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Reversal Reaction in Lepromatous Patients Induced by a Vaccine Containing Killed ICRC Bacilli—A Report of Five Cases[1]

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In the clinical trial of a vaccine containing killed ICRC bacilli, we observed that a single dose of the vaccine brought about lepromin conversion in about 50% of lepromatous (LL) and 90% of borderline (BB/BL) leprosy patients ([3]). In some LL patients the skin biopsies showed evidence of regressive changes and tissue bacillary clearance. These observations suggest that the vaccine-induced lepromin conversion may be linked with activation of host immune responses specific against *Mycobacterium leprae*. This contention is strengthened by our preliminary observations of "reversal" reactions in two LL patients. Since then, we have encountered three additional cases of "reversal" reaction with upgrading of lesions. The present paper describes the clinico-pathological observations, including follow up, in all five of the patients.

MATERIALS AND METHODS

Patients. A total of 55 LL patients volunteered for the study: 46 were vaccinated and 9 served as controls. All patients were on treatment with dapsone (DDS), and some also had received rifampin. Clinico-pathological features of the patients, including their responses to vaccination, have been described in detail elsewhere ([3]).

Five patients (all males, aged 12–48 years) developed, in the course of the study, "reversal" reaction. They were high-index LL cases who were on DDS that was taken somewhat irregularly, and treatment was continued throughout the study. The duration of the disease varied from 5–25 years. As expected, all were lepromin (Mitsuda) negative before vaccination.

ICRC vaccine. As described elsewhere ([3]), the vaccine was prepared from ICRC bacilli, strain C-44, killed by gamma irradiation manufactured for the Phase I and II trials under license numbers 1435 and 1594 from the Food and Drug Administration, Maharashtra State, India, under the advice of and clearance from the Drug Controller of India. Each patient received 0.1 ml of the vaccine (equivalent to 50–90 μg) in a single dose intradermally in the left deltoid region. The ICRC bacillus is a slow-growing mycobacterium that has been cultivated repeatedly from human lepromas since 1958 ([1]). It shows antigenic crossreactivity with *M. leprae* with reference to both serologic and cell-mediated immunity (CMI) antigens ([3]). On the basis of the analysis of sero-antigens, it has been classified as belonging to the *M. avium-intracellulare* group of organisms. Strain C-44 was isolated in 1969 from an LL patient and is now in the 89th passage.

Lepromin test. The Mitsuda reaction was carried out using 0.1 ml of standardized lepromin A (Lot No. AB-22) generously supplied by Dr. R. W. J. Rees, Head, Laboratory for Leprosy and Mycobacterial Research, Medical Research Council, London, England. It contained 1.6×10^8 bacilli per ml. The reaction was read between 3–4 weeks. Induration of more than 3.0 mm denoted a positive reaction. The Mitsuda reaction was performed before and after vaccination, as and when the patient reported to the hospital.

Bacillary Index (BI). Smears were pre-

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pared from earlobes and skin lesions and stained with Ziehl-Neelsen. Grading was done according to Ridley's classification.

Histopathology. Biopsies were obtained from skin lesions present on the patient's upper arm and back before vaccination, and from the sites of the "reversal" reaction and lepromin test. The biopsies were fixed in 10% Zenker-formol. Paraffin sections, cut at 5 μ, were routinely stained with hemotoxylin and eosin (H&E) and Fite's stain for acid-fast bacilli (AFB).

RESULTS

Pre-vaccination

All patients had clinical features of lepromatous leprosy and a BI of 5+ to 6+ at the time of admission. Patient No. 1 exhibited infiltrative lesions, and others had scars and wrinkled skin lesions, thickened nerves (ulnar and peroneal), and bilateral peripheral anesthesia. Only one patient (No. 4) showed deformity, presenting with a unilateral claw hand (right side). All were Mitsuda negative before vaccination.

