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Adoptive Transfer of Tolerance Induced by ICRC Bacilli Against *Mycobacterium leprae* in Mice[1]

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It is a well-established fact that high intravenous doses of certain mycobacteria can induce a state of anergy and an inability to respond to the same as well as other related antigens in mice ([11, 26, 27]). The route of administration of *Mycobacterium leprae* influenced the type of immune response in mice; the intradermal (i.d.) route gave rise to delayed-type hypersensitivity (DTH) and the intravenous (i.v.) route led to immunological tolerance ([33]). These authors have also demonstrated that *M. leprae* does not lose its immunogenicity even after heat-killing at 100°C, to evoke *M. leprae*-specific DTH response ([32]).

In our earlier studies, we have demonstrated that the strain ICRC bacilli (leprosy-derived mycobacteria, LDM), unlike other cultivable mycobacteria, did not lose their immunogenicity in mice after heat-killing at 100°C—a characteristic similar to *M. leprae* ([2, 5, 18]). The ICRC bacilli evoked a DTH response against *M. leprae* as assayed by foot pad swelling ([2]), a test commonly used for other mycobacteria by several workers ([25, 30, 31, 33]). It was also found that the immunity induced by the ICRC bacilli against *M. leprae* could be adoptively transferred to normal mice by sensitized splenocytes ([2]).

The present investigation was further designed to study a) the effect of the route of immunization to ICRC bacilli, b) the possible "transfer" of tolerance induced by i.v. injection of ICRC bacilli/*M. leprae,* c) the ability to break the *M. leprae*-induced tolerance by immunization with ICRC bacilli or BCG, and d) to examine the antigenicity of another ICRC strain, C-75 ([18]).

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MATERIALS AND METHODS

The method of sensitization of mice and the measurement of foot-pad thickness are essentially the same as described earlier ([2, 30, 31, 33]).

Mice

Inbred strains of female BALB/c mice 7–10 weeks of age, bred at the Cancer Research Institute, Bombay, India, were used for the experiments.

Antigens

Origins of ICRC bacilli—strains C-44 and C-75. The ICRC (Indian Cancer Research Centre) strains were derived from human lepromatous nodules and grown in Dubos modified medium ([6]). The strain number corresponds to the biopsy processed and successfully grown "*in vitro.*" The strain C-44 was isolated in 1969 from a lepromatous patient (no. 65801), while strain C-75 was isolated in 1982 from the lepromatous nodule of a "DDS resistant" patient (no. 2724). Strain C-44 was used to prepare the antileprosy vaccine (irradiated) and was found to evoke lepromin conversion in lepromatous leprosy patients and healthy volunteers. The skin test antigen of this strain evoked negative Mitsuda responses in these patients, like lepromin ([4, 5, 12, 13]). Biochemically, both of these strains have been found to show *M. avium intracellulare* characteristics ([19]).

Method of preparation of antigens. *M. leprae.* Heat-killed (Hk) *M. leprae* (2×10^7 bacilli/mouse) was used for both the immunization and tolerance experiments and was prepared from the stock concentrate kindly supplied by Dr. R. J. W. Rees, Head, Laboratory for Leprosy and Mycobacterial Research, Medical Research Council, London. The eliciting antigen, lepromin (Mitsuda), was prepared by appropriate dilution (3×10^7/foot pad) of stock concentrate in normal saline.

ICRC bacilli. The two strains of ICRC

bacilli (C-44 and C-75) used for immunization or for inducing tolerance were irradiated at 150 Krads in a ^{60}Co-gamma cell. The immunizing dose was 2×10^7 bacilli/mouse. The eliciting antigens of the two strains (ICRCins) were prepared by autoclaving the bacilli, and were processed in the same manner as for Mitsuda-type lepromin. The dose of ICRCins injected was 3×10^7 bacilli/foot pad.

Experimental protocols

Assessment of effect of various routes of injection of ICRC on the DTH response to ICRCin and lepromin. Four groups of not less than six mice each were injected with 3×10^7 bacilli (C-44 irradiated, Irr.) in a volume of 0.1 ml by four different routes, i.e., intradermal (i.d.), intravenous (i.v.), intraperitoneal (i.p.), and subcutaneous (s.c.), at day 0. The i.d. and s.c. injections were given on the flank. The i.v. injection was given through the tail vein. Mice were challenged in the foot pad separately with lepromin and the two ICRC antigens on day 28, and the foot pad enlargement (FPE) was measured with dial calipers just before and 24 hr and 48 hr after challenge. There was a maximum FPE after 48 hr and the corrected (Corr.) FPE was calculated by subtracting the mean FPE in nonimmunized mice from that of the immunized mice.

