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Lepromin Conversion in Repeatedly Lepromin Negative BL/LL Patients After Immunization with Autoclaved *Mycobacterium w*[1]

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A number of vaccines are under development against leprosy. The WHO-IMMLEP Task Force conceived the use of irradiated *Mycobacterium leprae* on grounds of the protection conferred by killed *M. leprae* against challenge with live *M. leprae* in the mouse foot pad ([27, 30]). Convit, *et al.* ([3, 4]) employ killed *M. leprae* with BCG for immunization of Mitsuda negative contacts and leprosy patients. They observed conversion of subjects of lepromin positivity, and histological and clinical improvement in a significant number of leprosy patients. A vaccine based on a cultivable mycobacterium, ICRC, has been suggested by Bapat, *et al.* (personal communication, 1980[2]) with reports of conversions and upgrading reactions in lepromatous leprosy patients.

We proposed two candidate vaccines, one consisting of acetoacetylated *M. leprae* ([34]) and the other of autoclaved *Mycobacterium w* ([33]). In both cases, the underlying rationale is to break tolerance to *M. leprae* antigens for cell-mediated immunity functions, given that one of the immunological defects in lepromatous leprosy is the long-term anergy of response to *M. leprae* antigens ([14]). As done successfully in other experimental systems, the immunological tolerance is proposed to be overcome by the use of either a hapten modified antigen (acetoacetylated *M. leprae*) or through a bacterium which is immunologically partially but not fully crossreactive with *M. leprae.*

Mycobacterium w was among the two atypical mycobacteria screened for its similarity to *M. leprae* from 16 mycobacterial species, on the basis of antigen-driven blast transformation and leukocyte migration inhibition with cells from tuberculoid leprosy patients ([21]). It induced crossreactive skin reactions with *M. leprae* in guinea pigs ([22]), gave strong delayed hypersensitivity (DTH) response in mice ([10]), and enlarged draining lymph nodes in mice ([23]). It produced Dharmendra and Mitsuda reactions similar to *M. leprae* lepromins in tuberculoid leprosy patients, while it differed from *M. leprae* in

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also evoking positive reactions in lepromatous leprosy patients ([13, 15, 17, 20, 24, 29]).

The objective of the present probing field study was to see whether immunization with *Mycobacterium w* can convert lepromin negative lepromatous leprosy patients to positivity status. To determine whether the lepromin conversion was stable or transient, skin tests with lepromin A were undertaken several months after the first test observations. It was further pertinent to enquire whether leukocytes from converted cases reacted with *M. leprae* antigens *in vitro* to give leuckocyte migration inhibition.

MATERIALS AND METHODS

Mycobacteria. *Mycobacterium w,* an isolate from a sputum sample of a tuberculosis patient, is a rapid grower. It is niacin negative, and semiquantitative catalase and heat-resistant catalase positive. It lacks the ability to hydrolyze Tween 80 up to ten days but degrades sodium salicylate. It reduces nitrates to nitrites. It grows on Löwenstein-Jensen, Middlebrook, and Stanton media. The organism does not grow on nutrient agar or McConkey agar. It does not form pigment either in the dark or in the light. Its properties were described by Saxena, *et al.* ([28]) and subsequently by Katoch ([18]). Many of these have also been counterchecked by Dr. Kristina Wickman at the National Bacteriological Laboratory, Stockholm, Sweden. On the basis of its growth metabolic characteristics, it is classifiable in Runyon's group IV. However, it differs from other strains previously included in this group.

Mycobacterium w was grown in Middlebrook's media (Difco Laboratories, Detroit, Michigan, U.S.A.) with albumin, dextrose, and casein (ADC) enrichment. The mycobacterial cell suspensions were washed with sterile normal saline, autoclaved at 15 pounds pressure for 15 min, and washed thrice with normal saline. Bacilli were suspended aseptically in nonpyrogenic physiological saline (0.9% w/v NaCl) at a concentration of 5×10^8 bacilli/ml. Each batch of vaccine was checked for sterility in thioglycolate, blood agar, and meat broth. Pyrogenicity was tested in rabbits injected with 5×10^7 bacilli per rabbit, and the temperature measured every 12 hr for a week. For toxicity, 50 times human doses were injected intradermally in ten male and ten female Swiss mice and the animals observed for five weeks for general condition, weight, and mortality. No loss of weight and no mortalities were observed. The vaccine preparations used in these studies were sterile, nonpyrogenic, and nontoxic, as tested by the above-mentioned criteria.

