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Sensitization or Tolerance to *Mycobacterium Leprae* Antigen by Route of Injection

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Aqueous suspensions of heat-killed *Mycobacterium leprae* in a dose of 10^7 organisms were highly immunogenic when injected intradermally (i.d.). The same dose of bacteria did not sensitize when given intraperitoneally (i.p.) or intravenously (i.v.), and did so only minimally at best when given subcutaneously. The i.d. route was the most immunogenic for sheep erythrocytes also. *M. leprae* injected i.p. or i.v. stimulated immune tolerance to *M. leprae* challenge i.d. In older mice (≥8 weeks), the i.v. injections gave more complete tolerance. Mice that had been rendered tolerant by i.v. injections maintained their tolerance for at least 168 days. Prior UV irradiation of intact mice prevented sensitization by the i.d. route. In normal mice, living *M. bovis* BCG given i.d. produced good sensitization to *M. leprae*. Mice that had been made tolerant by i.v. injection of *M. leprae* could be partially sensitized to *M. leprae* by i.d. immunization with BCG; mixtures of living BCG and heat-killed *M. leprae* were no more effective than BCG alone. These findings appear to have relevance to the pathogenesis of lepromatous leprosy and its immunoprophylaxis.

Immune tolerance for delayed-type hypersensitivity (DTH) against antigenic determinants of the etiological agent may be present temporarily in a number of infectious diseases, but it is a constant and important feature of lepromatous leprosy. In this more severe form of leprosy, the dermal involvement is general rather than local, and bacteremia is present. An immunological nonreactivity to *Mycobacterium leprae* can be regularly demonstrated by (negative) Mitsuda skin tests. This reaction is read 28 days after the injection of a suspension of intact, autoclaved *M. leprae*, standardized for bacterial content. The reaction is positive in tuberculoid patients and in nearly all normal adults in endemic and nonendemic regions. Lepromatous leprosy, when it is not under effective therapeutic control, is the chief or sole source of new infections. Thus, this ability of *M. leprae* to escape the immune response seems essential for its survival as a species.

The mechanism leading to the immune responsiveness is far from clear, however. A genetic deficiency in the response, based on a specific deficiency in T lymphocytes or antigen-presenting cells, is widely accepted, but the evidence is not compelling, and other factors could be involved, e.g., the route of infection (25). No experimental methods for the production of immune unresponsiveness to *M. leprae* have been reported.

In recent years, in the course of study of antileprosy vaccines in mice, we have found the intradermal (i.d.) route to be more immunogenic than the subcutaneous (s.c.) or footpad routes for the administration of *M. leprae* (26, 28), as judged by protection against infection and by sensitization measured by footpad enlargement (FPE) after challenge with *M. leprae* suspension. Aqueous suspensions of intact *M. leprae* are highly immunogenic, somewhat more so after heating to 100 to 121°C (31).

We have now made a systematic comparison of routes of injection and confirm that the i.d. route is highly effective for sensitizing mice under conditions when the other routes are ineffective. The intravenous (i.v.) route was one of the ineffective routes for sensitizing mice, but it was the best, under most conditions, for inducing immune tolerance to *M. leprae*. Long-lasting immune tolerance could be produced by i.v. injections of aqueous suspensions of intact *M. leprae* cells.

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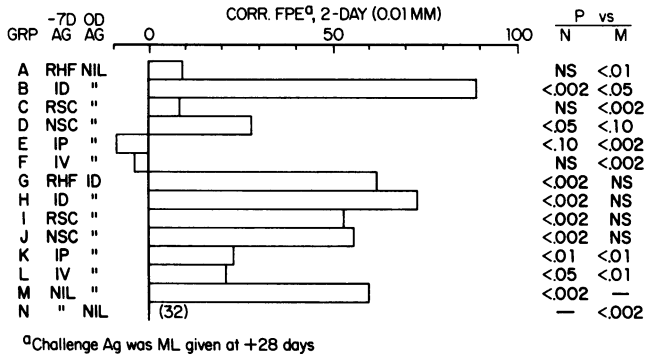


