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Immunotherapy with *Mycobacterium w* vaccine decreases the incidence and severity of Type 2 (ENL) reactions

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Summary Immunotherapy with *Mycobacterium w* (*M.w*) vaccine was given to 45 patients with multibacillary (MB) leprosy; 41 similarly classified patients served as controls. All patients received standard multidrug therapy (MDT). Incidence, severity and frequency of type 2 (ENL) reactional episodes were monitored in both groups in a follow-up extending up to 4 years. Reactions were seen in fewer vaccinated (10/37) BL and LL patients than in the control group (12/34). A total of 20 episodes were recorded in the vaccine group as against 29 in the controls, 75% of reactions were mild in vaccinated and 51·72% were mild in the control group patients, and 3 patients in the control group had more than 3 reactional episodes. None of the vaccinated patients showed this. No additional incidence of neuritis were seen among vaccinated individuals during reactional episodes.

Introduction

Erythema nodosum leprosum (type 2 reaction) affects patients with multibacillary BL and LL types of leprosy. Over 50% of LL and 25% of BL patients experience type 2 reactions.[1] There is a sudden development of crops of small, tender, subcutaneous nodules which persist for a few days and then subside. Other clinical features include fever, neuritis and polyarthralgia.[2] Histological features consist of 3 distinct changes: 1, intense infiltration of neutrophilic granulocytes within the lepromatous macrophage granuloma; 2, abundant presence of fragmented and granular bacilli; and 3, oedema of endothelium of vessel walls with granulocyte infiltration and local vasculitis.[3] The aetiopathogenesis of this symptom complex is the formation of immune complexes which are deposited in selective tissue sites. These complexes consist of mycobacterial antigens,

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IgG or IgM antibodies and complement.[4] It has been postulated that in ENL, there is an imbalance of immunoregulatory T-cell subsets. This is manifested as an increased ratio of helper (CD4+) to suppressor/cytotoxic (CD8+) T cells in blood.[5] Decrease in CD8+ T-cells may favour formation and deposition of immune complexes.[6]

Mycobacterium w (*M.w*) was selected from a panel of 16 mycobacteria on the basis of its immunological potentials.[7] It is a nonpathogenic, atypical, rapid growing mycobacterium.[8] It has undergone Phase I trials in Calcutta[9] and is currently in Phase III trials in Delhi and large-scale field trials in District Kanpur Dehat of Uttar Pradesh, State of North India. Results on the 1st series of multibacillary patients have been reported and have evidenced some immunotherapeutic benefits of combining this vaccine with standard MDT. The vaccine effectively brought about: 1, rapid BI fall; 2, faster clinical improvement; 3, lepromin conversion; and 4, histological upgrading. Due to a rapid overall improvement, treatment duration in vaccinated patients was shorter and more patients could be released from treatment (RFT) within the stipulated 2 treatment years.[10–13]

This report gives the incidence, frequency and severity of ENL (type 2) reactions in 86 patients who have been followed up for up to 4 years—45 have received MDT + *M.w* vaccine (vaccine group) and 41 of them are included in a group receiving MDT + micronized starch as placebo.

Materials and Methods

Patients inducted in this study form part of the large-scale Phase III immunotherapeutic trials being conducted at the Urban Leprosy Centres (ULCs) of Safdarjung and Dr Ram Manohar Lohia Hospitals of New Delhi.

Patients falling in a, BB, BL and LL spectrum of disease (Ridley–Jopling Scale); b, showing evidence of bacilli in slit-skin smears; c, negative in their Mitsuda reaction to lepromin-A; and d, having histological features of BB, BL or LL type disease are enrolled. Standard MDT as recommended by NLEP of India was given to all those inducted.[14] Half the patients, in addition, received intradermally *M.w* vaccine (containing 1×10^9 killed bacilli per dose as the 1st dose and 5×10^8 killed bacilli as the 2nd and subsequent doses every 3 months up to a maximum of 8 doses). The other half of the patients received a placebo injection of micronized starch at the same time intervals. Allotment of patients into the vaccine and control groups was done in a randomized manner according to a code supplied to the clinician.