Protocol of study. The protocol employed had the approval of the Drug Controller of India and of the institutional authorities. The study was conducted in confirmed lepromatous leprosy patients rendered bacteriologically negative by prolonged chemotherapy. The subjects were retested for negativity to both Dharmendra and Mitsuda lepromins before recruitment to the study. Immunization was carried out with a single intradermal injection of 5×10^7 autoclaved *Mycobacterium w* in 0.1 ml of sterile normal saline. A retest with lepromin was conducted 4–8 weeks after immunization.

Subjects. Thirty-two lepromatous leprosy patients diagnosed on the basis of clinical, histopathological, and bacteriological criteria were the subjects in the study. The Bacteriologic Index of each patient was originally graded into four degrees of positivity, based on review of 4–6 smears, as follows:

1+ = Bacilli not present in every field but in occasional fields; count not more than 2–5 bacilli/field when 50 fields were screened.

2+ = Bacilli present in every field but not more than 10 per field when 50 fields were screened.

3+ = Bacilli present in every field and more than 10 per field with globi when 50 fields were screened.

4+ = Innumerable bacilli and globi in every field of the 50 fields screened.

The patients had been treated at the School of Tropical Medicine for several years, and all were bacteriologically negative at the time of study.

Active cases were excluded on grounds of possible flare up and concomitant nerve damage. Most patients in earlier years were tested with lepromin and were recorded as negative. Before their recruitment for this study, a fresh testing with both Dharmendra and Mitsuda lepromin was carried out and only those persistently negative to both were taken for immunization. Their consent to

TABLE 1. *Characteristics of the subjects.*

Patient	Age (yr)	Sex	Date of registration	His-tology	Bacterial Index (BI)		Lepromin		Diagnosis
					Date	BI	Date	DL&ML[a]	
1. J.C.B.	73	M	8/31/59	LL	1959 1970	+++ Neg	8/31/59	Neg	BL
2. R.L.	55	M	1/7/54	LL	1954 1977	+++ Neg	1/7/54 1/30/57 12/29/80	Neg Neg Neg	LL
3. R.R.C.	45	M	12/12/47	LL[b]	1947 1955	+++ Neg	12/18/47 12/18/50 1/12/81	Neg Neg Neg	LL
4. R.S.B.	38	M	1971	LL[b]	1971 1975	+++ Neg	12/2/81	Neg	BL
5. S.C.K.	60	M	5/21/49	LL	1949 1958	++ Neg	5/2/49 2/2/50 4/26/51 5/31/51 6/14/51 2/13/52 12/29/80	Neg Neg Neg Neg Neg Neg Neg	LL
6. G.G.K.	55	M	3/15/76	LL	1976 1979	+++ Neg	3/13/76 3/12/81	Neg Neg	BL
7. M.B.	48	M	5/15/73	LL[b]	1973 1979	+++ Neg	9/17/75 9/29/80	Neg Neg	BL
8. D.M.	30	M	4/12/75	LL	1975 1977	+++ Neg	4/16/75 1/3/81	Neg Neg	BL
9. N.K.R.	52	M	9/17/69	LL[b]	1969 1972	++ Neg	7/25/69 12/19/80	Neg Neg	LL
10. H.M.	41	M	2/14/67	LL	1967 1978	++ Neg	7/14/67 12/19/80	Neg Neg	LL[c]
11. M.B.	32	M	5/20/76	LL	1976 1977	+++ Neg	5/20/76 2/12/81	Neg Neg	LL
12. B.H.	50	M	6/24/72	LL	1972 1979	+++ Neg	6/24/72 11/17/80	Neg Neg	BL
13. M.K.	40	M	10/3/70	LL[b]	1970 1976	+++ Neg	10/3/70 12/29/80	Neg Neg	BL
14. S.N.G.	52	M	8/11/69	LL[b]	1969 1976	+++ Neg	8/11/69 9/27/80	Neg Neg	BL
15. G.N.	56	M	3/29/71	LL[b]	1971 1973	+ Neg	3/29/71 9/29/80	Neg Neg	BB
16. S.M.A.	60	M	3/3/64	LL[b]	1964 1972	++ Neg	3/3/64 1/12/81	Neg Neg	LL
17. G.D.N.	50	M	9/19/76	LL[b]	1976 1979	++ Neg	9/19/76 12/29/80	Neg Neg	BL
18. N.R.N.	25	M	4/7/75	LL	1975 1977	++ Neg	9/19/76 12/29/80	Neg Neg	LL
19. W.A.	22	M	2/20/76	LL	1976 1980	++ Neg	11/29/76 12/28/80	Neg Neg	LL
20. M.S.	52	M	1/12/56	LL	1956 1976	+++ Neg	1/20/56 7/14/76 2/16/81	Neg Neg Neg	LL
21. S.K.B.	47	M	9/26/69	LL[b]	1969 1972	++ Neg	12/29/69 7/14/76 1/5/81	Neg Neg Neg	LL
22. A.A.	37	M	6/1/76	LL[b]	1976 1980	+++ Neg	6/2/76 12/1/80	Neg Neg	BL
23. J.P.	45	M	3/17/72	LL[b]	1972 1979	++ Neg	8/24/72 2/2/80	Neg Neg	LL