FIG. 1. Tolerance according to route of injection. Mice were injected with *M. leprae* cells by various routes at −7 days, challenged at 0 days by i.d. injection, and tested for sensitivity at +28 days by footpad injection. Corr. FPE, Corrected foot-pad enlargement. The "0D AG" column gives the routes of injection. RSC designates the right flank; NSC designates the nuchal area. Groups A to L had 6 mice each; M and N had 12. In parentheses, the uncorrected FPE is given for the negative control (M). The probability values (*P*) for each group versus groups M and N are shown in the last two columns.

MATERIALS AND METHODS

Descriptions of the techniques have appeared; they are induction and measurement of FPE (28), i.d. injection (29), and measurement of regional (inguinal) lymph node enlargement (29). The mice, all females aged 8 to 12 weeks unless otherwise stated, were CFW (a now outbred line maintained at the Centers for Disease Control, Atlanta, Ga.) or CBA/J or C57BL/6J from the Jackson Laboratory, Bar Harbor, Maine. Unless otherwise stated, the *M. leprae* suspensions were purified by the Percoll-gradient method of Draper (Protocol 1/79, *in* Report of the Enlarged Steering Committee Meeting, 7–8 February 1979, WHO Document TDR.IMMLEP-SWG (S) 80.3) from experimentally infected armadillo livers that had been gamma irradiated with 2.5 Mrad. The dose of *M. leprae* injected was 10^7 acid-fast bacteria (AFB), and the volume for injections was 0.01 ml for the i.d., 0.03 ml for footpad and s.c., and 0.20 ml for the intraperitoneal (i.p.) and i.v. (tail vein) routes. The i.d. and s.c. injections were given in the flank near, but not over, the inguinal lymph node. The i.d. and footpad injections were given with 30-gauge needles. There was very little leakage (after the footpad injection, the needle was rotated a half turn during withdrawal). Unless otherwise stated, the s.c. injections were given in the right flank at the same site as the i.d. injections, so that the lymph drainage would be to the same regional lymph node. The final diluent was Hanks balanced salt solution with 0.1% Tween 80. The *M. leprae* antigens used for inducing tolerance were unheated, whereas those for immunization and footpad challenge were heated for 30 min to 100C in their final dilutions, unless otherwise specified. With some suspensions, there is some agglutination on heating, and the agglutinates might be removed in the pulmonary capillaries. Experiments have shown that heated suspensions can be effective in inducing tolerance, however. To measure FPE, the thickness of the footpad was measured with dial calipers just before injection and 24, 48, and 72 h later. FPE was maximal at 48 h. Corrected (corr.) FPE signifies that the average FPE in nonimmunized controls has been subtracted. In the tables and figures, the value shown is the average for that group of mice. At 28 days, the induration at the i.d. site of injection and the size of the regional lymph nodes were usually measured.

Differences between groups were analyzed statistically by the two-sample rank test. The *P* values are for the two-tailed test, unless otherwise specified. The formula used to calculate tolerance was [(corr. FPE in positive control group) − (corr. FPE in suppressed group)]/(corr. FPE in positive control group).

UV irradiation was carried out with a bank of six FS40 lamps at a distance of 20 cm from the back of the mouse. The output of the FS40 lamp is almost entirely UV-B. At the target distance, irradiation was measured to be 0.52 mJ/cm^2 per s with a IL443 radiometer (International Light, Inc., Newburyport, Mass.) with a UVB-passing filter on the sensor. The mice were closely shaven on the back and sides, treated for 30 min each day for 6 consecutive days, and immunized i.d. 2 days later.

