Clin. exp. Immunol. (1985) **61**, 336–342.

Adoptive cell transfer of resistance to *Mycobacterium leprae* infections in mice

C. LOWE, S. J. BRETT & R. J. W. REES *National Institute for Medical Research, London NW7 1AA, UK*

(Accepted for publication 6 March 1985)

SUMMARY

Cells were transferred from mice intradermally vaccinated with killed *Mycobacterium leprae* to sublethally irradiated recipients. Unseparated cells from lymph nodes or spleens of *M. leprae* vaccinated mice were found to cause significant inhibition of the growth of a subsequent *M. leprae* challenge in mouse footpads for up to 26 weeks after vaccination. Vaccination with live BCG and cells transferred from BCG-vaccinated mice caused no significant inhibition of *M. leprae* growth in mouse footpads. Cell separation into fractions containing predominantly B and T lymphocytes showed that the inhibition of growth was due to *M. leprae*-sensitized T lymphocytes. *M. leprae* vaccinated mice were also skin tested with soluble *M. leprae* antigen and showed maximum delayed hypersensitivity responses 4 weeks after vaccination.

Keywords *M. leprae* protective immunity cell transfer delayed hypersensitivity

INTRODUCTION

It has been shown that killed armadillo-derived *Mycobacterium leprae* given in aqueous solutions can both sensitize mice (Shepard, 1976) and guinea pigs (Mehra & Bloom, 1979; Rees & Lowe, 1983) to *M. leprae* antigen and can also confer protection against *M. leprae* challenge in the mouse footpad (Shepard, 1976; and unpublished results from this laboratory). The degree of protection seems to be most complete when the immunizing dose of *M. leprae* is given by the intradermal route (Shepard *et al.*, 1982). Patel & Lefford (1978) have shown that vaccination with dead *M. leprae*, in this case given subcutaneously, could confer protection against challenge with *Mycobacterium tuberculosis* and BCG in mice and that this protection could be transferred by cell transfusion from vaccinated donors to recipient mice provided that the latter had been sublethally irradiated before transfer. Adoptive transfer of immunity to *M. leprae* following immunization with live, heat-killed or autoclaved *M. leprae* administered subcutaneously has also recently been reported (Graham & Navalkar, 1984). In the present study we have demonstrated that immunity to *M. leprae* can be transferred following intradermal immunization with gamma-irradiation killed *M. leprae*. We have also studied the kinetics of development of immunity, and partially characterized the cells involved.

MATERIALS AND METHODS

Animals. Specific pathogen-free CBA mice, 8–12 weeks of age, bred at the National Institute for Medical Research, were used throughout the studies. Five to 14 mice of one sex only were used for each experimental group.

Correspondence: Celia Lowe, National Institute for Medical Research, London NW7 1AA, UK.

M. leprae *vaccine and vaccination.* The tissues of experimentally infected armadillos were exposed to gamma-irradiation (2·5 Mrad, Cobalt-60 source) and the killed *M. leprae* were extracted and purified (Draper, 1979). They were used either as a freshly prepared suspension, or freeze dried and resuspended. Mice were vaccinated by intradermal injection in the shaved flank with 5×10^8 or 10^9 acid-fast bacilli (AFB) or 50 or 100 μg of freeze-dried *M. leprae* suspended in 0·02 ml of 0·05% Tween 85 in saline.

BCG vaccine. This was supplied as a freshly prepared suspension by Glazo Laboratories at 5 mg wet wt/ml with a viable count of 5×10^8 AFB/ml. It was given undiluted as 10^7 viable units in a 0·02 ml intradermal injection.

In each experiment one group of vaccinated mice was used to assess resistance to direct infectious challenge, the remainder were used as cell donors in cell transfer experiments.

Soluble M. leprae *antigen (SML).* This was prepared from purified armadillo derived *M. leprae* by ultrasonic disruption (Smelt, Rees & Liew, 1981). SML was standardized for protein content (Lowry *et al.*, 1951) and used as a skin-test antigen at a dose of 5 μg protein.