Evaluation of reactions

A detailed history of the patient experiencing any previous reactions (type, severity, frequency and duration) was taken at the time of enrollment. A reaction proforma was filled for each reactional episode during the 2 or more years of treatment and subsequent follow-up. This proforma included eliciting a history of fever, joint pains, epistaxis, conjunctivitis, pedal oedema, lymphadenopathy and epididymo-orchitis. Examination of skin for ENL lesions, ulceration or desquamation was looked for. Pain/tenderness of peripheral nerves, recent development of sensory and motor deformities were especially

Table 1. Patients under study

Type of leprosy (No. of patients)	Vaccine group (MDT + *M.w* vaccine)	Control group (MDT + placebo)	Total
BB	8	7	15
BL	14	14	28
LL	23	20	43
Total	45	41	86

elicited. Grading of reaction was as 'mild', 'moderate' or 'severe'.[15] Treatment details during reactional episode were recorded separately on the proforma. Mild reaction was managed with rest and nonsteroidal anti-inflammatory drugs (NSAID). Moderate cases were managed on an outdoor basis on oral steroids and NSAID. Severe cases were hospitalized and oral steroids were given.

Results

Of the 86 patients enrolled in the study, 45 were inducted in the vaccine group and 41 in the control group (Table 1).

A total of 11 patients in the vaccine group had a previous history of Type 2 reaction and 10 of these showed subsequent reactions during the 2 years or more of chemoimmunotherapy; 1 patient with a previous history of 2 reactional episodes before treatment had no ENL during treatment. In the control group, 8 patients had a previous history of ENL. During 2 or more years of treatment with MDT, 12 patients had reactions. Thus, 4 patients with no previous history of ENL had first-time reactions after treatment in this group.

During the course of 2 or more years of follow-up, 20 reactional episodes in 10 (out of 37) BL and LL patients were recorded in the vaccine group (Table 2). The number of episodes were higher in the control group where 29 were seen in 12 (out of 34) BL and LL patients.

With regard to the onset of Type 2 reactions (Table 3), in the vaccine group, reactions were seen throughout 2 or more years, although most occurred within the 1st year (13 out

Table 2. Incidence of reactional episodes during treatment

Group	BL	LL
Vaccine	4 (2/14)	16 (8/23)
Control	5 (2/14)	24 (10/20)

Figures in parentheses give the number of patients experiencing reactional episodes by total number of patients; 11 patients in the vaccine group and 8 patients in the control group had a history of reaction prior to initiation of therapy.

Table 3. Onset of reactional episodes (in months) and their severity

	Group							
	0–3	3–6	6–12	12–24	> 24	Mild	Moderate	Severe
Vaccine	3	4	6	4	3	15	4	1
Control	8	2	3	10	6	15	11	3

Table 4. Frequency of reactional episodes

	Group			
	Once	Twice	Thrice	> Thrice
Vaccine (No. of patients)	3	4	3	—
Control (No. of patients)	3	4	2	3

Table 5. Changes in BI values (mean ± SEM)

Type of disease	Group	No. of patients	Initial	Months			
				6	12	18	24
LL	Vaccine	23	3·90 ± 0·27	2·78 ± 0·25	2·21 ± 0·25	1·35 ± 0·28 ($p < 0·03$)	0·92 ± 0·28 ($p < 0·06$)
	Control	20	3·67 ± 0·25	3·02 ± 0·27	2·59 ± 0·24	2·34 ± 0·27	1·78 ± 0·29
BL	Vaccine	14	2·51 ± 0·29	1·22 ± 0·20	0·53 ± 0·15 ($p < 0·003$)	0·24 ± 0·07 ($p < 0·001$)	0·02 ± 0·01 ($p < 0·003$)
	Control	14	2·02 ± 0·30	1·66 ± 0·26	1·41 ± 0·26	1·06 ± 0·19	0·63 ± 0·20

All statistical analyses were done using analysis of variance. Significant decreases in BI in vaccinated patients were seen in BL and LL types of leprosy. The p values are indicated in parentheses.

of 20); 3 episodes occurred beyond 2 years. In the control group, 13 out of 29 reactional episodes occurred in the 1st year and 16 out of 29 beyond 1 year; 6 episodes were recorded after 2 years.

With reference to severity of reactional episodes, reactions were mild in 15 out of 20 episodes in the vaccine group and 15 out of 29 in the control group; 14 reactions were moderate to severe in the control group (Table 3).

Frequency of reactional episodes in vaccine and control groups were: vaccine, 3 patients (30%) once, 4 (40%) twice and 3 (30%) thrice; and control, 3 (25%) once, 4 (33%) twice, 2 (16·6%) thrice and 3 patients (25%) more than 3 episodes (Table 4).