RESULTS

Route of injection. A systematic comparison of the various routes had not been performed, so this was done in the first experiment. CBA and C57BL mice were injected with 10^7 *M. leprae* cells i.d., s.c., i.p., i.v., and by the footpad route. The resultant sensitization was tested 28 days later. The only significant sensitization in both lines of mice followed i.d. immunization (data not shown).

To test whether the lack of response in the first experiment could be attributed to tolerance, CBA mice were injected with heated antigens by various routes (Fig. 1) and, 7 days later, were immunized i.d. After another 28 days, sensitization was tested by footpad injections. Again the i.d. route was by far the most effective in stimulating sensitization (group B). Tolerance, as measured by reduction in FPE versus the

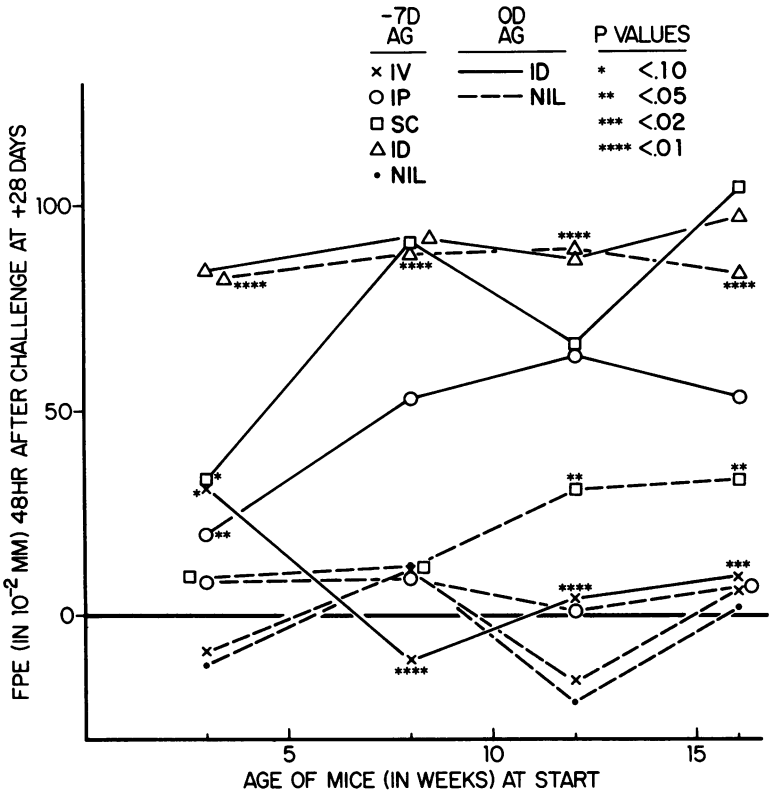


FIG. 2. Age of mice and immune response. Mice of the ages shown were injected with *M. leprae* cells by the various routes. After 7 days, the groups marked with solid lines were challenged intradermally; those marked with dashed lines were left unchallenged. After another 28 days, the resultant sensitization was measured by footpad injection. There were five mice per group. FPE, Footpad enlargement.

positive controls (group M), was seen only with mice injected i.p. and i.v. The other routes for the antigens given at −7 days did not cause detectable unresponsiveness, even when stimulating no sensitization.

We wondered whether the superiority of the i.d. route was a peculiarity of *M. leprae* as an antigen, perhaps in connection with its pathogenic predilection for skin. We were not able to find a comparison in mice in the literature that involved the i.d. route. La Grange et al. (11) had compared several routes other than the i.d. route with sheep erythrocytes, an unrelated and more widely used antigen. Accordingly, we immunized CBA mice with 10^7 sheep erythrocytes by various routes and tested the resulting sensitization by footpad injections of 10^8 sheep erythrocytes at intervals up to 12 days. FPE was read at 24 h. The i.d. route was more immunogenic than the footpad route. As expected (11), the i.v. and i.p. routes were less effective than the footpad route. The maximum with all routes of immunization came at 4 days.