Post-vaccination

Two to three weeks after vaccination, all patients developed a local ulcer and enlargement of the regional lymph nodes. The ulcer healed with local treatment. Mild erythema nodosum leprosum (ENL) was observed in two out of five patients 3–4 weeks after vaccination. Two patients (Nos. 3 and 4), who had received a higher dose (90 μg) of the vaccine, exhibited "reversal" reaction within 4 months. In the rest of the patients, the reaction occurred between 5–10 months post-vaccination. Following vaccination, the lepromin reaction, which could be repeated in only four patients, was positive (The Table).

Clinical features of the "reversal" reaction were distinct and consisted of a single (Patient No. 2), multiple (Patients Nos. 1, 3, and 5), localized raised erythematous, well-demarcated plaques, or numerous small papules over the extremities and back (Patient No. 4). The lesions disappeared and complete recovery was observed in about 1–3 months without additional treatment. None of the patients exhibited fresh nerve lesions. Only one case (Patient No. 3) complained of transient pain in the right ulnar and peroneal nerves but this patient recovered fully with corticosteroids within 1 week. All patients showed some clinical improvement as judged by a lowering of the BI. Examination of the biopsies of the reaction sites showed that four out of five exhibited granulomas consisting of epithelioid cells, giant cells, and a moderate number of lymphocytes, a picture consistent with BT. In one patient, granulomas showed collection of epithelioid cells and ill-defined giant cells but very few lymphocytes, a picture suggestive of BB.

Clinico-pathological features of patients

Clinico-pathological features of the patients, including their Bacillary Index (BI) and Morphological Index (MI), are summarized in The Table. Special features of each case, including histopathological changes, are described below.

Patient No. 1: LF (Reg. No. 88978). He presented with infiltrative lesions on the face, body, and limbs with shiny skin, thickened nerves, and peripheral anesthesia but no deformity. Five months post-vaccination, he developed a few well-demarcated, localized, raised, as well as flat, erythematous plaques on the abdomen. After the biopsy of the reaction site was performed, he received lepromin but went to his village and reported only 6 months later, presenting a picture of remarkable recovery. The skin lesions had disappeared.

The pre-vaccination biopsy showed marked atrophy of the epidermis. A clear subepidermal zone free of cellular infiltrate was present. In the dermis there was marked, diffuse, chronic, granulomatous inflammation consisting chiefly of foamy and spindly macrophages laden with AFB (Figs. 1 and 2). The lymphocytes were conspicuous by their absence. The morphologic characterisitcs of the infiltrates were consistent with the diagnosis of histoid, although a clear-cut nodular appearance was not seen.

The biopsy from the reaction site exhibited normal epidermis. The dermis showed granulomatous inflammation consisting of epithelioid cells, giant cells, and a moderate number of lymphocytes (Figs. 3 and 4). Occasional beaded AFB were seen in macrophages.

Patient No. 2: GD (Reg. No. 2818). At the time of vaccination, he showed only old

THE TABLE. *Pre-vaccination and post-vaccination clinico-pathological features of the patients.*

Patient no./ initials/age (yr)/duration of disease (yr) (all males)	Disease type/BI at admission	Pre-vaccination				Vacc. batch and dose[a] / Date of vaccination	Post-vaccination responses				BI/MI after recovery
		Mitsuda reaction (mm)	ENL	Histo-path. (grading)	BI/MI		ENL	Reversal reaction (months post-vaccination)	Lepromin conversion (months post-vaccination)	Histo-path. (grading)	
1. LF/12/5	LL/6+	0	Nil[b]	LL	5+/1	V2—67[a] / 7/17/80	Nil	5	N.D.[c]	BT	N.D.
2. GD/31/9	LL/5+	0	+	LL	4+/1	V3—50[a] / 3/31/80	Nil	10	10 (4)[e]	BT	2+/0
3. HSP/40/25	LL/6+	0	+	LL	3+/0.5	V5—90[a] / 8/6/80	+	2	4[d] (5)[e]	BT	2+/0
4. GPK/27/6	LL/5+	0	+	N.D.	2+/0	V5—90[a] / 8/6/80	+	4	4 (4)[e]	BT	0/−
5. RK/48/15	LL/5+	0	Nil	LL	5+/1	V5—60[a] / 8/18/80	Nil	10	14[d] (4)[e]	BB	4+/0

[a] Dose of the vaccine expressed as μg bacillary protein.
[b] Nil = None.
[c] N.D. = Not done.
[d] Lepromin reaction could be performed only after patient recovered from "reversal" reaction.
[e] Figures in parentheses denote average diameter of induration in mm.