Skin tests. These were given as a subcutaneous injection of 5 μg SML in 0·02 ml borate buffered saline into the left hind footpads of five mice per group. The thickness of the foot was measured with a screw gauge micrometer (Moore and Wright, Sheffield) immediately before injection and 24, 48 and 72 h later. The results were expressed as the mean increase in footpad thickness of sensitized mice at each time interval, corrected by subtracting the respective mean increases of five unimmunized control mice similarly skin tested.

Adoptive cell transfer. Mice were killed by cervical dislocation and spleens, cervical, inguinal and popliteal lymph nodes were removed aseptically at various times after vaccination. Similar tissues were obtained from age-matched unvaccinated control mice. Cell suspensions were obtained by teasing the spleens and lymph nodes through a nylon sieve into Dulbecco's modified Eagle's medium (DMEM) containing 5% heat-inactivated foetal calf serum. Large pieces of debris were allowed to sediment; the suspensions were then decanted and centrifuged twice at 250*g* for 10 min. The cells were then counted and assessed for viability with trypan blue. Cells from each group were either left unseparated or enriched for nylon wool non-adherent T-cells by passage through nylon wool columns (Julius, Simpson & Herzenberg, 1973). Alternatively the cells were treated with anti-Thy 1:2 (Olac) diluted 1/5000 and with rabbit complement (Buxted Rabbit Company, Buxted, Sussex, UK) diluted 1/6. Following these treatments the cells were again counted and their viability assessed.

Mice were injected with 10^7 viable cells suspended in 0·2 ml DMEM intravenously into the tail vein. On the same day the recipient mice had received 600 rad total body irradiation from a Cobalt-60 source.

Experimental design

1. In the first experiment cells were obtained from the inguinal and popliteal lymph nodes 6 weeks after vaccination. This cell suspension was checked for the presence of *M. leprae*, and found to contain less than the detectable limit ($2{\cdot}5 \times 10^4$ AFB/ml).
2. In a second experiment the inguinal lymph nodes from the vaccinated side were excluded to avoid transfer of undetectable numbers of killed *M. leprae* or live BCG from the initial vaccination sites. Cells from the other lymph nodes were mixed with nylon wool non-adherent cells from the spleen. Again cells were transferred 6 weeks after vaccination.
3. In the third experiment cells were harvested and pooled from the lymph nodes and spleens 4 weeks after vaccination and then divided into three groups, (a) unseparated, (b) T-cell enriched nylon wool non-adherent, and (c) anti-Thy 1:2 and complement treated.
4. The fourth experiment was designed to study the kinetics of cell transfer immunity by transfusing unseparated spleen cells at intervals of 1, 2, 4 and 26 weeks after vaccination. Groups of five vaccinated mice were also skin tested with SML at the same time intervals.

Challenge infections. Protective immunity was assessed against a foot-pad infection in mice to an infectious challenge of 10^4 live *M. leprae* in 0·03 ml 0·1% bovine albumen in saline inoculated into the right hind footpad. Suspensions of live *M. leprae* were prepared freshly either direct from biopsies of skin from lepromatous patients or from footpads following their serial passage in mice.

Assessment of protective immunity. This was assessed by applying standard methods for

determining the multiplication of *M. leprae* in mouse footpads, based on the procedures used for harvesting the footpads and counting the total yields of AFB (Rees, 1964). By these methods yields of $< 2{\cdot}5 \times 10^4$ AFB/footpad were considered negative. Protection afforded by vaccination or cell transfer was determined by the growth of *M. leprae* in the footpads of individual mice from these groups compared with that obtained in unvaccinated mice *or* in mice transfused with cells from unvaccinated mice. Growth of *M. leprae* with yields of more than 5×10^5 AFB/footpad was obtained in the control mice 26–28 weeks after challenge, at which time half of the mice from the groups in the first three experiments were killed and their footpads counted. The remainder were counted 35–40 weeks after challenge. In the fourth experiment all groups were assessed at 26 weeks.