Induction of DTH tolerance to ICRC bacilli and *M. leprae* and its adoptive transfer. The protocol of this experiment is shown in Figure 1. The adoptive transfer of tolerance was carried out by the method described earlier ([1]). A previous experiment on the effect of routes of administration of the ICRC bacilli showed that the i.v. route produced maximum tolerance in mice. Therefore, the spleen cells from mice injected by the i.v. route with *M. leprae* and ICRC bacilli (C-75 strain) were used in an adoptive transfer study in presensitized mice. There were 3 recipient groups consisting of 7 mice each, out of which 2 groups were sensitized by the i.d. route with 2×10^7 ICRC bacilli (Irr. C-75 strain) and 1 group was injected with saline. The donors were two groups of mice made tolerant by i.v. administration of either 2×10^7 ICRC bacilli (Irr. C-75 strain) or *M. leprae* (Hk). At the end of 28 days, the donor mice were killed by cervical dislocation, the spleens dissected, and a cell suspension was prepared in normal saline ([20, 24]). The viability of the cells was estimated by the 0.2% trypan blue dye exclusion method, and cells with 90% viability were used. The spleen cells (1×10^8) suspended in 0.1 ml saline were injected through the tail vein into recipients. They were then challenged separately with lepromin and the two ICRC antigens immediately (within 2 hr of cell transfer). The foot pad swelling was measured just before and also 24 hr and 48 hr after challenge. In these experiments, a maximum foot pad swelling was observed at 24 hr and, hence, we report these values. The transfer of spleen cells from control (nonsensitized) to intradermally sensitized recipient mice provided a positive control for the experiment. Similarly, spleen cells were also transferred from nonsensitized donors to nonsensitized recipients.

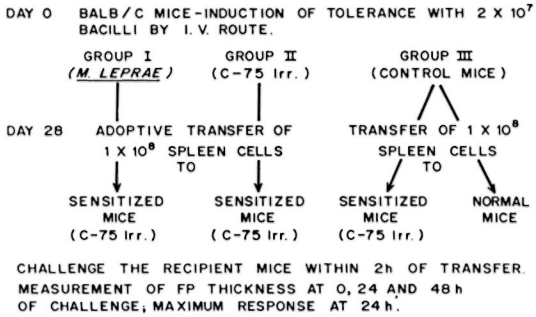

FIG. 1. Adoptive transfer of tolerance. The tolerance induced by i.v. injection of both Hk *M. leprae* and irradiated C-75 strains of ICRC bacilli were adoptively transferred by spleen cells (1×10^8) to recipient mice sensitized 28 days before with C-75 (Irr.) bacilli. Similarly, spleen cells from nonsensitized (saline injected) mice were also transferred to presensitized (C-75 Irr.) as well as nonsensitized recipients. All of the recipients were challenged in the foot pads with lepromin, C-44 ICRCin or C-75 ICRCin within 2 hr of cell transfer.

Induction of tolerance by *M. leprae* and recovery by ICRC bacilli, BCG, and *M. leprae*. The method described by Shepard, *et al.* ([33]) was followed. Four groups of mice were injected i.v. with 2×10^7 *M. leprae* (Hk) at day 0. Seven days later, these mice were immunized i.d. with either 2×10^7 *M. leprae* (Hk), ICRC bacilli (Irr. C-44 or C-75), or 6×10^5 live BCG. At day 7, a new set of mice were also injected i.v. with *M. leprae*. They were all challenged in the foot pad

TABLE 1. *Tolerance according to routes of injection.*[a]

Groups	Routes	Test antigens	2-Day Corr. FPE ($\times 10^{-2}$ mm) median values	% Tolerance	p[b]
A	i.d.	Lepromin	24	—	—
		C-44 ICRCin	26	—	—
		C-75 ICRCin	36	—	—
B	i.v.	Lepromin	6	75	0.00009
		C-44 ICRCin	15	42	0.023
		C-75 ICRCin	17	53	0.0016
C	i.p.	Lepromin	9	63	0.0003
		C-44 ICRCin	19	27	0.003
		C-75 ICRCin	19	47	0.005
D	s.c.	Lepromin	15	38	0.0012
		C-44 ICRCin	16	38	0.0012
		C-75 ICRCin	14	61	0.002

[a] Mice were injected with Irr. ICRC C-44 bacilli (2×10^7 bacilli) by i.d., i.v., i.p., and s.c. routes at day 0, and challenged at day 28 with lepromin, C-44 ICRCin and C-75 ICRCin (3×10^7 bacilli) in the foot pads.
[b] Probability values for each group against i.d. injected group; values are for a two-tailed test.

with lepromin and the two ICRC antigens 28 days after i.d. immunization, and the foot-pad thickness was measured as before.

In all of these experiments, the nonsensitized mice on challenge showed more or less similar foot-pad thickness to all three test antigens and, hence, we represent only the lepromin values in all the graphs.

The differences between the groups were assessed by the two-tailed Mann-Whitney U test (with exact allowance for tied values) using a computer program derived from Hill and Peto ([16]).