Histological location of injected material. To help to understand why i.d. injections were so much more immunogenic, and to check that the locations of the injections were those intended, a suspension of carbon particles (India ink washed in Hanks balanced salt solution) was injected i.d., s.c., or into the footpad. The mice were killed in a few minutes, and the tissues were removed for histological sections, which were cut through the center of the blackened area. After i.d. injections, the carbon particles were sharply localized, principally in the dermis extending all the way from the basal layer of the epidermis to the panniculus carnosus. Close contact with the epidermis and the hair follicles and dermal glands appeared to provide for contact with Langerhans cells. The volume of the deposit (4 to 9 mm^3) and the diffuse infiltration of the dermal fibrillar network pointed to a process of filtration and concentration. After the s.c. injections, the carbon lay in the loose areolar tissue beneath the panniculus carnosus in diffuse and more widespread deposits. After the footpad injections, the carbon lay partly in the dermis and partly in the areolar tissue of the muscles, vessels, and nerves. Lymphatic drainage was seen as blackened popliteal nodes in

TABLE 1. Tolerance induced with *M. leprae* purified by different methods[a]

Group	Antigen[b]			Corr. FPE,[c] 2 days	Tolerance (%)	*P* vs	
	−14 days	0 days	+28 days			G	Control
A	39A	39A	AB40	17	71	<0.02	<0.01
B	39A	AB40	AB40	31	60	<0.01	<0.05
C	AB40	39A	AB40	13	80	NS[e]	<0.01
D	AB40	AB40	AB40	5	94	NS	<0.002
E	Nil	39A	AB40	59	—	<0.002	—
F	Nil	AB40	AB40	77	—	<0.002	—
G	Nil	Nil	AB40	(13)[d]		—	

[a] The suspensions were injected i.v. at −14 days, i.d. at 0 days, and into the left hind footpad at +28 days. CFW mice were used, 10 mice per group.
[b] The *M. leprae* was purified by the short trypsin method (39A) or the Percoll method (AB40).
[c] FPE, Foot pad enlargement
[d] Uncorrected average FPE.
[e] NS, Not significant.

about 90% of the mice inoculated in the footpad, but not at this interval in the regional lymph nodes after i.d. and s.c. inoculation.

Age of mice and route of injection. Under many conditions, young mice are made tolerant more easily than are older mice (3), so in the next experiment (Fig. 2), CFW mice of selected ages were injected by various routes and, after 7 days, challenged i.d. The results (Fig. 2) confirmed those obtained in CBA and C57BL mice. For sensitization (dashed lines), the i.d. route was more effective at all ages. The only other route giving sensitization of any significance was the s.c. route in the 12- and 16-week-old mice. For tolerance (solid lines), the i.v., i.p., and s.c. routes were partially effective in 3-week-old mice, but in 8-, 12-, and 16-week-old mice, only the i.v. and i.p. routes were effective. In these older mice, the i.v. route gave nearly complete tolerance (calculated as 91, 88, and 84%, respectively), and the i.p. route gave partial tolerance (38, 27, and 24%, respectively).

Preparation methods. Initially, only one lot of *M. leprae* antigen had been used in the experiments in which tolerance was demonstrated. To rule out a "preparation artifact," that is, a peculiarity of the particular lot or an effect of the preparation technique, *M. leprae* was prepared from different starting material by another method. The short trypsin method (27) was used. It is known to yield immunogenic *M. leprae* without detectable sensitization to armadillo proteins (28), and there is no possible contamination with Percoll. The preparation received 2.5 Mrad of gamma irradiation. The results (Table 1) showed that *M. leprae* produced by the short trypsin method was also tolerogenic. It was somewhat less potent as a tolerogen and an immunogen; whether this arose from the lesser degree of purity of the preparation, a strain difference in *M. leprae*, or the trypsin treatment could not be said.