The difference between groups were analysed statistically by the Wilcoxon two-sample rank test (Colquhoun 1971) and the *P* values were calculated for a two-tailed test.

RESULTS

Adoptive transfer of immunity to M. leprae *following intradermal vaccination with gamma-irradiation killed* M. leprae *or live BCG*

Figure 1a shows the individual AFB counts in the footpads 26 weeks after a 10^4 infectious challenge in mice transfused with 10^7 lymph node cells from unvaccinated mice and from mice vaccinated with 10^9 *M. leprae*. A group of the vaccinated mice was directly challenged and significant protection ($P < 0{\cdot}01$) was obtained in both directly challenged mice and in the group transfused with sensitized cells compared with the group given unsensitized cells. Results at 39 weeks after challenge (Fig. 1b) were very similar.

In a second experiment using mixed lymph node and nylon wool non-adherent spleen cells, again there was a significant difference from the unsensitized cell controls. However, in mice vaccinated with 10^7 BCG (Glaxo) there was no significant protection to the *M. leprae* challenge either in the directly vaccinated mice or in mice transfused with 10^7 spleen and lymph-node cells from BCG-vaccinated mice (see Table 1).

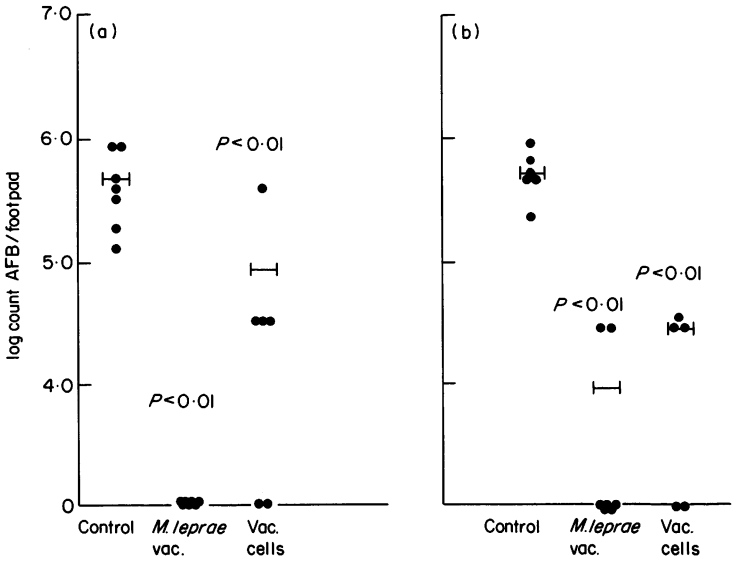

Fig. 1. Multiplication of *M. leprae* in the footpads of mice at 26 weeks (1a) and 39 weeks (1b) following a 10^4 infectious challenge. Mice were either directly vaccinated with 10^9 *M. leprae* (*M. leprae* vac) or transfused with 10^7 unseparated cells from *M. leprae* vaccinated mice. Bars represent the means of seven footpad counts.

Table 1. The effect of BCG and *M. leprae* vaccination or cell transfer upon the multiplication of *M. leprae* in mouse footpads

Vaccine or cell transfer	Log count AFB* (26 weeks)	Log count AFB* (35 weeks)
10^7 cells from unvaccinated controls	6·39 (5·88–6·73)	6·33 (6·04–6·45)
10^7 BCG (ID)	6·17 (5·64–6·56; $P>0·1$; NS)	6·09 (5·72–6·38; $P>0·1$; NS)
10^7 cells from BCG vaccinated mice	6·06 (5·82–6·29; $P>0·1$; NS)	6·07 (5·84–6·24; $P>0·1$; NS)
10^9 killed *M. leprae* (ID)	5·77 (<4·4–6·34; $P=0·01$)	5·88 (4·92–6·24; $P=0·01$)
10^7 cells from *M. leprae* vaccinated mice	5·88 (5·29–6·23; $P=0·01$)	5·90 (5·05–6·16; $P=0·01$)

* Log counts are the mean of seven footpad counts. Figures in parentheses are the range of counts and P values for difference from the controls.
ID; Intradermal vaccination.