TABLE 1. *Continued.*

Patient	Age (yr)	Sex	Date of registration	Histology	Bacterial Index (BI)		Lepromin		Diagnosis
					Date	BI	Date	DL&ML[a]	
24. L.M.M.	45	M	9/30/72	LL[b]	1972	+++	10/6/72	Neg	BL
					1979	Neg	2/19/81	Neg	
25. S.K.D.	31	M	9/8/75	LL	1975	+++	9/8/75	Neg	LL
					1979	Neg	1/12/81	Neg	
26. K.P.	40	M	8/24/71	LL	1971	+++	3/13/72	Neg	LL
					1974	Neg	2/2/81	Neg	
27. L.M.G.	52	M	10/9/53	LL	1953	++	11/10/53	Neg	BL
					1976	Neg	11/29/57	Neg	
							10/3/80	Neg	
28. H.P.	40	M	11/19/62	LL	1962	+++	11/19/62	Neg	LL
					1979	Neg	2/16/81	Neg	
29. A.P.	52	M	5/5/61	LL[b]	1961	++	2/26/62	Neg	LL
					1966	Neg	3/9/70	Neg	
							1/3/81	Neg	
30. R.S.	25	M	4/27/76	LL[b]	1976	++	5/3/76	Neg	BL
					1979	Neg	1/12/81	Neg	
31. B.N.B.	42	M	3/30/71	LL[b]	1971	++	6/7/71	Neg	BL
					1976	Neg	1/15/81	Neg	
32. V.M.	66	M	7/28/61	LL[b]	1961	+++	8/14/61	Neg	LL
					1969	Neg	3/2/70	Neg	
							3/2/81	Neg	

[a] Dharmendra lepromin and Mitsuda lepromin.
[b] Histology of the patient's lesions were done earlier and diagnosed, but were not available for fresh review at the commencement of the present study.
[c] Patient also had pulmonary tuberculosis.

participate in this study was obtained. The particulars of the subjects studied are summarized in Table 1.

Lepromin testing. Dharmendra lepromin was made and administered essentially by the method of Dharmendra ([8]). It was of human biopsy origin (1 mg bacillary powder/10 ml); 0.1 ml of this suspension was administered intradermally. An erythematous reaction of 10 mm or more at 48 hr was taken as positive.

Mitsuda lepromin was prepared from human leproma nodules as per the WHO protocol ([36]). An induration of 3 mm or more at four weeks was scored as positive.

Biopsies of the lepromin sites were taken from patients showing lepromin conversion, fixed in formalin, sectioned, and stained with both hematoxylin-eosin and modified Ziehl-Neelsen.

Skin retest. The retest was carried out by one of us (A.F.) six months after the initial investigations without knowledge of the former results. S.C. had carried out the initial investigations. Blood samples were coded and the code was opened after analysis was completed.

Lepromin A (3×10^8 bacilli/ml) was made available by the WHO-IMMLEP through the Indian Council of Medical Research; 0.1 ml of lepromin A was injected intradermally at a site distant from the previous lepromin retest in the same arm. The other arm had received immunization. The size of erythema at 48 hr was determined. A 10 mm reading was considered positive.