Duration of immune tolerance. In some experimental models, tolerance lasts only a few weeks, a period that is probably related to the time required for cellular responses in the tolerant animals (3). To determine the kinetics of the *M. leprae* tolerance, the experiment shown in Table 2 was performed. Groups of mice were injected i.v., and other uninoculated mice were caged as controls. After different intervals, groups were challenged i.d., and 28 days later they were tested for sensitization by footpad injections. When the challenge (secondary, i.d.) injection was given on the same day as the i.v. injection, the amount of tolerance was 55%. From 14 to 168 days, it was in the range 79 to 107%. A second i.v. injection at 84 days gave no apparent increase in tolerance.

In a similar experiment carried out in CBA mice after tolerance was induced by i.p. injections of *M. leprae*, 5% tolerance was observed after challenge at 0 days, 19% at 7 days, and 36 to 44% at 14, 28, 56, 84, and 112 days.

Dosage and route of induction of tolerance. Rubin et al. (22) have reported that tolerance to type 1 reovirus can be induced by oral administration of UV-inactivated virus in high doses (10^9 particles) or in low doses (10^3 particles). At intermediate doses, minimal suppression was seen. We measured the tolerance-inducing effect of oral and i.v. *M. leprae* in doses ranging in 10-fold dilutions from 10^8 to 10^2 AFB, given 14 days before i.d. challenge with 10^7 AFB. The resultant sensitization was measured 28 days later. Consistent tolerance was seen only with the i.v. doses of 10^7 and 10^8; the oral route was ineffective.

Effect of UV treatment. Although there is some disagreement in the literature, several recent publications have stated that UV exposure of shaved mice decreased the density of Langerhans cells in the epidermis and depressed the ability to become sensitized with contact anti-

TABLE 2. Duration of tolerance after i.v. injections of *M. leprae*[a]

Day 1° Ag given i.v.	Day 2° Ag given i.d.	FPE[b] (0.01 mm)			Suppression	
		1° Ag + 2° Ag	2° Ag only	Neither	%	*P*
0	0[c]	50	86	20	55	>0.10
0	+14	27	124	32	105	<0.01
0	+28	38	120	16	79	<0.01
0	+56	35	131	15	83	<0.02
0	+84	28	93	10	78	<0.02
0, +84	+84	14			95	<0.01
0	+112	2	111	9	107	<0.002
0, +84	+112	6			103	<0.002
0	+168	18	126	4	89	<0.01
0, +84	+168	29			80	<0.01

[a] The primary (1°) antigen (Ag) was given i.v. at 0 and +84 days, as indicated. The secondary (2°) antigen was given i.d. at the time shown, and 28 days later the resultant sensitization was measured by footpad injection. CFW mice were used, five mice per group.

[b] FPE, Footpad enlargement

[c] A few minutes after the i.v. injection.

gens (14, 34). Topical application of corticosteroids was also reported to depress this ability locally (14).

We found that UV treatment in a daily dose of 940 mJ/cm^2 depressed the ability to become immunized with *M. leprae* for at least 14 days (Fig. 3). Treatment with betamethasone ointment had little effect in the dosage that had been found to block contact sensitization to dinitrofluorobenzene (14). In experiments in progress, the minimal locally effective dose of UV irradiation has been less than 5 mJ/cm^2.

Tolerance challenged by *Mycobacterium bovis* BCG. Live BCG is effective (much more so than other cultivable mycobacteria tried) in sensitizing mice to FPE challenge with *M. leprae* and in protecting them against infective challenge (30). Thus, an opportunity is provided to study the specificity of the *M. leprae*-induced tolerance, especially as it bears on the choice of a vaccine for prevention of leprosy (see Discussion). With other antigens, tolerance can often be broken by injections of related antigens with the appropriate degree of cross-reaction (3).

