\log_{10}$) of organisms/footpad throughout infection. ——— BALB/c; ●———● C57BL. Each point is the mean ± SE of the readings of six mice.

significantly ($P < 0{\cdot}001$) more organisms were recovered from the feet of BALB/c mice than from the feet of C57BL mice.

Histological examination of livers, spleens and draining popliteal lymph nodes

Infiltration and acid fast bacilli: Macrophages were present in the paracortical areas of the draining popliteal lymph nodes of C57BL mice 6 weeks after infection. Some AFB were present in these macrophages. The macrophage infiltrate in the popliteal lymph node of BALB/c mice was less than that of C57BL mice at 6 weeks, but similar numbers of AFB were detectable. The lymph nodes were homogenized in 1 ml of 0·1% albumin water and smears were prepared for organism counts (Hart & Rees, 1960). The lower limit of organisms countable by this technique is 10^5 organisms/ml and the popliteal lymph nodes of neither strain contained countable numbers of organisms.

At 6 months after infection, there was a large amount of macrophage infiltrate in the paracortical areas of the popliteal lymph nodes of both strains. In BALB/c mice, large numbers of AFB were present in these macrophages—an average of $4{\cdot}7 \times 10^8$ organisms/lymph node. No organisms were detectable in the popliteal lymph nodes of C57BL mice.

Histological examination of the livers from 6 week infected mice revealed that the livers of C57BL mice contained mononuclear cell infiltration in the periportal areas and elsewhere, which was often associated with mononuclear cells (Kupffer cells) containing acid-fast material. The liver sections of BALB/c mice contained a few collections of mononuclear cells with the presence of some acid-fast material.

At 6 months there was a similar amount of periportal infiltration of lymphocytes in the livers of C57BL mice as was present at 6 weeks. In some places there were macrophages in the periportal infiltrate and occasional granulomas of macrophages and lymphocytes were present elsewhere. These macrophages contained no detectable organisms. The livers of BALB/c mice contained more lymphocyte infiltration at 6 months than at 6 weeks. Granulomas of macrophages and lymphocytes were present (on average four/section), but acid-fast bacilli were rarely seen. The spleens of both strains contained no detectable AFB at 6 weeks, but at 26 weeks some AFB were detectable in macrophages in the white pulp of the spleens of BALB/c mice.

Table 2. IgG antibody levels in serum of *M. lepraemurium* infected mice

	Parkes*	BALB/c			C57BL		
	5m i.v.	n.m.s.	6w s.c.	26w s.c.	n.m.s.†	6w s.c.	26w s.c.
O.D. 405 n.m.	0·903	0·054	0·080	0·854	0·028	0·095	0·078
% of known +ve					3·1	10·5	8·6

†Normal mouse serum.
*Outbred mice which are used for passaging *M.Lepraemurium* organisms.

Cellular activity

The draining lymph nodes of both strains at 6 weeks showed some B-cell activity with more germinal centres and more plasma cells in the nodes of BALB/c mice than in C57BL nodes. By 6 months this difference was much greater with lymph nodes of BALB/c mice containing large numbers of germinal centres and large numbers of plasma cells in the medullary cords and in the paracortex interspersed between the infiltrating macrophages. There were few germinal centres in the lymph nodes of C57BL mice, but moderate numbers of plasma cells were present in the paracortex and some in the medullary cords. At 6 weeks after infection, the spleens of C57BL mice contained many germinal centres (on average ten/section) in the white pulp and quite a few plasma cells, mostly in the red pulp. There were few plasma cells in the spleens of BALB/c mice and fewer germinal centres (about three/section) than in C57BL spleens. By 26 weeks, there were similar numbers of germinal centres in the spleens of the two strains (two–three/section) and large numbers of plasma cells were present, many in the white pulp. Very little T-cell activity was detectable in the spleens at any time.