Characterization of cells involved in adoptive transfer of immunity

The effect of transfusing unseparated, T-cell enriched and T-cell depleted cells was compared. Again there was a significant difference in footpad counts from the controls using sensitized unseparated cells from lymph nodes and spleens at both the 26 week and the 39 week harvests. At 26 weeks the T-cell enriched cells gave no significant protection owing to very high footpad counts in two out of seven mice, but at 39 weeks there was significant protection in those mice receiving T-cell enriched cells from immunized mice compared to those receiving T-cell enriched cells from control mice (Fig. 2). At both harvest times a transfusion of T-cell depleted cells gave no significant protection.

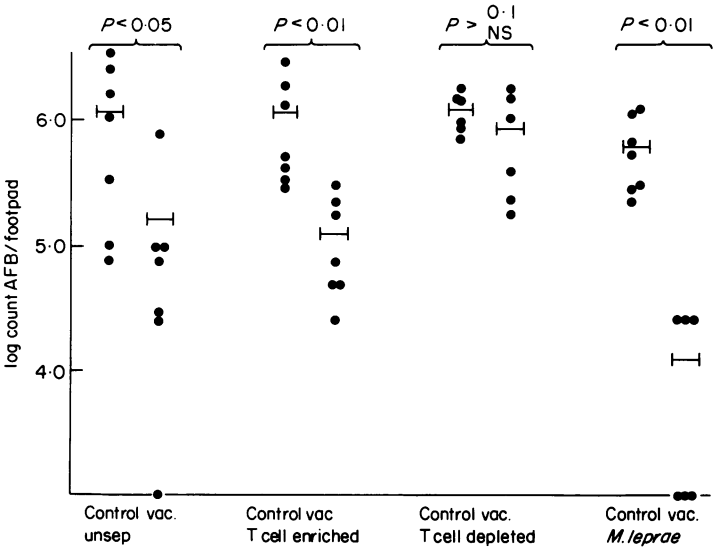

Fig. 2. The effect of transfusing 10^7 cells (column 1 = unseparated; column 2 = T-cell enriched; and column 3 = T-cell depleted) on the multiplication of *M. leprae* in mouse footpads 39 weeks after a 10^4 infectious challenge. The effect of direct vaccination with *M. leprae* is also shown (column 4). Bars represent the means of seven footpad counts. Each column compares the protective effect of cells from vaccinated mice (vac) with that of identically treated cells from unvaccinated mice (control).

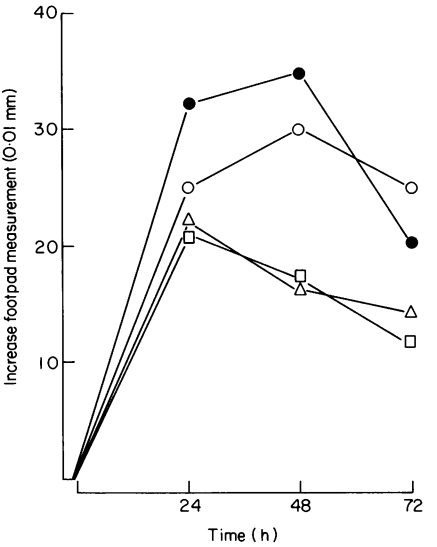

Fig. 3. Corrected mouse footpad enlargement in mice skin tested with SML △ 1 week, □ 2 weeks, ● 4 weeks and ○ 26 weeks after vaccination with irradiation-killed *M. leprae*. Points represent the means of five footpad enlargements minus five unvaccinated control measurements.

Kinetics of development of DTH and immunity to M. leprae

In the first three experiments described above we used a time interval of 4–6 weeks between vaccination and cell transfer following our previous observations that after 4 weeks a granuloma had formed at the vaccination site and the skin test response to SML is strongest at this time interval and peaks at 48 rather than at 24 h. This is illustrated in Fig. 3 where experimental groups of vaccinated mice were skin tested for delayed-type hypersensitivity responses at intervals of 1, 2, 4 and 26 weeks after a 10^9 *M. leprae* vaccination.