Leukocyte migration inhibition assay (LMI). The technique of Soberg and Bendixen ([31]) as modified by Myrvang, *et al.* ([25]) was employed. Washed cells were adjusted to a concentration of 40×10^6 cells/ml of the medium. The cell suspension was packed in a 20 μl capillary, which was cut at the interface and placed in the migration chamber. The chambers were filled with either RPMI (GIBCO Laboratories, Grand Island, New York, U.S.A.) medium with 10% fetal calf serum (GIBCO) alone or with antigen (10^8 *M. leprae*/ml). The chambers were sealed and incubated at 37°C for 14–18 hr.

TABLE 2. *Results of retesting with Dharmendra lepromin (DL) and Mitsuda lepromin (ML) after immunization.*

Patient	Date of imjunization with *Mycobacterium w*	Lepromin retest		
		Date	Reading (mm)	
			DL	ML
1. J.C.B.	2/23/81	3/23/81	12	7
2. R.L.	3/30/81	4/29/81	15	7[a]
3. R.R.C.	3/16/81	4/16/81	10	8
4. R.S.B.	2/23/81	3/26/81	12	10
5. S.C.K.	2/23/81	3/26/81	15	8
6. G.G.K.	3/12/81	4/2/81	10	7
7. M.D.	3/9/81	4/6/81	20	10
8. D.M.	2/26/81	3/23/81	10	Neg
9. N.K.R.	2/16/81	3/23/81	Neg	Neg
10. H.M.	1/29/81	3/26/81	15	12[b]
11. M.B.	3/13/81	4/14/81	Neg	Neg
12. D.H.	2/16/81	3/23/81	Neg	Neg
13. M.K.	2/2/81	3/9/81	Neg	Neg
14. S.M.G.	3/16/81	5/4/81	Neg	Neg
15. G.N.	1/29/81	2/26/81	15	10
16. S.M.A.	2/15/81	3/12/81	12	5
17. G.D.N.	2/29/81	–	10	5
18. N.N.	3/19/81	4/14/81	Neg	Neg
19. W.A.	12/29/80	2/2/81	Neg	Neg
20. M.S.	3/2/81	4/2/81	18	10
21. S.K.B.	2/2/81	3/3/81	20	10[a]
22. A.A.	3/2/81	4/2/81	Neg	Neg
23. J.P.	2/23/81	3/26/81	12	8
24. L.M.M.	2/26/81	3/26/81	15	8
25. S.K.D.	2/12/81	3/25/81	10	5
26. K.P.	3/30/81	4/20/81	12	7
27. L.M.G.	3/16/81	4/20/81	10	5
28. H.P.	4/20/81	5/21/81	Neg	Neg
29. A.P.	4/20/81	5/21/81	Neg	Neg
30. R.S.	2/23/81	4/2/81	15	8
31. B.N.B.	3/25/81	4/9/81	Neg	Neg
32. V.M.	4/11/81	5/18/81	15	7

[a] Ulcer at ML site.
[b] Severe local reaction and ulcer at site of 1 mm.

The area of migration was measured by planimetry and the migration index (MI) calculated. Cells were checked at the end of the assay for viability. Under the conditions employed the viability was more than 97%. A migration index of more than 80 was considered negative.

RESULTS

Table 2 gives the results of the investigations. It will be noted that 20 out of the 32 subjects converted to lepromin positivity status after a single intradermal injection of autoclaved *Mycobacterium w*. The induration of the Mitsuda reaction in converted cases was 5 mm or above, which was well over the cut-off point of 3 mm. Three of the converted cases showed ulceration at the lepromin (Mitsuda) site. The histopathology of the positive Mitsuda relepromin sites showed massive mononuclear infiltration in all cases; granuloma formation was seen in only 12 and associated giant cells were discernible in only two cases. Acid-fast staining did not demonstrate bacilli within the granuloma. Figures 1–4 give the representative histopathology of the lepromin converted cases.

Side reactions. One case, H.M., showed severe local reaction ulcer at the site of immunization. H.M. was a patient with pulmonary tuberculosis. Three converted cases had ulceration at the site of the Mitsuda lepromin injection. In others no apparent side effects, local or systemic, were seen.

Retest studies. In order to ascertain whether the conversions caused by immunization with *Mycobacterium w* were stable, a retest for lepromin reactivity was carried out after 7–11 months. Table 3 gives the results. Ten cases recorded in the initial test as converted to lepromin positivity gave positive skin test responses to lepromin A seven months or more after immunization. Two cases were marginally positive in the current skin tests (M.S. and J.P.). M.S. however gave a strongly positive leukocyte migration index, but J.P.'s leukocytes produced an almost negative migration index. The rest of the converted cases gave clearly positive LMI tests, as well as reacted clearly positively in skin response to lepromin A.