Antibody measurement

The IgG antibody levels in the serum of *M. lepraemurium*-infected mice at 6 weeks and 26 weeks after infection were compared with the antibody level in pooled serum (the 'known positive' serum) from Parkes strain mice infected for 5 months with 10^9 *M. lepraemurium* organisms administered intravenously (Table 2). The serum tested was a pool from ten mice of each strain. The serum from BALB/c mice 26 weeks after infection contained high levels of antibody.

DISCUSSION

Resistance to subcutaneous infection with *M. lepraemurium* is usually assessed by counting the organisms present at the infection site and/or in the draining lymph node at various times after infection (Closs, 1975a; Lagrange & Hurtrel, 1978; Alexander & Curtis, 1979). C57BL mice were able to limit the multiplication of *M. lepraemurium* in the footpad and to limit the spread of organisms from this site to the draining lymph node. The number of organisms in the feet of BALB/c mice increased throughout the period studied and at 6 months their draining lymph nodes contained large numbers of organisms. Histological examination of the livers and spleens at 6 months revealed that few intact organisms were detectable in these organs in either strain. Thus only limited dissemination of organisms had occurred in the low resistance BALB/c mice during the period of infection studied. Eventually the organisms disseminate throughout the body and cause the death of these mice at about 12 months (J. Alexander—personal communication). High resistance C57BL mice survive for at least 15 months following subcutaneous infection with 10^7 organisms (Turcotte, 1980). In spite of the marked difference in local immunity between the two strains during the period of infection studied, the changes in cellular reactivity to specific antigen were similar in high and low resistance mice.

By 8 days after infection, there was an increase in antigen-reactive cells in the draining lymph node and a positive delayed footpad response in the contralateral footpad in both strains. However, there was no histological evidence of a lymphocytic response at the infection site. The 24-hr footpad reaction decreased at 2 and 3 weeks, there was a drop in the antigen-stimulated response of the popliteal lymph node cells at 2 weeks and there was still no lymphocytic infiltration at the infection site. By 4 weeks, the infected footpads of both strains were infiltrated with a mixture of lymphocytes and histiocytes and there was an increased delayed response in the contralateral footpad. The 24-hr delayed response of C57BL mice was highest at 6 weeks when the infected footpad was at its maximum

size. Thereafter, the delayed footpad response and the size of the infected footpad both decreased and the delayed footpad responses at 21 and 25 weeks were within the range of the responses of uninfected C57BL mice. The delayed footpad responses of BALB/c mice were very high at 8 and 16 weeks of infection, but also fell to control levels at 21 weeks with a slight rise at 25 weeks. Except at 4 and 6 weeks the 24 hr responses of BALB/c mice were significantly higher than the 24 hr responses of C57BL mice, but the responses of uninfected BALB/c mice were also significantly higher than those of uninfected mice of the C57BL strain. The proliferative responses to specific antigen of popliteal lymph node cells from both strains were higher than those of uninfected mice for the first 8 weeks of infection, and were similar to those of uninfected mice at 12 weeks and 16 weeks. The responses of BALB/c mice increased at 21 and 25 weeks, but the response of C57BL mice was similar to the responses of uninfected mice at 21 weeks, and there was a slight increase in the response at 25 weeks. The antigen-stimulated proliferative responses of the popliteal lymph node cells of BALB/c mice were higher than those of C57BL mice throughout, except at 8 days after infection, but again the responses of uninfected BALB/c mice were higher than those of uninfected C57BL mice.

Thus, neither the size nor the persistence of these two criteria of specific cellular reactivity correlated with resistance. The loss of cellular reactivity of C57BL mice was not followed by a loss of resistance. Alexander (1979) using the same strains of mice, the same source of organisms and the same infective dose as used here, has shown that even after 40 weeks of infection there are still about 10^7 organisms/footpad in C57BL mice. In BALB/c mice, the lack/loss of local resistance is accompanied by high cellular reactivity to specific antigen for at least 6 months.