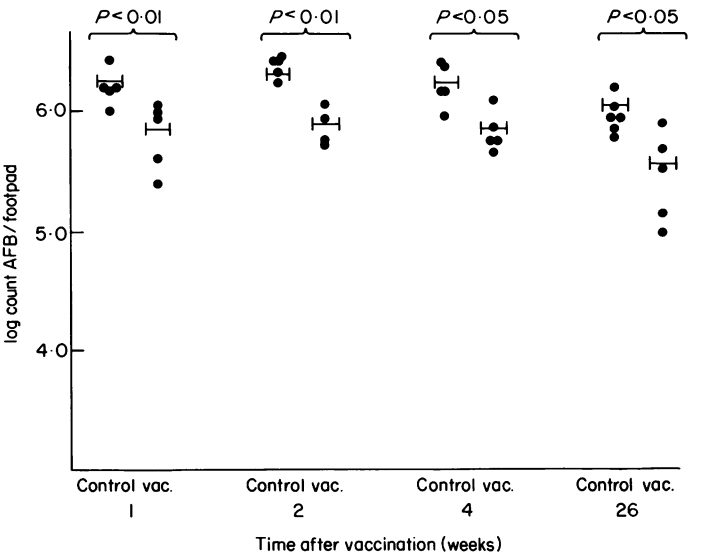

Figure 4. Multiplication of *M. leprae* in the footpads of mice transfused with 10^7 unseparated cells from control mice (left columns) or from mice vaccinated with 10^9 killed *M. leprae* (right columns) at the stated intervals after vaccination. Bars represent the means of five AFB counts 26 weeks after challenge.

In the same experiment we also transfused 10^7 spleen cells from the vaccinated mice into recipient mice at the same time intervals, and assessed protection against a challenge with viable *M. leprae*. The results (Fig. 4) indicate that there was significant protection in all groups, indicating that *M. leprae* immune cells were present by 7 days after immunization, and persisted for at least 26 weeks.

DISCUSSION

It is clear from our results that the vaccination of mice with whole *M. leprae* in saline produces a population of sensitized T-lymphocytes which can be transferred to sublethally irradiated recipient mice and then inhibit the growth of an *M. leprae* footpad challenge. Since Patel and Lefford's (1978) results indicate that *M. leprae* vaccination and cell transfer in mice can also inhibit the growth of *M. tuberculosis* and BCG infections it may be that sensitization is predominantly to common mycobacterial antigens and not to an *M. leprae*-specific antigen. Shepard, Van Landingham & Walker (1980) have however tried vaccination in mice with a number of other mycobacterial species but, with the exception of BCG there was no significant inhibition of growth of *M. leprae* in footpads. In this laboratory we have previously obtained a significant degree of protection to *M. leprae* challenge in mouse footpads using either the Glaxo or the Pasteur strain of BCG 10^7 live bacilli given intradermally (unpublished results). Such protection was, however, never as complete as with the 10^9 dead *M. leprae* vaccination. In this series of experiments, however, we were unable to demonstrate any significant protection with BCG (Glaxo) either by direct vaccination or by cell transfer (see Table 1).

The results presented in Fig. 2 indicate that successful transfer of immunity against *M. leprae* is associated with the transfer of T-cells, and further experiments to determine the Lyt phenotype of these cells are in progress. It is also clear from Fig. 2, and also from our previous experience, that the protection afforded by transfused sensitized lymphocytes is never so complete as that obtained by using direct vaccination. This is probably, at least partly due to technical reasons such as the number of cells transfused, and the distribution and survival of transfused cells in the recipient mice. In a chronic infection such as leprosy, such factors are likely to be of even greater importance.