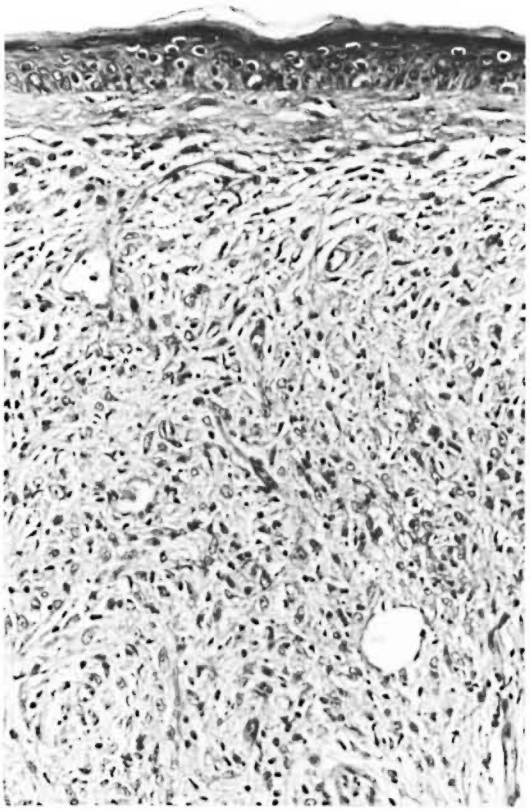

FIG. 1. Biopsy of skin lesion of Patient No. 1, showing atrophy of epidermis, a clear subepidermal zone, and marked diffuse infiltration of the dermis with histiocytes. The cellular exudate has very few lymphocytes (H&E ×175).

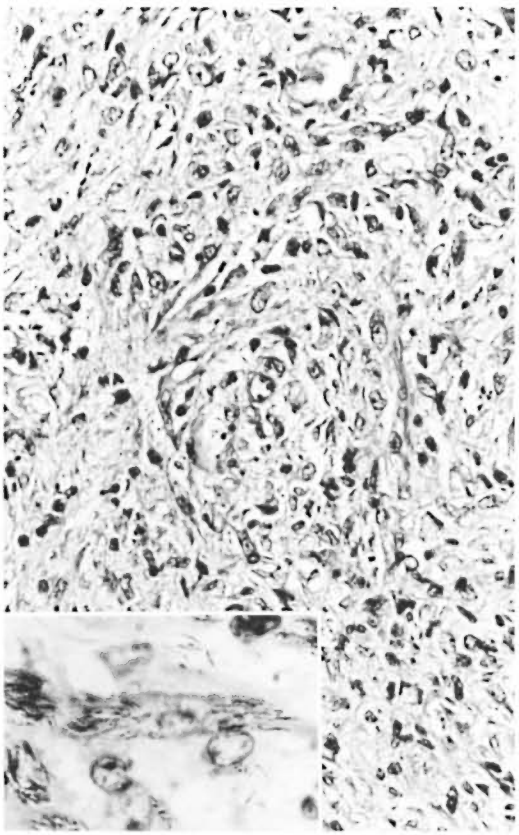

FIG. 2. High power magnification of Figure 1, showing vacuolated, foamy and spindly macrophages (H&E ×440). Inset exhibits AFB-laden macrophages (Fite's stain ×1120).

scars and wrinkled skin lesions, thickened nerves, and peripheral anesthesia without any deformity. He developed a "reversal" reaction 10 months post-vaccination in the form of a single, localized, hypopigmented skin patch with raised erythematous, well-demarcated margins on his back. He recovered from the reaction in about 4 weeks and his BI was reduced to 2+.