The loss of delayed footpad reactivity and positive antigen-specific lymphocyte transformation and leucocyte migration inhibition in mice with progressive mycobacterial infections has been described (Poulter & Lefford, 1978; Rook, 1975a, b). Both sets of authors attributed the loss of cellular reactivity to high levels of persistent mycobacterial antigen. In the experiments reported here there were large numbers of organisms in the infected foot and draining lymph nodes of BALB/c mice at 6 months after infection and the spleens and livers contained a few organisms detectable in histological sections. The systemic load of intact organisms may have been sufficient to cause the loss of the delayed footpad response, and large amounts of soluble antigen may have been present in the circulation. The organism load of C57BL mice was low throughout the period studied and appeared to be confined almost entirely to the infected foot. It is not known whether the lack of increase in organism numbers at this site is due to bacteriostasis or due to a balance between bacterial multiplication and bacterial killing. However, examination of sections of the feet revealed the presence in macrophages of acid-fast fragments, which may indicate that killing of bacteria was occurring. If so, soluble antigen released from killed organisms might lead to suppression of lymphocyte reactivity. Histological examination of the livers and spleens of 6-week infected C57BL mice revealed a large amount of cellular activity (lymphocytic infiltration in the liver; B-cell activation in the spleen) in the absence of detectable intact organisms. This may also indicate that soluble antigen was being released into the circulation.

The difference in kinetics of the delayed footpad response of BALB/c and C57BL mice infected subcutaneously with *M. lepraemurium* has previously been reported (Alexander & Curtis, 1979). This difference in kinetics appears to correlate with resistance in this system. However, C57BL and BALB/c mice injected with the non-pathogen BCG (Glaxo) also give delayed footpad responses with the kinetics given by *M. lepraemurium*-infected mice of the same strains for up to a month after the injection of BCG (Adu, Curtis & Turk—manuscript in preparation). Both strains of mice are equally and highly resistant to BCG and so the difference in kinetics of the delayed response does not correlate with resistance in the BCG system.

It appears from the data presented here that in a chronic infection with *M. lepraemurium* the 24-hr delayed skin-test reponse and the antigen-specific lymphocyte transformation response are not indicators of resistance. The question arises whether resistance in this infection is in fact cell-mediated or even immunologically-mediated. The data given hereon specific antibody production suggests that resistance is not antibody-mediated. Alexander (1979) has shown that T-cell-enriched spleen cell fractions taken from C57BL mice after 6 weeks of subcutaneous infection with *M. lepraemurium* are able to transfer resistance to irradiated donors, though transfer does not always occur. On the other hand, nylon wool non-adherent (T-cell-enriched) spleen cells from 6-week infected BALB/c mice consistently transfer resistance to irradiated donors and this transferred resistance is abrogated by the co-transfer of nylon

wool adherent (B-cell-enriched) spleen cells or serum from infected BALB/c mice. A small amount of data on increased resistance of C57BL mice to reinfection (Closs, 1975b) and on increased organism counts in the draining lymph nodes of nu/nu C57BL/6 mice compared with nu/+ C57BL/6 (Lagrange & Hurtrel, 1978), suggest that resistance in this strain is probably mediated by immunologically active cells. The data of Alexander (1979) indicate that these cells may be T cells. However, the role of B cells in producing both a delayed footpad response (Henderson, Parker & Turk, 1980) and lymphocyte transformation should not be overlooked. This may be particularly relevant at 21 and 25 weeks of infection when histological examination of BALB/c popliteal lymph nodes shows marked B-cell activity which is accompanied by a high lymphocyte transformation response.

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REFERENCES

ADU H.O., CURTIS J. & TURK, J.L. (1981) Differences in cell-mediated immune responses of 'high resistance' and 'low resistance' mice to a non-athogenic mycobacterium. (Manuscript in preparation.)

ALEXANDER J. (1979) Adoptive transfer of immunity and suppression by cells and serum in early *Mycobacterium lepraemurium* infections in mice. *Parasite Immunol.* **1,** 159.