RESULTS

Effect of the route of administration on the degree of sensitization. The magnitude of DTH in mice sensitized with the ICRC bacilli by different routes of administration is shown in Figure 2. The foot-pad responses against the test antigens, lepromin and ICRCins (C-44/C-75), showed that there was significant sensitization against all of the antigens in i.d. injected mice. The median FPE against lepromin was 24 ($\times 10^{-2}$ mm); that against C-44 ICRCin was 26; and for the C-75 strain, it was 36. On the other hand, there was significant reduction in the foot-pad swelling in the remaining three groups of mice injected i.v., i.p., or s.c., indicating suppression of DTH responses. In the i.v. injected mice, the median FPE for the three test antigens ranged from 6–17; in the i.p. injected mice it was 9–19; and in s.c. injected mice it ranged from 14–16. The percent tolerance for the three test antigens, as calculated by Shepard's formula ([33]), ranged from 42%–75%, 27%–63%, and 38%–61% in i.v., i.p., and s.c. routes, respectively (Table 1).

Adoptive transfer of tolerance: mice injected with C-75 Irr. This series consisted of four sets of experiments, and the results are shown in Figure 3. It was observed that transfer of normal spleen cells to C-75 (Irr.) i.d. sensitized mice had no effect on FPE to lepromin, ICRCin C-44, or ICRCin C-75; the median FPE being 33, 43, and 35×10^{-2} mm, respectively. On transfer of spleen cells from C-75 i.v. injected mice to C-75 i.d. sensitized mice, the FPE on challenge was significantly decreased to 5, 6, and 4×10^{-2} mm to lepromin, C-44, and C-75 antigens (suppression effect was 88%, 88%, and 91%), respectively.

In the third group, spleen cells from *M. leprae* (i.v.) tolerized mice were transferred to C-75 i.d. sensitized mice and here again, there was reduction in the foot-pad swelling. The median values for lepromin, C-44, and C-75 antigens were 19, 16, and 18×10^{-2} mm with percent suppressions of 42%, 60%, and 49%, respectively. On transfer of spleen cells from nonsensitized donors to nonsensitized recipients, the FPE produced against all three antigens was less than 10×10^{-2} mm as median values.

The experiment has demonstrated that spleen cells from i.v. tolerized mice, on transfer to sensitized recipients, have effec-

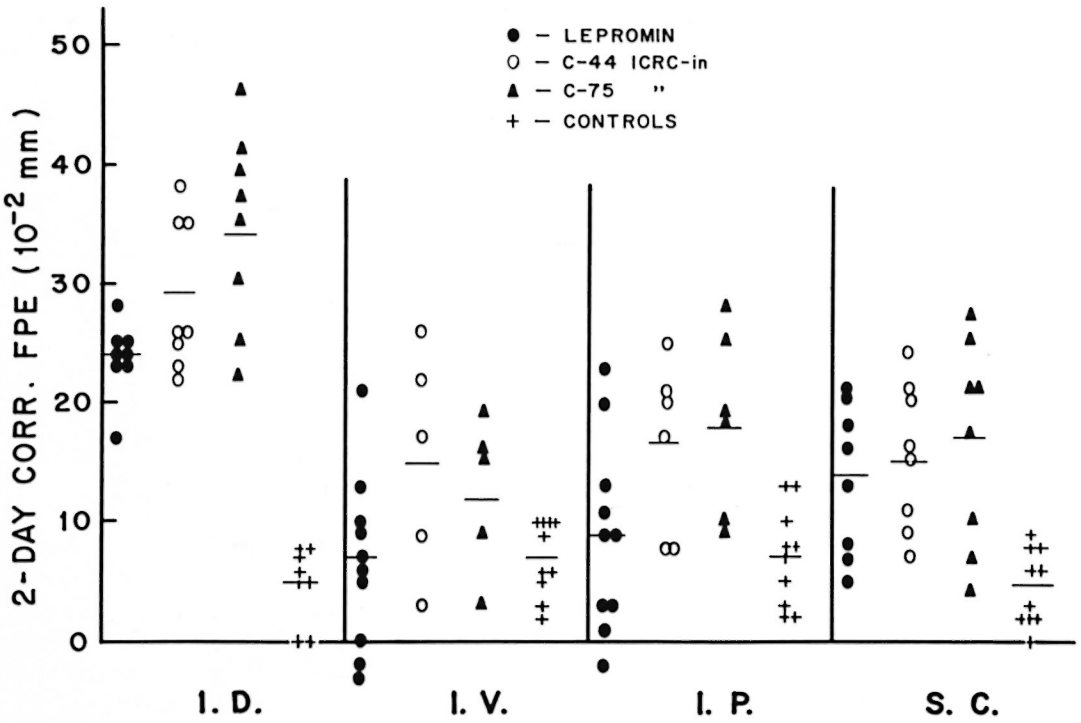

FIG. 2. FPE in mice: comparison of routes of injection of C-44 Irr. bacilli (2×10^7). Four sets of mice were injected each with 2×10^7 C-44 bacilli (Irr.) by the i.d., i.v., i.p., and s.c. routes at day 0. At day 28, they were challenged separately by lepromin and the two ICRC antigens (C-44 and C-75) in the foot pads (3×10^7/foot pad). The 48 hr corrected FPE against the three antigens is shown. Controls are mice injected with saline and tested for sensitivity against the three test antigens. Since the responses in saline-injected mice to the three antigens were more or less the same, only foot pad responses to lepromin are represented in the graph. The line in each scatterogram indicates the mean FPE value.

tively suppressed the DTH response. Thus, the suppressor effect brought about by i.v. injection of both *M. leprae* and ICRC strains could be adoptively transferred in the mouse system.