All cases from the nonconverted category in the earlier investigation gave negative LMI tests during this investigation. One of them, however, gave a positive skin test (D.M.). D.M. was negative in the LMI test. The others remained negative in the skin test.

DISCUSSION

These investigations indicate that a fairly large number of confirmed cases of lepromatous leprosy, repeatedly negative to lepromin, were converted to lepromin positivity status after a single intradermal injection of *Mycobacterium w*. The conversions assume significance since the delayed hypersensitivity reactions were clearly above the borderline. Histopathological study of the

TABLE 3. *Evaluation after several months of cell-mediated immunity* in vivo *and* in vitro *to* Mycobacterium leprae *antigens in patients immunized with* Mycobacterium w.

Patient	Post-immunization relepromin test date	Lepromin A		Migration Index (MI)[a]	
		Date	48 hr erythema (mm)	Date	MI
Converted cases					
1. R.L.	4/29/81	11/3/81	11	11/3/81	18
2. R.S.B.	3/26/81	11/3/81	14	11/3/81	44
3. G.G.K.	4/2/81	11/3/81	11	11/3/81	41
4. S.M.A.	3/12/81	11/3/81	12	11/3/81	39
5. M.S.	4/2/81	11/3/81	9	11/3/81	17
6. S.K.B.	3/3/81	11/5/81	12	11/5/81	9
7. S.K.D.	3/25/81	11/7/81	12	11/7/81	19
8. K.P.	4/20/81	11/7/81	N.A.[b]	11/7/81	61
9. J.P.	3/26/81	3/1/82	10	3/1/82	77
10. L.M.M.	3/26/81	2/5/82	15	2/5/82	33
Nonconverted cases					
11. B.N.B.	4/9/81	11/7/81	N.A.[b]	11/7/81	81
12. A.A.	4/2/81	11/7/81	Neg[c]	11/7/81	91
13. D.M.	3/23/81	11/7/81	13	11/7/81	115
14. S.N.G.	5/4/81	1/22/82	Neg[c]	1/22/82	80

[a] MI (migration index) = $\frac{\text{Area of migration with antigen} \times 100}{\text{Area of migration without antigen}}$.

[b] N.A. = not available.

[c] Neg = not discernible.

positive Mitsuda lepromin sites showed mononuclear infiltration in all converted cases, which could have occurred only if T cells recognized the antigen and released mediators. Granuloma formation with epithelioid cells was seen in 12 of the converted cases. This could have occurred as a consequence of the activation of macrophages by lymphokines, leading to the clearance of mycobacteria and the concomitant transformation of macrophages to epithelioid cells in a granuloma. The absence of the acid-fast bacilli in the lepromin site in converted cases further supports the contention that the immune system cells were activated by *M. leprae* antigens and were involved in bacterial clearance. If it be so, then immunization with a heterologous, crossreactive mycobacteria brought in the breakage of tolerance to *M. leprae* antigens, as far as cell-mediated immunity responses such as delayed-type hypersensitivity (DTH) and LMI are concerned.

The conversions brought about by immunization with *Mycobacterium w* were not of transient character but were stable for several months, as indicated by the retest investigations. Leukocytes from these patients also gave LMI. LMI seemingly correlates with DTH skin responses and not with antibody responses against an antigen ([6, 7]). Lymphokines (T cell derived) determine the fate of intracellular pathogens such as *Listeria, Toxoplasma* and *Leishmania* in macrophages. Myrvang, *et al.* ([25]) showed that in lepromatous leprosy, in correlation with disseminated disease, there was negative DTH skin test and depressed LMI. In tuberculoid leprosy, a high cell-mediated immune response could be shown by positive skin tests and elevated LMI responses.

The correlation between previous skin test positivity and the present skin test and LMI test was generally good in the converted cases. Two cases, M.S. and J.P., gave marginal skin test responses. M.S. was paradoxically strongly positive in the LMI test, while J.P. was borderline in the LMI test.