The pre-vaccination biopsy of an old skin lesion showed marked atrophy of the epidermis and a clear subepidermal zone free of inflammatory cells. The dermis exhibited, throughout its thickness, chronic granulomatous inflammation consisting predominantly of vacuolated and foamy macrophages, a varying number of plasma cells, and very few lymphocytes. Diffusely scattered neutrophils were also observed. In the deeper dermis, there was a focus of neutrophilic infiltrate with necrosis indicating ENL. AFB staining was negative.

During the post-vaccination period, the patient was biopsied twice. The first biopsy was taken from a skin lesion 10 weeks before the patient developed a clinically expressed "reversal" reaction which was also biopsied. The morphologic picture of the two biopsies was more or less similar. They are, therefore described together here. The epidermis was normal and no clear subepidermal zone was seen. The dermis showed a number of well-formed granulomas consisting of epithelioid cells, giant cells, and a moderate number of lymphocytes. The picture was suggestive of a BT granuloma. The biopsy also showed edema and focal neutrophilic infiltration, expecially around blood vessels in the upper dermis. Neutrophils were seen even within the granulomas.

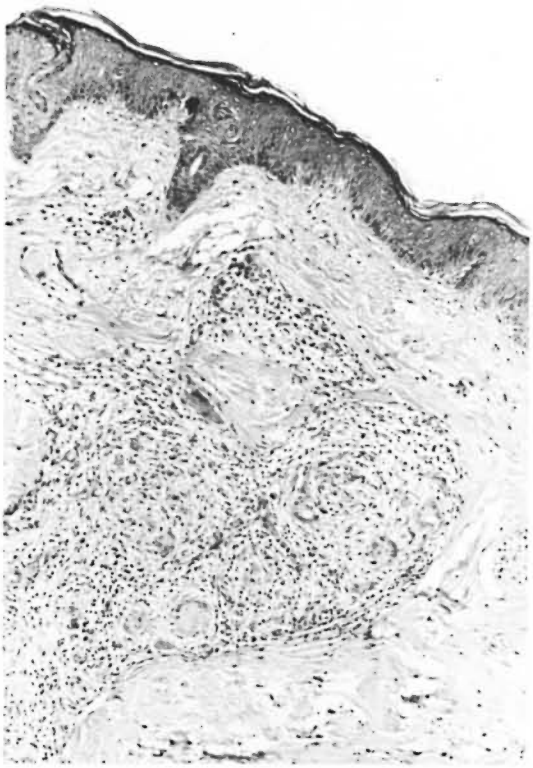

FIG. 3. Skin biopsy of reversal site in the same patient as in Figure 1, showing normal epidermis. The inflammatory infiltrate in some places reaches almost to the epidermis. The dermis shows a well-formed granuloma consisting of epithelioid cells, giant cells, and lymphocytes (H&E ×110).

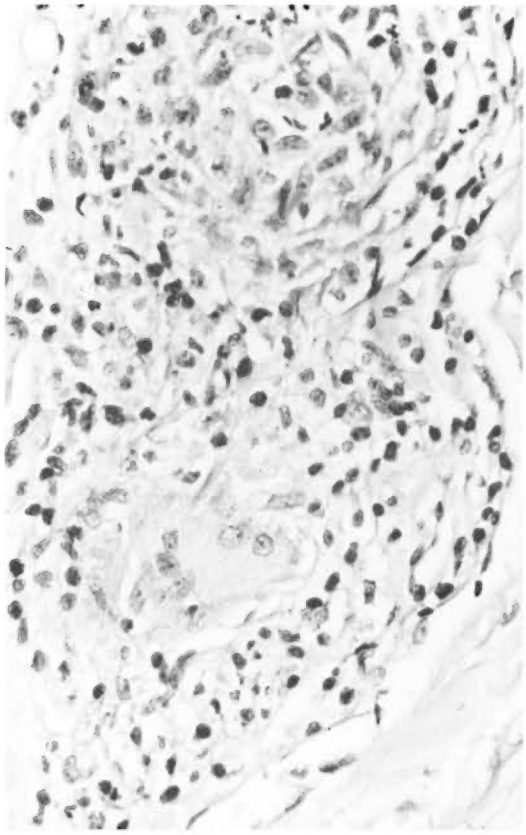

FIG. 4. High power magnification of Figure 3, showing cellular details of the granuloma (H&E ×440).