Induction of tolerance by *M. leprae* and its recovery by ICRC strains (C-44/C-75), BCG, and *M. leprae*. This experiment was done in order to find out whether the *M. leprae*-induced tolerance could be broken by i.d. sensitization with ICRC bacilli, BCG, or *M. leprae* itself.

Figure 4 shows the results of this experiment. In the first group, mice received only *M. leprae* (Hk) i.v. at day 7 and the median values of FPE at 48 hr remained low (lepromin = 3, C-44 ICRCin = 6, and C-75 ICRCin = 3×10^{-2} mm), indicating suppression of DTH response following i.v. injection of *M. leprae*. The three groups of mice made tolerant at day 0 by i.v. injection of *M. leprae* and sensitized i.d. at day 7 with either of the two ICRC strains or with BCG showed increases in FPE upon challenge with the test antigens. Thus, in the C-44-sensitized mice the FPE to lepromin had increased from 3 to 14. In the C-75-sensitized mice, it increased from 3 to 29, and in the BCG-sensitized mice, it increased from 3 to 28, indicating that the two ICRC strains and BCG were equally effective in partially breaking *M. leprae*-induced tolerance. In the last group, it was demonstrated that the i.d. sensitization with *M. leprae* had no effect on *M. leprae*-induced tolerance, and the FPE values to the three test antigens were below 10. The probability (p) values for the differences between the *M. leprae*-sensitized group and the C-44/C-75- and BCG-sensitized groups are presented in Table 2. The recoveries from tolerance after C-44 immunization to lepromin, C-44

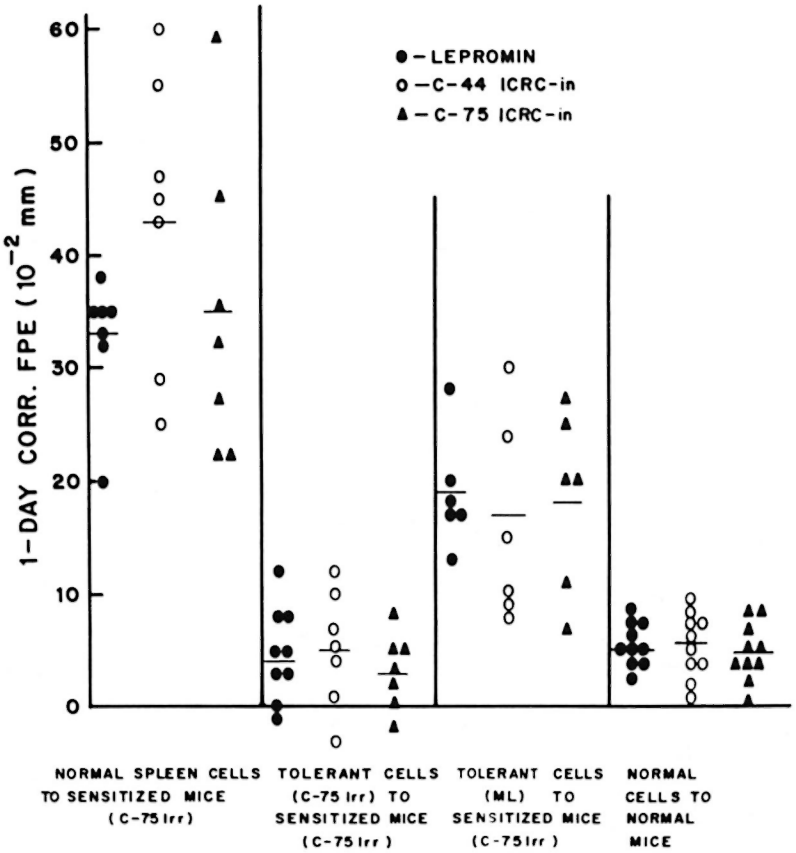

FIG. 3. FPE after adoptive transfer of tolerance to sensitized mice. About 1×10^8 spleen cells in saline, obtained from nonsensitized and tolerant mice, were injected through the tail vein in a volume of 0.1 ml into sensitized as well as nonsensitized mice. The recipients were challenged separately with lepromin, C-44 ICRCin, and C-75 ICRCin (3×10^7) within 2 hr of cell transfer. Maximum FPE was obtained at 24 hr. Controls are mice injected with saline.