The correlation in the nonconverted group was clear except in the case of D.M. D.M. had produced a marginally positive response to Dharmendra lepromin during the initial investigation following immunization. He was negative to Mitsuda lepromin at this stage. The positive lepromin A skin test in the retest correlated to the Dhar-

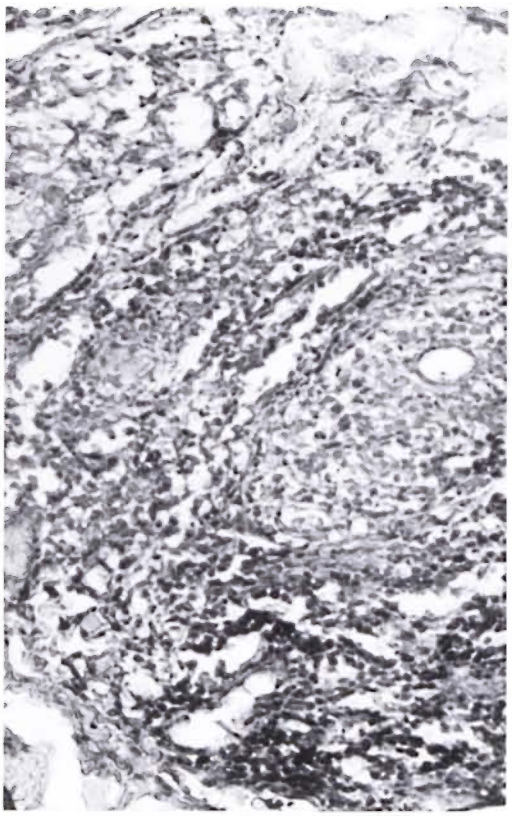

FIG. 1. Epitheloid cell granulomas surrounded by mononuclear cell infiltration in the dermis in a positive Mitsuda lepromin reaction in a lepromatous patient who was immunized with *Mycobacterium w* (Hematoxylin and eosin ×160).

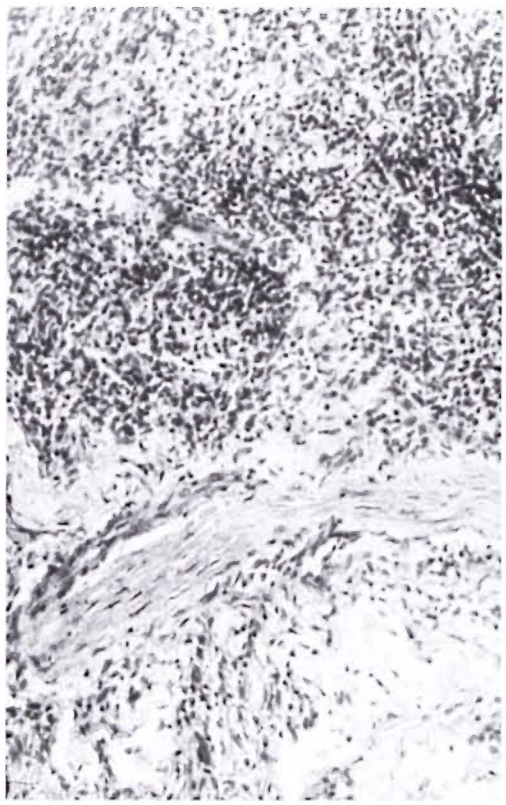

FIG. 2. Marked mononuclear cell infiltration in the superficial dermis in a positive Mitsuda lepromin reaction in a lepromatous patient who was immunized with *Mycobacterium w* (Hematoxylin and eosin ×160).

mendra reading, while Mitsuda negativity is apparently correlating with the negative LMI response. The results suggest the conversion from a state of negativity to positivity in DTH responses to *M. leprae* administered as lepromins, as a result of immunization.

The skin response was correlated to the leukocyte migration inhibition assay, indicating the ability of leukocytes to produce mediators (lymphokines) influencing migration of leukocytes when challenged with *M. leprae* antigens.

Immune tolerance could be due to two types of mechanisms. It could be due to either central or peripheral unresponsiveness. In central unresponsiveness the lymphocytes are clonally deleted or rendered irreversibly inactive ([26, 35]). If central unresponsiveness is the mechanism of the immune unresponsiveness in leprosy, the lepromin conversion caused by the antigenically crossreactive *Mycobacterium w* can only be explained by assuming a split tolerance, in which case the regulatory T helper cells are tolerant but the delayed hypersensitivity T cells specific for *M. leprae* continue to be functional. Thus *Mycobacterium w* could break tolerance by providing crossreactive T cell help for anti-*M. leprae* DTH responses.