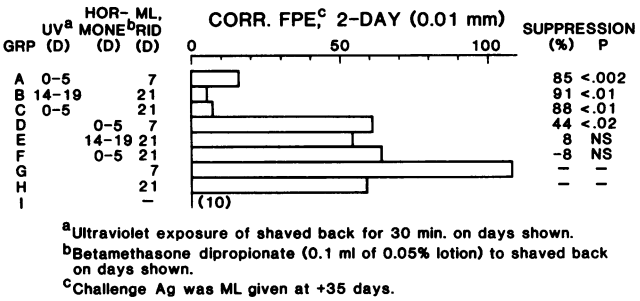
An experiment was carried out to determine whether BCG alone or in mixture with *M. leprae* could sensitize mice made tolerant to *M. leprae*. Fourteen days after i.v. injections of *M. leprae*, CFW mice were challenged with live BCG, heat-killed *M. leprae*, a mixture of the two, or the two antigens given separately in the left and right flanks to avoid possible adjuvant effect of one antigen on the other by drainage to a common regional lymph node. After a further 28 days, sensitization was tested by footpad injections of *M. leprae*.

FPE was distinct in all of the normal (nontolerant) mice that received i.d. vaccines (Fig. 4). In the tolerant mice receiving *M. leprae* vaccine alone, FPE was profoundly decreased, as expected. In the tolerant mice receiving BCG alone or BCG plus *M. leprae*, FPE was diminished only 50 to 70%. The probability (*P*) values for the differences between group B (*M. leprae* i.d.) and groups A, C, and D (which received BCG i.d.) were <0.002 in each case. In other words, the effect of BCG in tolerant mice was significant. The local reactions to vaccine and regional lymph node enlargements 28 days after i.d. vaccine were largely in concordance with the FPE, and *M. leprae* vaccination of tolerant mice did not cause significant local reaction or regional lymph node enlargement (data not shown).

In other parts of this experiment, similar groups of mice were challenged with living *M. leprae* cells to measure protection against infection. The results show that the i.v. injections largely nullified the protection afforded by the i.d. vaccines (manuscript in preparation).

DISCUSSION

The i.d. route and induction of DTH. That the i.d. route is superior for induction of DTH to such antigens as bacterial supsensions was reported long ago (10). This situation appears to have been neglected in recent years, however, and DTH is now generally induced in mice with s.c. injections. Our results show marked superiority of the i.d. route for saline suspensions of *M. leprae*. Saline suspensions of *M. leprae* given i.d. are more effective than preparations in incomplete Freund adjuvant given by other routes in mice (28) and in guinea pigs (15). Patel and Lefford reported that the footpad route was effective for immunizing mice with *M. leprae*, as indicated by several measures of cell-mediated immunity, including FPE to a supernatant antigen (20). They did not compare the i.d. route,



[a]Ultraviolet exposure of shaved back for 30 min. on days shown.
[b]Betamethasone dipropionate (0.1 ml of 0.05% lotion) to shaved back on days shown.
[c]Challenge Ag was ML given at +35 days.

FIG. 3. Effect of UV and corticosteriod treatment on i.d. sensitization to *M. leprae*. Mice were treated as shown on days 0 through 5 or 14 through 19 and injected i.d. 2 days later.

however, and the dose of vaccine they used was roughly 25 times as great as that we used here (28). We have observed protection against infection afforded by s.c. or footpad vaccination that was only moderately less than that provided by i.d. immunization (28). Close comparison of the results in FPE and protection tests requires, however, allowance for differences in timing and dose-response curves.

The superiority of the i.d. route for sensitization and of the i.v. route for inducing tolerance, and the sensitivity of the i.d. vaccination site to UV-B irradiation, are reminiscent of the situation in experimental contact sensitivity. In this phenomenon, the epidermal Langerhans cells appear to play an important role as efficient antigen-presenting cells (21, 24, 32, 33), in the course of which they traverse the dermis in lymphatic vessels to reach the regional lymph node. Other possible factors include the fibrillar structure of the dermis, which can act as a filter to concentrate and hold i.d.-injected particles in position.