ICRCin, and C-75 ICRCin were 58%, 81%, and 56%, respectively. After C-75 immunization, it was 66%, 69%, and 78%, and after BCG immunization it was 78%, 76%, and 86%, respectively. In the tolerant mice sensitized with *M. leprae,* the recovery of tolerance to the three test antigens was only 13%, 17%, and 13%, respectively. These results indicate that, like BCG, the cultivable leprosy-derived mycobacteria (C-44 and C-75 ICRC strains) were equally effective in breaking *M. leprae*-induced tolerance. Thus, efficacy in recovery from *M. leprae*-induced tolerance was of the order: BCG > C-75 > C-44 strains of ICRC bacilli.

DISCUSSION

The route of injection of an antigen markedly affects the type and magnitude of the immune response, as observed by many workers ([8, 10, 22, 23]). The intradermal administration of *M. leprae* antigens induced delayed-type hypersensitivity in mice, while the intravenous route led to immunological tolerance or unresponsiveness as determined by the foot pad enlargement method ([33]). ICRC bacilli show the same characteristics as *M. leprae* in inducing DTH when administered by the i.d. route, and in inducing tolerance when given i.v., i.p., or s.c. Similarly, ICRC bacilli, like *M. leprae* and unlike many other cultivable mycobacteria, do not lose their immunogenicity in mice even after heat-killing at 100°C ([2, 18]).

The superiority of the i.d. route for sensitizing mice against bacterial antigens was reported long ago ([17]). Whether the antigen-presenting cells, such as Langerhans' cells

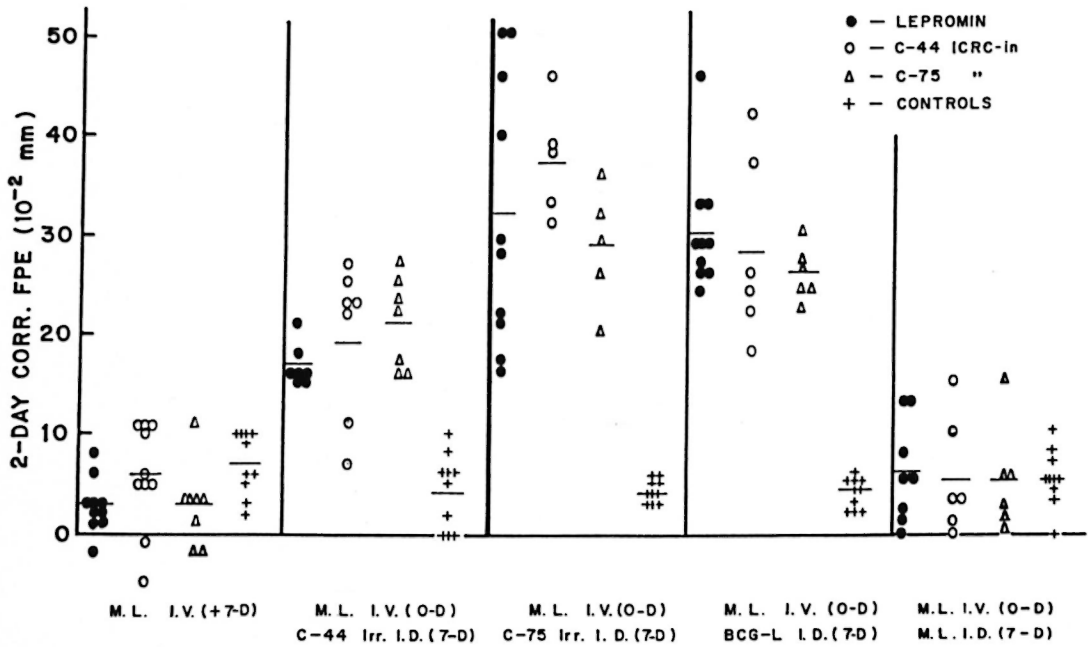

FIG. 4. FPE after *M. leprae* tolerance and the challenge of tolerance with various antigens. Initially four sets of mice were selected, and all received Hk *M. leprae* (2×10^7 bacilli) i.v. at day 0. At day 7, all mice were sensitized i.d. by any of the four antigens, i.e., irradiated C-44 bacilli, Irr. C-75 bacilli, live BCG, or Hk *M. leprae*. A new set of mice received only *M. leprae* i.v. at day 7 as shown in the first column. All mice were injected into the foot pads with lepromin and the two ICRC antigens 28 days after sensitization. The 48 hr foot pad responses are shown. In controls (saline injected initially i.v. and then i.d.), only response to lepromin is represented.

(LC), have any role in these immune responses is not clearly evident. However, a reduction in the number of LC in lepromatous patients has been reported ([21, 24]), and the observation is believed to be related to *M. leprae*-specific immunosuppression.

The adoptive transfer experiments revealed that the tolerance induced by i.v. injection of Hk *M. leprae* or irradiated ICRC bacilli could be adoptively transferred to sensitized as well as nonsensitized mice through splenocytes. In other words, i.d. sensitization could be completely abrogated by tolerized spleen cells i.v. There is no report in the literature on the transfer of *M. leprae*-induced tolerance to sensitized or nonsensitized mice. Our findings are the first attempt in this direction. We have demonstrated that, like *M. leprae*, ICRC bacilli also induced tolerance in mice by the i.v. route, and that the suppression induced by these two antigens could be adoptively transferred to sensitized and nonsensitized mice. The transfer of suppression induced by *M. lepraemurium* (*Mlm*) was recently reported ([1]). It was observed that spleen cells from BALB/c mice, injected s.c. with *Mlm*, when transferred, resulted in significantly reduced resistance to *Mlm*; recipients in these experiments, however, were irradiated. In contrast, the recipients in our series were neither treated with cyclophosphamide nor exposed to sub-lethal irradiation.