On the other hand, the immune tolerance against *M. leprae* could also be caused by a peripheral unresponsiveness mechanism. This would include unresponsiveness due to inhibition of immune reactivity by T cell products ([11]), inhibition by B cell products ([5, 16]), or receptor blockade ([1, 9]). A cross-

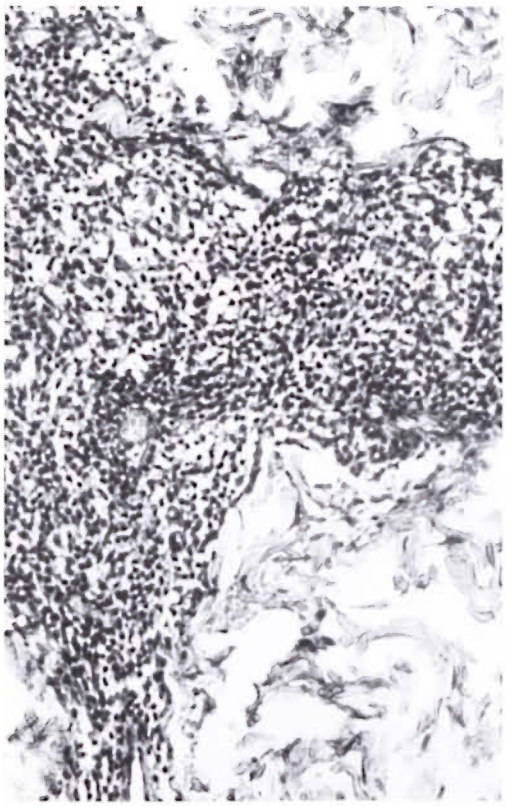

FIG. 3. Marked mononuclear cell infiltration in the superficial dermis in a positive Mitsuda lepromin reaction in a lepromatous patient who was immunized with *Mycobacterium w* (Hematoxylin and eosin ×160).

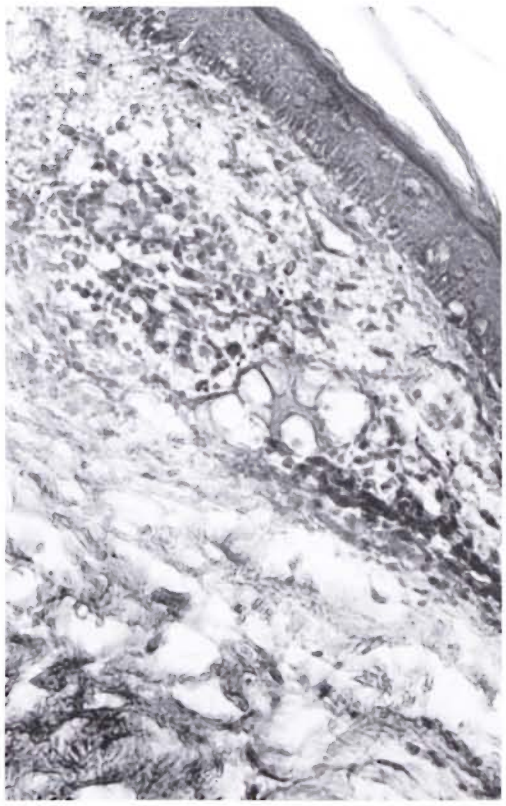

FIG. 4. Formed granulomas surrounded by some mononuclear cells in the subepidermal location in a positive Mitsuda lepromin reaction in a lepromatous patient who was immunized with *Mycobacterium w* (Hematoxylin and eosin ×160).

reactive antigen like *Mycobacterium w* can break tolerance to *M. leprae* in such an immune defect by a variety of ways. It can do so by either stimulating helper activity or aborting suppression. Gershon ([12]) has defined the phenotype of a contrasuppressor cell which has suppressor cell aborting activity. Using idiotypic probes in the phosphorylcholine system, the role of anti-idiotypic suppressor cells to abort idiotype-bearing suppressor T cells has been documented ([19]). This kind of circuitry could be involved in alleviation of the immune suppression, basis of the anergic lepromatous state.

A pertinent question that arises is whether the lepromin conversion of the type observed here signifies protection. The experimental protocol does not address itself to this question. It may, however, be mentioned that another attenuated atypical mycobacterium, BCG, which had similar traits, i.e., partial crossreactivity and partial differences with *M. leprae*, was protective in a clinical trial in Uganda. A cautionary note needs to be made however: live BCG confers protective immunity against *M. leprae* in the mouse foot pad, while *Mycobacterium w* does not. BCG was not protective against leprosy in a trial carried out in Burma. There are no good reasons to explain these apparent contradictions but a hypothesis on the role of environmental mycobacteria in modulating the protective efficacy of antileprosy vaccines has been advanced ([32]).