In the vaccine group 5 patients (2-BL, 3-LL) had neuritis during episodes of ENL. This resulted in a left ulnar claw in a BL patient and a bilateral claw in an LL patient. In

Table 6. Analysis based on 95% Confidence interval

Group	Incidence (%)	Confidence interval (%)
Incidence of reactional episodes		
Vaccine	27	−13·2 to 29·8
Control	35	
Reactions occurring early (within 1st year)		
Vaccine	65	−7·5 to 47·8
Control	44·8	
Reactions being mild		
Vaccine	75	−3 to 49·57
Control	51·7	

the control group neuritis was seen in 6 patients (2-BL, 4-LL); 1 BL patient had a left ulnar claw and facial palsy.

BI CHANGES

Changes in the BI of patients in the study are given in Table 5. The fall in LL patients receiving the vaccine was from 3·90 (±0·27 SEM) to 0·92 (±0·28 SEM) after 2 years of immunochemotherapy, whereas in the control group corresponding values were 3·67 (±0·25 SEM) to 1·78 (±0·29 SEM). In BL type leprosy the fall over 2 years was 2·51 (±0·29 SEM) to 0·02 (±0·01 SEM) in the vaccine group and 2·02 (±0·30 SEM) to 0·63 (±0·20 SEM) in the control group.

Discussion

Erythema nodosum leprosum (ENL) is triggered when there is an abundance of fragmented or granular bacilli in tissues. These 'nonviable' bacillary forms act as antigens and in the presence of precipitating antibodies and complement factors result in formation of immune complexes.[4]

Data regarding the incidence of Type 2 (ENL) reactions with use of MDT is scarce. In 2 studies, MDT seems to have caused no worsening or development of fresh reactions over monotherapy (in a 2-year follow-up).[16,17] In another study in Madras, 62 out of 177 (35%) BL or LL patients showed evidence of Type 2 reactions.[18] Some of the patients in this study showed reactions even up to 60 months of follow-up. In our experience, the incidence of reactions in the control group (receiving MDT alone) BL and LL patients was 12/34 (35·29%), which is quite comparable with reports in the literature. After immunization with *M.w* vaccine, patients had accelerated bacteriological clearance. The fall at the end of 2 years of treatment was statistically significant in both LL ($p < 0{\cdot}06$) and BL ($p < 0{\cdot}003$) vaccine group patients. In fact, the BI after 12 months of treatment in vaccine group BL patients was lower than that achieved by control group patients at 24

months (Table 5). Several earlier reports have also documented a rapid clearance of bacilli from slit-skin smear sites[19,20] and from biopsy tissues[13] in *M.w* vaccinated patients. Some patients with high BI of 5–6 have shown clearance of AFB within 18–24 months.[20] BI fall per 12 treatment months in LL patients in the vaccine group was reported to be 1·84 (±0·18 SEM) compared to 0·98 (±0·11 SEM) in the control group. In BL patients, it was 1·64 (±0·20 SEM) (vaccine group) and 0·63 (±0·14 SEM) (control group).[19] This rapid clearance of AFB from the body may explain: 1, less incidence of reactions in the vaccine *vs* control group (10 patients/20 episodes *vs* 12 patients/29 episodes); and 2, most reactional episodes occurring early in the vaccine group (13 out of 20 in the 1st year (65%), 17 out of 20 by the 2nd year (85%) and only 3 episodes beyond 2 years (15%)). In the control group figures were 13/29 (44·82%) in the 1st year, 23/29 (79·31%) by the 2nd year and 6 episodes beyond 2 years (20·6%). The reactions were also less severe in the vaccine group; 15 of 20 reactions (75%) were mild, 4 (20%) were moderate and only 1 severe (5%). In contrast, patients in the control group had 15 (51·72%) mild, 11 (37%) moderate and 3 (10·3%) severe reactional episodes. Frequency of reactional episodes was quite comparable in the 2 groups. The difference was that in the control group 3 patients (25%) had more than 3 episodes, which was not seen among vaccinated patients. Statistical analysis of data using test of proportions showed insignificant differences between the vaccine and control groups for incidence of reactional episodes ($z = 0{\cdot}7525$), early occurrence of reactions (within the 1st year) ($z = 1{\cdot}39$) and being milder in the vaccinated patients ($z = 1{\cdot}6435$). As can be seen by the *'z'* values the differences were at the margin/border of being significant. One reason for this could be the small sample size.