Tolerance can often be broken by immunizing the tolerant animals with antigens which crossreact with the tolerogen. BCG has been used to break *M. leprae* tolerance in mice ([33]). It has also been reported that live BCG was the most effective among cultivable mycobacteria in sensitizing mice to foot pad challenge with *M. leprae* ([31]). We have earlier demonstrated that ICRC bacilli, even in killed form, could sensitize mice against *M. leprae* ([2,18]). We have examined this quality of sharing of "antigen behavior" of *M. leprae* and ICRC strains in mice, also by an attempt to break *M. leprae*-induced tolerance. Like BCG, both of the ICRC

TABLE 2. *Challenge of* M. leprae *tolerance with i.d. sensitization with various antigens.*

Groups[a]	Immunizing antigen i.d. + 7 days[b]	Eliciting antigens[c]	2-Day Corr. FPE ($\times 10^{-2}$ mm) median values	% Recovery	p[d]
A	C-44 Irr.	Lepromin	14	58	0.0003
		C-44 ICRCin	21	81	0.008
		C-75 ICRCin	20	56	0.001
B	C-75 Irr.	Lepromin	29	66	0.00005
		C-44 ICRCin	38	69	0.004
		C-75 ICRCin	29	78	0.004
C	BCG live	Lepromin	28	78	0.00005
		C-44 ICRCin	25	76	0.002
		C-75 ICRCin	25	86	0.002
D	*M. leprae* (Hk)	Lepromin	5	17	—
		C-44 ICRCin	3	13	—
		C-75 ICRCin	4	13	—

[a] The four groups of mice were injected with Hk *M. leprae* (2×10^7 bacilli) by the i.v. route at day 0.
[b] At day 7, mice were sensitized by the i.d. route with Irr. C-44 ICRC bacilli, C-75 ICRC bacilli, live BCG, or Hk *M. leprae.*
[c] Sensitivity was tested by foot pad injection of lepromin, C-44 ICRCin or C-75 ICRCin 28 days after sensitization.
[d] Probability values are for a two-tailed test.

strains in killed form are effective in breaking *M. leprae* tolerance. As observed by Shepard, *et al.* ([33]), i.d. immunization with *M. leprae* could not terminate (abolish) the tolerance induced by the same antigen i.v.

Suppressor cells are often implicated in T-cell tolerance in many infections ([7, 14]), but there is no evidence to show an active role of suppressor T cells in the immune tolerance in leprosy. However, an increase in the suppressor T-lymphocyte subpopulation has been reported by many workers ([3, 15]). An adherent suppressor activity in lepromatous leprosy patients, presumably due to monocytes, has also been reported ([28, 29]). In experimental mycobacteroses, i.v. injections have been made of antigen-produced suppressor cells consisting of both T cells and macrophages ([9, 10, 34]) and, in the case of *M. lepraemurium,* it was observed that the route of infection produced T cells of different phenotypes mediating two types of cellular immune responses ([22, 23]).

On the basis of the present observations, it may be pointed out that the i.v. injection of *M. leprae* and ICRC bacilli generated suppressor cells, and this could be confirmed by the suppression of DTH which these cells brought about in the recipients after adoptive transfers. Whether these suppressor cells are T cells or a mixture of T cells and macrophages is yet to be worked out. The presence of specifically sensitized cells in the spleen after i.d. injection of ICRC bacilli was already confirmed by adoptive transfer of immunity to nonsensitized mice ([2]).

SUMMARY

ICRC bacilli, the cultivable leprosy-derived mycobacteria, isolated from lepromatous nodules of leprosy patients were found to be immunogenic in BALB/c mice at a dose of 2×10^7 acid-fast bacilli when injected by the intradermal (i.d.) route. The sensitization to lepromin and ICRC antigens was measured by the foot pad enlargement (FPE) method. The same dose of bacilli when injected by intravenous (i.v.), intraperitoneal, and subcutaneous routes induced immune tolerance in mice as indicated by reduction in the FPE to the test antigens. The spleen cells obtained after i.v. injection of ICRC bacilli/*Mycobacterium leprae* after adoptive transfer brought about suppression of delayed-type hypersensitivity in sensitized as well as nonsensitized recipients, indicating production of suppressor cells after i.v. injection. Similarly, the tolerance induced by i.v. injection of *M. leprae* in mice could be partially converted to immunity by i.d. sensitization with live BCG and two strains of ICRC bacilli (C-44 and C-75).