Although the results of experiments to follow the kinetics of development of DTH responses to *M. leprae* indicate that the response is maximal at 4 weeks after vaccination (Fig. 3), the cell transfer data suggests that *M. leprae*-responsive lymphocytes are present within 7 days (Fig. 4). This might be interpreted to mean that there is a dissociation between cell populations mediating DTH and protective immunity, similar to that reported for *M. tuberculosis* and *M. bovis* vaccinated mice (Orme & Collins, 1984). However, it could also reflect the fact that it is much easier to measure small differences in DTH responses compared to similar differences in protective immunity against *M. leprae*. Thus, in Fig. 4, although we could detect significant differences between mice transfused with control cells and those transfused with immune cells, the system was probably not sufficiently sensitive to detect differences between the groups of mice transfused with immune cells at different time intervals after vaccination. One interesting finding is that immune cells were still present and able to confer immunity to recipient mice for at least 26 weeks after vaccination, suggesting that intradermal vaccination with killed *M. leprae* produces a state of prolonged immunological responsiveness which could be of practical significance for the use of such a vaccine in man.

We thank Dr M. J. Colston for helpful discussion and revision of the manuscript and Mr R. Butler for expert technical assistance. Purified *M. leprae* and SML were supplied by the IMMLEP component of the UNDP/World Bank/WHO Special Programme.

REFERENCES

COLQUHOUN, D. (1971) *Lectures on Biostatistics: an Introduction to Statistics with Applications in Biology and Medicine*. p. 137. Clarendon Press, Oxford.

DRAPER, P. (1979) Annex 1, Protocol 1/79 Report of the Enlarged Steering Committee Meeting for Research on the Immunology of Leprosy

(IMMLEP). World Health Organisation, Geneva, Switzerland.

GRAHAM, L.J.R. & NAVALKAR, R.G. (1984) Evaluation of *Mycobacterium leprae* immunogenicity via adoptive transfer studies. *Infect. Immun.* **43,** 79.

JULIUS, M.H., SIMPSON, E. & HERZENBERG, L.A. (1973) A rapid method for the isolation of functional thymus derived murine lymphocytes. *Eur. J. Immunol.* **3,** 645.

LOWRY, O.H., ROSEBROUGH, N.S., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265.

MEHRA, V. & BLOOM, B.R. (1979) Induction of cell-mediated immunity to *Mycobaterium leprae* in guinea pigs. *Infect. Immun.* **23,** 787.

ORME, I.M. & COLLINS, F.M. (1984) Adoptive protection of the *Mycobacterium tuberculosis*-infected lung cell. *Immunology*, **84,** 113.

PATEL, P.J. & LEFFORD, M.J. (1978) Specific and nonspecific resistance in mice immunized with irradiated *M. leprae*. *Infect. Immun.* **20,** 692.

REES, R.J.W. (1964) Limited multiplication of acid fast bacilli in the footpads of mice inoculated with *M. leprae*. *Br. J. exp. Pathol.* **45,** 207.

REES, R.J.W. & LOWE, C. (1983) Biological activities in guinea-pigs and in man of bacteria and cell-free extracts of purified armadillo-derived (PAD) *Mycobacterium leprae*. In *The Immunology of Leprosy* (ed. by G. P. Talwar, J. L. Turk & R. J. W. Rees) p. 43, Arnold-Heinemann, New Delhi.

SHEPARD, C.C. (1976) Vaccination of mice against *M. leprae* infection. *Int. J. Lepr.* **44,** 216.

SHEPARD, C.C., VAN LANDINGHAM, R. & WALKER, L. L. (1980) Searches among mycobacterial cultures for antileprosy vaccines. *Infect. Immun.* **29,** 1034.

SHEPARD, C.C., WALKER, L.L., VAN LANDINGHAM, R.M. & SHUNZHANG Y.E. (1982) Sensitization or tolerance to *Mycobacterium leprae* antigen by route of injection. *Infect. Immun.* **38,** 673.

SMELT, A.H.M., REES, R.J.W. & LIEW, F.Y. (1981) Induction of delayed-type hypersensitivity to *Mycobacterium leprae* in healthy individuals. *Clin. exp. Immunol.* **44,** 501.