SUMMARY

Thirty-two clinically, histopathologically confirmed cases of BL/LL leprosy were ren-

dered bacteriologically negative by prolonged chemotherapy. All of them were negative to Mitsuda and Dharmendra lepromin at the start of study. They were immunized with a single intradermal injection of 5×10^7 autoclaved *Mycobacterium w* and were retested for lepromin reaction 4–6 weeks later. Twenty subjects gave at this time a positive reaction with both Dharmendra and Mitsuda lepromins. The histology of biopsies from converted cases showed mononuclear infiltration in all and granuloma formation in 12 of the 20 positive cases.

The stability of the conversion of the patients' lepromin positivity was investigated 6–11 months after immunization with *Mycobacterium w*. Patients who were earlier converted to a positivity status remained positive in the skin test response to *M. leprae*. The leukocytes of these patients produced lymphokines on culture with lepromin, causing leukocyte migration inhibition. Patients who did not convert earlier continued to remain anergic to lepromin. These results suggest a conversion, stable for several months, to lepromin positivity caused by immunization with *Mycobacterium w* in about 60% of BL/LL leprosy patients.

RESUMEN

Treinta y dos pacientes con lepra BL/LL confirmada clínica e histopatológicamente, se tornaron bacteriologicamente negativos por quimioterapia prolongada. Todos ellos fueron negativos a las leprominas de Mitsuda y de Dharmendra al inicio del estudio. Los pacientes fueron inmunizados con una sóla inyección intradérmica de 5×10^7 *Mycobacterium w* muertos por calor, y su reactividad a la lepromina se determinó 4 a 6 semans después. A este tiempo, 20 pacientes dieron una reacción positiva con ambas leprominas. La histología de las biopsias de los casos convertidos mostró un infiltrado mononuclear en todos los casos y la formación de granulomas en 12 de los 20 casos positivos.

La estabilidad de la conversión positiva a la prueba de la lepromina se investigó 6 to 11 meses después de la inmunización con *Mycobacterium w*. Los pacientes que fueron tempranamente convertidos a positivos, permanecieron positivos en su respuesta dérmica al *M. leprae*. Los leucocitos de estos pacientes produjeron linfocinas cuando se cultivaron en presencia de lepromina y causaron la inhibición de la migración de leucocitos. Los pacientes que no se convirtieron tempranamente, continuaron siendo anérgicos a la lepromina. Estos resultados indican que el 60% de los pacientes BL/LL inmunizados con *Mycobacterium w*, desarrollaron una positividad a la lepromina que fue estable por varios meses.

RÉSUMÉ

Trente-deux cas de lèpre BL/LL confirmés cliniquement, et histologiquement, ont été négativés bactériologiquement à la suite d'une chimiothérapie prolongée. Tous ces cas étaient négatifs aux lépromines de Mitsuda et de Dharmendra au début de l'étude. Ils ont été immunisés par une injection unique intradermique de 5×10^7 de *Mycobacterium w* autoclavé; ils ont été soumis à nouveau à une réaction à la lépromine 4 à 6 semaines plus tard. A ce moment, vingt sujets ont témoigné de réaction positive tant à la lépromine de Dharmendra qu'à celle de Mitsuda. L'étude histologique des biopsies prélevées chez les cas ayant viré leur épreuve à la lépromine a révélé une infiltration mononucléaire chez tous les malades; la formation de granulomes a été constatées chez 12 des 20 cas positifs.

La stabilité du virage positif de la lépromine chez les malades a été suivie 6 à 11 mois après l'immunisation par *Mycobacterium w*. Les malades qui avaient montré auparavant un virage positif ont continué à montrer un résultat positif de l'épreuve cutanée à *M. leprae*. Les leucocytes de ces malades produisaient des lymphokines sur des cultures avec lépromine, entraînant l'inhibition de la migration des leucocytes. Les malades qui n'avaient pas viré leur test ont continué à être anergique à la lépromine. Ces résultats suggèrent que le virage positif à la lèpromine, persistant pour plusieurs mois, est causé par l'immunisation avec *Mycobacterium w* chez approximativement 60% des malades BL/LL.

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