UV irradiation. We found that UV treatment of intact mice profoundly depressed the ability to become sensitized with *M. leprae*. This evidence does not exclusively implicate Langerhans cells, however, because other superficial antigen-presenting cells, including dermal Ia-positive dendritic cells (9) could also be inactivated. Even the antigen-presenting function of splenic adherent cells disappears after heavy UV-B treatment of an intact mouse (12).

Our i.d. injections placed the *M. leprae* principally in the dermis, down to a depth of about 0.3 mm, or about the same as the depth of penetration of 95% of UV irradiation of wavelength 300 nm (19). A UV dose of 10 mJ/cm^2 for 4 days is sufficient to lower the number of stainable Langerhans cells locally in the skin of mice and to block locally the induction of contact sensitization on application of dinitrofluorobenzene (34). The human minimal erythemal dose in fair-skinned Caucasians is about 30 mJ/cm^2 (19). That exposure to UV plays a role in the immunology of human skin diseases is suggested by the report (18) that sun-damaged skin (manifested as solar elastosis in persons with chronic occupational exposure to sun) is difficult to sensitize with 2,4-dinitrochlorobenzene and is less reactive than normal skin in the same person to i.d. injected skin test antigen (*Candida albicans*, mumps, and purified protein derivative).

The i.v. route and DTH tolerance. Tolerance to DTH as a result of i.v. injection of an antigen that is given at the same time as or before the sensitizing injection has been described many times. In many of the reported examples, the i.v. antigen was injected in a less immunogenic form. For example, i.v. tuberculoprotein preceding i.d. viable BCG inhibited sensitization to the tuberculoprotein in guinea pigs (4). Similarly i.v.-injected proteins prevent sensitization to the same proteins injected s.c. in Freund complete adjuvant (1, 7). Such approaches have been used frequently since, and in general the i.v. antigen is given in rather large amounts (1 mg or more). In unpublished work, we have observed tolerance irregularly following s.c., i.p., and i.v. injection of disrupted *M. leprae* given before i.d. sensitization with intact *M. leprae*. (Disrupted *M. leprae* is not immunogenic, but it serves well as an eliciting antigen in a sensitized mouse [26]). Tolerance based on i.v. injection of the same form of antigen that is found sensitizing by another route (usually s.c.) has also been described, e.g., for haptenated syngeneic cells (reviewed in reference 8), for viral antigens (reviewed in reference 17), and for sheep erythrocytes (13). The results with the *M. leprae* system described here compare favorably with those of the other systems in the literature, in that (i) the tolerance-inducing *M. leprae* was injected in the normally sensitizing form, in a dose that was no greater than that needed to sensitize (about 3 μg), (ii) the resultant tolerance was nearly complete, and (iii) it lasted for at least 168 days. Furthermore, the sensitization that was prevented was of the long-lasting type

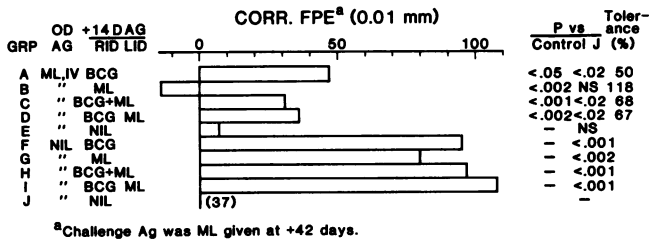


FIG. 4. Comparison of the i.d. immunizing activity of *Mycobacterium bovis* BCG and *M. leprae* in *M. leprae*-tolerant mice. At 0 days, some of the mice were injected i.v. with *M. leprae*. At 14 days, they and untreated controls were challenged i.d. in the right flank with BCG (groups A, D, H, and I), *M. leprae* (groups B and G), or a mixture of BCG and *M. leprae* (groups C and H). In addition, groups D and I received *M. leprae* in the left flank. After another 28 days, sensitization was tested by footpad injection of *M. leprae*.

rather than the evanescent form lasting only a few days. Whether the efficiency of this system arises from properties of *M. leprae* itself or merely from a fortunate choice of experimental conditions is not clear at this time.