RESUMEN

Se encontró que los bacilos ICRC aislados de los nódulos lepromatosos de pacientes con lepra, son inmunogénicos en ratones BALB/c a dosis de 2×10^7 bacilos por la via intradérmica (i.d.). La sensibilización a la lepromina y al antígeno ICRC se midió por el método del engrosamiento de la almohadilla plantar. La misma dosis de bacilos inoculada por la via intravenosa (i.v.), intraperitoneal, o subcutánea, indujo una tolerancia inmunológica en los ratones que pudo ser medida por la reducción en el engrosamiento plantar al inyectar los antígenos de prueba. Las células de bazo obtenidas de ratones inoculados con bacilos ICRC o con *Mycobacterium leprae* indujeron, por transferencia adoptiva, supresión de la hipersensibilidad tardía tanto en los recipientes sensibilizados como en los no sensibilizados, indicando que la inyección i.v. de estos bacilos indujo la producción de células supresoras. La tolerancia inducida en los ratones por la inyección i.v. de *M. leprae* pudo ser parcialmente convertida a inmunidad, por la administración i.d. de BCG vivo y de 2 cepas de los bacilos ICRC (C-44 y C-75).

RÉSUMÉ

On a observé que les bacilles ICRC, qui sont des mycobactéries dérivées de la lèpre mais cultivables, isolées à partir de nodules lépromateux de malades, étaient immunogéniques chez les souris BALB/c à la dose de 2×10^7 bacilles acido-résistants injectés par voie intradermique. L'induction d'une sensibilité à la lépromine et aux antigènes ICRC a été mesurée par la méthode d'empâtement du coussinet plantaire de la souris (FPE). La même dose de bacilles, lorsqu'elle était injectée par voies intraveineuse, intrapéritonéale, ou sous-cutanée, entraînait une tolérance immunitaire de la souris, ainsi qu'on a pu le mettre en évidence par la réduction de l'empâtement du coussinet plantaire. Les cellules spléniques obtenues peu après injection intraveineuse de bacilles ICRC/*Mycobacterium leprae* après transfert, a entraîné la quasi suppression de l'hypersensibilité de type retardé, et ceci aussi bien chez les animaux sensibilisés que chez ceux qui ne l'avaient pas été. Cette observation indique qu'il y a production de cellules suppressives après injection intraveineuse. De même, la tolérance induite par l'injection intraveineuse de *M. leprae* chez la souris peut être transformée partiellement en immunité par une sensibilisation intradermique avec du BCG vivant, de même qu'avec deux souches de bacilles ICRC (C-44 et C-75).

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REFERENCES

1. Alexander, J. Adoptive transfer of immunity and suppression by cells and serum in early *Mycobacterium lepraemurium* infections in mice. Parasite Immunol. **1** (1979) 159–166.
2. Amminikutty, J. and Bapat, C. V. Induction of delayed-type hypersensitivity by ICRC anti-leprosy vaccine and the adoptive transfer of cell-mediated immunity in mice. Indian J. Lepr. **56** (1984) 754–763.
3. Bach, M.-A., Chatenoud, L., Wallach, D., Phan Din Tuy, F. and Cottenot, F. Studies on T cell subsets and functions in leprosy. Clin. Exp. Immunol. **44** (1981) 491–500.
4. Bapat, C. V. Immunological properties of *M. leprae* culture isolates ICRC bacilli: hypothesis on relationship between *M. leprae* and ML-culture isolates. Acta Leprol. (Geneve) **2** (1984) 175–194.
5. Bapat, C. V., Modak, M. S., DeSouza, N. G. A. and Chulawalla, R. G. Comparative study of skin reactions in leprosy patients to *M. leprae* lepromin and to ICRCin, an antigen from cultivable acid-fast bacilli from *M. leprae* isolated from lepromatous nodules. Lepr. India **49** (1971) 472–484.
6. Bapat, C. V., Ranadive, K. J. and Khanolkar, V. R. *In vitro* cultivation of an acid-fast mycobacterium isolated from human lepromatous leprosy. Int. J. Pathol. Bacteriol. **1** (1958) 156–159.
7. Basten, A., Miller, J. F. A. P., Sprent, J. and Cheers, C. Cell-to-cell interaction in the immune response. A T-cell dependent suppression in tolerant mice. J. Exp. Med. **140** (1974) 199–217.
8. Bjune, G., Closs, O. and Barneston, R. St. C. Early events in the host parasite relationship and immune response in clinical leprosy: its possible importance for leprosy control. Clin. Exp. Immunol. **54** (1983) 289–297.
9. Bullock, W. E., Carlson, E. M. and Gershon, R. K. The evolution of immunosuppressive cell populations in experimental mycobacterial infection. J. Immunol. **120** (1978) 709–716.
10. Colizzi, V., Ferluga, J., Garreau, F., Malkovsky, M. and Asherson, G. L. Suppressor cells induced by BCG release non-specific factors *in vitro* which inhibit DNA synthesis and interleukin-2 production. Immunology **51** (1984) 65–71.
11. Collins, F. M. and Mackaness, G. B. The relationship of delayed hypersensitivity to acquired anti-tuberculosis immunity. I. Tuberculin sensitivity and resistance to infection in BCG vaccinated mice. Cell Immunol. **1** (1970) 253–265.
12. Deo, M. G., Bapat, C. V., Bhalerao, V., Chaturvedi, R. M., Bhatki, W. S. and Chulawalla, R. G. Anti-leprosy potentials of ICRC vaccine. A study in patients and healthy volunteers. Int. J. Lepr. **51** (1983) 540–549.
13. Deo, M. G., Bapat, C. V., Bhatki, W. S. and Chulawalla, R. G. Potential anti-leprosy vaccine