ALEXANDER J. & CURTIS J. (1979) Development of delayed hypersensitivity responses in *Mycobacterium lepraemurium* infections in resistant and susceptible mice. *Immunology*, **36,** 563.

BJUNE G., BARNETSON R. ST.C., RIDLEY D.S. & KRONVALL G. (1976) Lymphocyte transformation test in leprosy: correlation of the response with inflammation of lesions. *Clin. exp. Immunol.* **25,** 85.

CLOSS O. (1975a) Experimental murine leprosy. Growth of *Mycobacterium lepraemurium* in C3H and C57/BL mice after foot-pad inoculation. *Infect. Immunol.* **12,** 480.

CLOSS O. (1975b) Experimental murine leprosy: induction of immunity and immune paralysis to *Mycobacterium lepraemurium* in C57BL mice. *Infect.Immunol.* **12,** 706.

CURTIS J. & TURK J.L. (1979) Mitsuda-type lepromin reactions as a measure of host resistance in *Mycobacterium lepraemurium* infection. *Infect. Immunol.* **24,** 492.

HART P.D. & REES R.J.W. (1960) Effect of macrocylon in acute and chronic pulmonary tuberculosis infection in mice a shown by viable and total bacterial counts. *Br. J. exp. Pathol.* **41,** 414.

HART P.D., SUTHERLAND I. & THOMAS J. (1967) The immunity conferred by effective BCG and vole bacillus vaccines, in relation to individual variations in induced tuberculin sensitivity and to technical variations in the vaccines. *Tubercle*, **48,** 201.

HENDERSON D.C., PARKER D. & TURK J.L. (1980) Dissociation between tumour resistance and delayed-type hypersensitivity to tumour-associated antigens in the mouse. *Immunology*, **39,** 1.

LAGRANGE P.H. & HURTREL B. (1978) Local immune response to *Mycobacterium lepraemurium* in C3H and C57BL mice. *Clin. exp. Immunol.* **38,** 461.

MCMURRAY D.N. & ECHEVERRI A. (1978) Cell-mediated immunity in anergic patients with pulmonary tuberculosis. *Am. Rev. resp. Dis.***118,** 827.

MYRVANG B., GODAL T., RIDLEY D.S., FROLAND S.S. & SONG Y.K. (1973) Immune responsiveness to *Mycobacterium leprae* and other mycobacterial antigens throughout the clinical and hist-pathological spectrum of leprosy. *Clin. exp. Immunol.* **14,** 541.

POULTER, L.W. & LEFFORD M.J. (1978). Relationship between delayed-type hypersensitivity and the progression of *Mycobacterium lepraemurium* infection. *Infect. Immunol.* **20,** 530.

ROOK G.A.W. (1975a) The immunological consequences of antigen overload in experimental mycobacterial infections of mice. *Clin. exp. Immunol.* **19,** 167.

ROOK G.A.W. (1975b) The potentiating mitogenic and inhibitory effects on lymphocytes *in vitro* of macrophages in the lymph nodes of mice overloaded with mycobacterial products. *Clin.exp.Immunol.* **21,** 163.

TURCOTTE R. (1980) Influence of route of *Mycobacterium lepraemurium* injection on susceptibility to mouse leprosy and on lymphoblastic transformation. *Infect. Immunol.* **28,** 660.

TUBERCULOSIS PROGRAM USPHS (1955) Experimental studies of vaccination, allergy and immunity in tuberculosis. V Relation between degree of tuberculin sensitivity and resistance in the vaccinated individual. *Am. J. Hyg.* **62,** 185.

VOLLER A., BIDWELL D. & BARTLETT A. (1976) Microplate enzyme immunoassays for the immunodiagnosis of virus infections. *In*: (Ed. by N.R. Rose and H. Friedman), p. 506. *Manual of clinical immunology*, American Society for Microbiology, Washington, D.C.

YOUMANS G.P. (1975) Relation between delayed hypersensitivity and immunity in tuberculosis. *Am. Rev. resp. Dis.* **111,** 109.