Two examples of suppression of T-cell sensitization for mycobacteria have been described recently. Nakamura and Tokunaga (16) injected 10^7 BCG cells i.v. into a weakly responding mouse strain (C3H/He) and transferred spleen cells to syngeneic recipients that were treated with cyclophosphamide and challenged by footpad immunizations with BCG. The cyclophosphamide pretreatment was necessary for good DTH in controls. DTH was tested by injection of purified protein derivative into the other rear footpad. Watson and Collins (35) injected i.v. into mice 10^8 cells of BCG or *M. simiae*. These bacilli persist for a long time in the spleen. Lymphocyte transformation in vitro (of spleen cell suspensions exposed to added mycobacterial antigen) was distinct in the early stages (14 days) after infection but decreased later (≥28 days after infection). Mixing experiments involving early and late spleen cell suspensions showed responder cells in the early spleens and suppressor cells in late spleens.

Tolerance to *M. leprae* antigens in humans. The 28-day local skin reactions to *M. leprae* vaccine in the mouse are analogous to Mitsuda reactions in humans (28). In both tests, intact heat-killed *M. leprae* is injected i.d., and the reactions are read at 28 days, that is, an immunogenic product that persists in the tissues is injected by an immunogenic route, and the reactions are read after a time that allows generation of DTH and the development of a hypersensitivity granuloma around the persisting antigen. A positive reaction indicates the presence of DTH to *M. leprae*, either preexisting or newly generated as a result of the vaccination. A negative reaction signifies the presence of immune tolerance to *M. leprae*. (Negativity in a single test could of course result from faulty injection or perhaps UV inactivation of antigen-presenting cells). The 28-day i.d. reactions in the mouse are not as distinct as those in humans, however, and it is more satisfactory to test the mouse for DTH at 28 days by the injection of antigen into the footpad.

Termination of tolerance by injection of BCG. Live BCG has been by far the most effective of cultivable mycobacteria in sensitizing mice to FPE challenge with *M. leprae* and in protecting them against infective challenge (30). In doses of 10^7 AFB, live BCG and heat-killed *M. leprae* give roughly equivalent results. Field trials of BCG vaccine against leprosy have given contradictory results (2, 5, 23) for unknown reasons, so the usefulness of BCG as an antileprosy vaccine is not clear.

In the field use of an antileprosy vaccine, a point of particular concern is possible unresponsiveness of persons who are already infected with *M. leprae*. The incidence in endemic areas of immune unresponsiveness to *M. leprae*, as indicated by negative Mitsuda reactions, is low, although the exact figure is disputed. Convit et al. (6) have studied skin test conversions in three groups of Mitsuda-negative persons: family contacts, leprosy patients of the indeterminate type, and lepromatous patients rendered free of AFB in their skin smears by prolonged chemotherapy. All three categories were reported to be converted to Mitsuda positivity by i.d. injection of a mixture of heat-killed *M. leprae* and live BCG, but not by the injection of either component alone. Therefore, these authors have suggested the mixture as a vaccine (6).

Experimental models of tolerance to *M. leprae* have not been available previously. Our results with tolerant mice are different from those just summarized in Mitsuda-negative patients. We found that the mice made tolerant with *M. leprae* responded to vaccination with BCG by development of distinct, but somewhat reduced, sensitivity to *M. leprae* antigen. The addition of *M. leprae* to the BCG did not change

the result significantly. As expected, vaccination of the tolerant mice with *M. leprae* alone produced no significant sensitization. The 28-day skin reactions at the vaccination site paralleled the FPE in the various groups. That is, most of those injected with *M. leprae* had negative skin reactions, so they were, in effect, Mitsuda negative.

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