from killed ICRC bacilli: a clinico-pathological study. Indian J. Med. Res. **74** (1981) 164–177.
14. Gershon, R. K. and Kondo, K. Immunological tolerance. Immunology **21** (1971) 903–914.
15. Gupta, S. K., Bhutani, L. K. and Nath, I. The *in situ* characterization of mononuclear cell infiltrates in dermal lesions of leprosy. Int. J. Lepr. **50** (1982) 297–305.
16. Hill, I. D. and Peto, R. Probabilities derived from finite populations. Appl. Stat. **20** (1971) 99–105.
17. Jullanelle, L. A. Reactions of rabbits to intracutaneous injection on pneumococci and their products. VI. Hypersensitivity to pneumococci and their products. J. Exp. Med. **51** (1930) 643–657.
18. Kale, V. P. and Bapat, C. V. Antigenic cross-reactivity between ICRC bacilli and *M. leprae*—"*in vitro*" evaluation. Indian J. Lepr. **56** (1984) 219–231.
19. Kale, V. P., Bhat, A. V. and Bapat, C. V. Comparison of biochemical characterization of ICRC bacilli with *M. leprae*: effect of substrate alteration in the medium. Indian J. Lepr. **56** (1984) 212–218.
20. Lefford, M. J. Transfer of adoptive immunity to tuberculosis in mice. Infect. Immun. **11** (1975) 1174–1181.
21. Liu, J., Shi, Y., Kong, Q. and Ye, G. Y. Preliminary observation on Langerhans' cells in leprosy. Int. J. Lepr. **50** (1982) 316–318.
22. Mathew, R. C., Curtis, J. and Turk, J. L. T-cell proliferation in *Mycobacterium lepraemurium* infection. I. Lack of correlation between antigen-specific proliferation of Lyt $1+$ 23^- cells and resistance in lethal infections. Immunology **51** (1984) 185–192.
23. Mathew, R. C., Curtis, J. and Turk, J. L. T-cell proliferation in *Mycobacterium lepraemurium* infection. II. Characterization of cells that transfer resistance in subcutaneous infected mice. Immunology **51** (1984) 703–710.
24. Mathur, N. K., Mangal, H. N., Mathur, D., Bedwal, R. S. and Mathur, R. S. Langerhans' cell and leprosy. Lepr. India **55** (1983) 22–28.
25. Patel, P. J. and Lefford, M. J. Specific and non-specific resistance in mice immunized with irradiated *Mycobacterium leprae*. Infect. Immun. **20** (1978) 692–697.
26. Rook, G. A. W. Immune responses to mycobacteria in mice and man. Proc. R. Soc. Med. **69** (1976) 442–444.
27. Rook, G. A. W. Suppressor cells of mouse and man. What is the evidence that they contribute to the aetiology of the mycobacterioses? Lepr. Rev. **53** (1982) 306–312.
28. Salgame, P. R., Mahadevan, P. R. and Antia, N. H. Mechanism of immunosuppression in leprosy: presence of suppressor factor(s) from macrophages of lepromatous patients. Infect. Immun. **40** (1983) 1119–1126.
29. Sathish, M., Bhutani, L. K., Sharma, A. K. and Nath, I. Monocyte-derived soluble suppressor factor(s) in patients with lepromatous leprosy. Infect. Immun. **42** (1983) 890–899.
30. Shepard, C. C., Minagawa, F., Van Landingham, R. M. and Walker, L. L. Footpad enlargement as a measure of induced immunity to *Mycobacterium leprae*. Int. J. Lepr. **48** (1980) 371–381.
31. Shepard, C. C., Van Landingham, R. M. and Walker, L. L. Searches among mycobacterial cultures for anti-leprosy vaccines. Infect. Immun. **29** (1980) 1034–1039.
32. Shepard, C. C., Walker, L. L. and Van Landingham, R. M. Heat stability of *Mycobacterium leprae* immunogenicity. Infect. Immun. **22** (1978) 87–93.
33. Shepard, C. C., Walker, L. L., van Landingham, R. M. and Ye, S.-Z. Sensitization or tolerance to *Mycobacterium leprae* antigen by route of injection. Infect. Immun. **38** (1982) 673–680.
34. Turcotte, R. Evidence for two distinct populations of suppressor cells in the spleens of *Mycobacterium bovix* BCG sensitized mice. Infect. Immun. **34** (1981) 315–322.