However, analysis based on a 95% confidence interval[21] brought out distinct differences between the 2 groups more evocatively (Table 6). Confidence intervals indicated that at best the vaccine was a great advantage (29·8; 47·8; 49·57%) and at worst a slight disadvantage (−13·2; −7·5; −3%) (see Table 6).

Immunotherapy with *M. vaccae* in patients with chronic repeated ENL has shown resolution of reaction some 2–3 months later in about half the cases in an on-going study.[22] Using immunotherapy with killed *M. leprae* and live BCG, Convit *et al.*[23] reported that vaccination coincided with the disappearance of ENL lesions in some patients.

When using ICRC vaccine, Deo *et al.*[24] reported that half the vaccinated LL patients, with BI 3–5, developed ENL reactions 2–6 weeks postvaccination. These patients had no previous history of ENL. ENL in these patients, according to the authors, was precipitated by either the presence of appropriate antigens of ICRC bacillus or large quantities of antigens released through breakdown of *M. leprae* upon vaccination.[24] Effects of immunotherapeutic cytokines gamma interferon and interleukin 2 on ENL reactions have not been reported in the literature.

An important observation was that the use of *M.w* vaccine did not precipitate any additional incidence of neuritis or deformity during the course of Type 2 reactions. This has also been evidenced histologically where vaccinated patients did not show evidence of an increase in dermal nerve twig inflammation.[13]

Experiences using immunotherapeutic candidate vaccines, 1, *M. leprae* and BCG, and 2, *M. vaccae* indicate that there is a decrease in incidence of type 2 reactions postvaccination. This is similar to our experience using *M.w* vaccine, where a decrease in incidence, frequency and severity of reactions have been recorded. Contrary to these findings are the results with ICRC vaccine (where half of LL patients with BI 3–5 had

ENL reactions postvaccination). Based on our experience and that of others, it appears that any immunotherapeutic agent capable of inducing rapid bacterial clearance should also secondarily decrease the incidence of Type 2 reactions.

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L'immunothérapie par le vaccin *Mycobacterium w* réduit l'incidence et la sévérité des réactions de type 2 (ENL)

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Résumé L'immunothérapie par le vaccin *Mycobacterium w* (*M.w*) a été administrée à 45 patients atteints de lèpre multibacillaire; 41 patients classés de façon similaire ont servi de témoins. Tous les patients ont reçu la thérapeutique multidrogue standard (MDT). L'incidence, la sévérité et la fréquence des épisodes réactionnels de type 2 (ENL) ont été suivis dans ces 2 groupes pendant une période qui a duré jusqu'à 4 ans. On a observé des réactions chez un plus petit nombre de patients BL et LL vaccinés (10/37) que dans le groupe témoin (12/24). Au total, 20 épisodes ont été enregistrés dans le groupe des vaccinés contre 29 dans le groupe des témoins, 75% des réactions ont été faibles dans le groupe des vaccinés et 51,72% ont été faibles dans celui des témoins, et 3 patients du groupe des témoins ont eu plus de 3 épisodes réactionnels, ce que l'on n'a observé chez aucun des patients vaccinés. Aucune incidence supplémentaire de névrite n'a été observée parmi les individus vaccinés au cours des épisodes réactionnels.

La inmunoterapia con vacuna *Mycobacterium-w* reduce la incidencia y severidad de las reacciones (ENL) de tipo 2

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Resumen Se administró inmunoterapia con vacuna *Mycobacterium-w* (*M.w*) a 45 pacientes con lepra multibacilar (MB); 41 pacientes con clasificaciones similares actuaron de controles. Todos los pacientes recibieron la terapia multidroga (MDT) normal. Se controló la incidencia, severidad y frecuencia de episodios reaccionales (ENL) de tipo 2 en ambos grupos en un estudio posterior de hasta 4 años. Se observaron reacciones en menos pacientes vacunados BL y LL (10/37) que en el grupo de control (12/34). Se registraron un total de 20 episodios en el grupo vacunado comparado con 29 en el grupo de control. 75% de las reacciones eran leves en el grupo vacunado, y 51, 72% eran leves en el grupo de control, y 3 pacientes en el grupo de control tuvieron más de 3 episodios reaccionales. No se observó esto en ninguno de los pacientes vacunados. No se observó una incidencia adicional de neuritis entre los individuos vacunados durante los episodios reaccionales.