Patient No. 3: HP (Reg. No. 2656). He had complete loss of eyebrows, wrinkling of earlobes, and bilateral peripheral anesthesia with thickening of peripheral nerves but no deformities. Within a month after vaccination he developed mild ENL with fever and joint pains which subsided after 7 days of symptomatic treatment. Two months post-vaccination, he developed skin eruptions over the trunk, indicative of "reversal" reaction. The lesions consisted of small, well-demarcated, raised, erythematous papules with clear margins. At that time he had pain and tenderness in the right ulnar and peroneal nerves. He recovered completely without any residual motor deficit after treatment with a corticosteroid for 1 week.

The pre-vaccination biopsy revealed a normal epidermis. Collections, consisting chiefly of vacuolated, foamy mononuclear cells, occasionally containing a few beaded AFB, were seen around skin appendages, particularly in the upper dermis. Lymphocytes were conspicious by their absence. The picture was consistent with the diagnosis of LL under long-term treatment.

The biopsy from the reaction site showed normal epidermis. The dermis contained, throughout its thickness, granulomas consisting of epithelioid cells, well-formed giant cells, and a moderate number of lymphocytes. No AFB were seen.

Patient No. 4: GPK (Reg. No. 91044). The patient had no active lesions, but had wrinkled and scarred areas over the body and limbs. He had bilateral peripheral anesthesia and exhibited a single deformity—a unilateral (right side) claw hand. Four months after vaccination, he developed raised erythematous plaques with well-demarcated margins, and sensory impairment over the

chest, arm, and face. The patient recovered in 3–4 weeks, and there was no evidence of fresh nerve damage.

No pre-vaccination biopsy was available. This patient's diagnosis was based mainly on clinical features and history. The biopsy of the reaction site showed normal epidermis. The dermis contained granulomas consisting of epithelioid cells, giant cells, and a moderate number of lymphocytes. No AFB were seen.

Patient No. 5: RK (Reg. No. 1106). This was an old case of leprosy, showing loss of eyebrows, wrinkling of earlobes, and depression of the bridge of the nose. He had bilateral peripheral anesthesia with partial absorption of fingers and toes. Ten months post-vaccination, he developed multiple, flat, hypopigmented patches on the chest and abdomen consistent with "reversal" reaction. The lesions gradually became diffuse and less visible in a few months' time.

The pre-vaccination biopsy of the skin showed atrophy of the epidermis and a clear subepidermal zone free of inflammatory cells. The dermis showed diffuse infiltration with foamy histiocytes containing a few beaded AFB. There was a paucity of lymphocytes.

The biopsy of the reaction site showed a mild atrophy of the epidermis but no clear subepidermal zone. The dermis showed granulomatous inflammation consisting of epithelioid cells, a few lymphocytes, and ill-formed giant cells. No AFB were seen.

Lepromin reaction

Three out of four positive lepromin reactions were confirmed by biopsies which exhibited well-formed granulomas consisting of epithelioid cells, giant cells, and a moderate number of lymphocytes. Fite's stain revealed a few fragments of AFB in the macrophages.

DISCUSSION

"Reversal" reaction occurs mostly in borderline patients when the bacterial load has been markedly reduced by prolonged treatment ([8]). The reaction is virtually unknown in polar LL patients. In this study, four out of five patients had clinico-pathological features of LL. Although in the absence of a pre-vaccination biopsy a firm diagnosis could not be made for Patient No. 4, the fact that he had episodes of ENL would put him rather nearer the lepromatous end of the leprosy spectrum. As mentioned elsewhere ([3]), none of the controls exhibited lepromin conversion or "reversal" reaction which was, therefore, not just a consequence of prolonged treatment with DDS.

"Reversal" reaction has been reported in three out of four LL patients given multiple injections of transfer factor by Hastings and Job ([5]). Convit, *et al.* ([2]) observed "reversal" reaction, associated with lepromin conversion, in patients with indeterminate leprosy administered multiple injections of a vaccine containing heat-killed *M. leprae* (A) and BCG. The reaction was not seen if either *M. leprae* or BCG were given alone. However, there are reports which indicate that BCG can precipitate "reversal" reaction in borderline cases ([4, 7]).

"Reversal" reaction is generally associated with neurological lesions and thus poses a major clinical problem ([6]). The clinical presentation observed in this study was predominantly that of skin hypersensitivity. Since nerve damage is known to appear several weeks after the manifestation of skin lesions we have followed the patients for 1½ years. However, none of our patients developed fresh permanent nerve lesions. Only one patient (No. 3) complained of nerve pains but completely recovered on conservative therapy.

In this study there appears to be no relationship between the bacillary load and the occurrence of "reversal" reaction—even patients with a high BI of 6+ (Patient No. 1) developed the reaction within a few months of vaccination. To that extent our results are at variance with the belief that "reversal" reaction occurs mostly when the bacillary load is markedly reduced. Another interesting feature is that the vaccination-induced ENL and "reversal" reaction, which are generally observed in immunologically distinct and opposite types ([8]), have occurred in the same patient. However, this should not be surprising since the mechanisms of the reactions are different. Whereas ENL is probably due to depositions of antigen-antibody complexes, upgrading of CMI appears to be the underlying mechanism for the "reversal" reaction ([6, 8, 9]). As such, both forms of reactions could occur

in the same patient following an immunogenic stimulus.

There is global interest in the development of a vaccine for the prevention of leprosy, especially because drug resistance is occurring at an alarmingly high rate. Before immunoprophylactic efficacy is assessed in field trials, it would be essential to show that the vaccine is immunogenic. The ICRC vaccine induces lepromin conversion in a majority of LL patients ([3]). The occurrence of "reversal" reactions is yet another evidence of the immunogenic potentials of the vaccine.

SUMMARY

Clinico-pathological features of five cases of lepromatous leprosy exhibiting "reversal" reaction with upgrading of lesions following vaccination with ICRC vaccine have been described. Two patients also developed ENL. Associated with the "reversal" reaction, the patients exhibited lepromin conversion. No evidence of fresh nerve lesions was observed in any patient. Besides lepromin conversion, occurrence of "reversal" reaction is yet another evidence of immunogenicity of the ICRC vaccine.

RESUMEN

Se describen las caracteristicas clinico-patológicas de cinco casos de lepra lepromatosa en reacción "reversa" con lesiones en regresión inducidas por la aplicación de la vacuna ICRC. Dos de los pacientes vacunados desarrollaron ENL asociado a la reacción "reversa", los pacientes exhibieron conversión a la lepromina y en ningún caso se observaron evidencias de lesiones recientes en los nervios. Además de la conversión a la lepromina, la ocurrencia de reacción "reversa" es una evidencia más de la inmunogenicidad de la vacuna ICRC.

RÉSUMÉ

On décrit les caractéristiques cliniques et pathologiques observées chez cinq cas de lèpre lépromateuse montrant une réaction de réversion (reversal reaction), avec transformation correspondante des lésions, à la suite d'une vaccination par le vaccin de l'ICRC. Deux malades ont également développé un érythème noueux lépreux. En rapport avec cette réaction de "réversion", les malades ont également présenté un virage de la réaction à la lépromine. Aucune évidence de nouvelles lésions nerveuses n'a été observée chez ces malades. Outre, le virage de la lépromine, l'apparition de la réaction de "réversion" est encore un autre signe qui témoigne du pouvoir immunogénique du vaccin de l'ICRC.

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