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Response of *Mycobacterium habana* vaccine in patients with lepromatous leprosy and their household contacts. A pilot clinical study

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Summary Single dose vaccination was carried out with *Mycobacterium habana* vaccine, 31 lepromatous leprosy cases receiving 1·5 mg (1·5mg=$6{\cdot}27 \times 10^8$ bacilli) and 36 household contacts randomly receiving 1·5, 2·0, 2·5 mg vaccine intradermally. Duration of study was 18 weeks. Vaccination induced lepromin conversion in 100% of lepromatous leprosy cases and lepromin negative household contacts and augmentation of lepromin reactivity in 100% of lepromin positive household contacts, which was stable for the 15 weeks duration of follow-up. The maximum augmentation in lepromin reactivity was obtained with 1·5 mg of vaccine, which is probably the supramaximal dose. Overall, post-vaccination, those without prior BCG vaccination scars showed higher mean values of lepromin augmentation. Local vaccination site changes included induration, ulceration, itching, pain and uncomplicated regional lymphadenopathy, all of which remitted spontaneously by 15 weeks. Systemic side-effects noted were pyrexia, ENL and jaundice, and were seen with no greater frequency than that reported in other vaccine trials. Overall, systemic side-effects were easily controlled and were not accompanied by clinically detectable nerve or ocular damage. The safety profile investigations revealed an increase in the mean values of Hb%, RBC count and PCV in household contacts and of PCV in lepromatous patients, post-vaccination. Alterations in the liver function tests were also observed in patients of lepromatous leprosy. Thus, *M. habana* vaccine appears to be useful in stimulating specific CMI against *M. leprae* as evidenced by increased lepromin reactivity.

Introduction

Any vaccine potentially effective against *Mycobacterium leprae* should induce immune responsiveness to *M. leprae.* Various vaccine trials against leprosy have conclusively shown

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changes in disease subtype, reduction in bacteriological and morphological indices, and protection in contacts of leprosy patients, postvaccination.[1–7] The specific CMI against *M. leprae* and disease status correlate well with the degree of lepromin reactivity (delayed type hypersensitivity; DTH) of an individual.[1,7–9]

Mycobacterium habana (M. simiae serovar-1, TMC 5135), an atypical mycobacterium belonging to Runyan group I, was obtained from the TMC Collection Center, Saranac Lake, New York, USA. *M. habana* vaccine was treated by gamma-irradiation of the culture at 300 kRad from a ^{60}CO source. The immunoreactive fractions of the vaccine have been characterized by Chaturvedi *et al.*[10] *Mycobacterium habana* protects mice against infectious challenge with *Mycobacterium leprae*[11–13] and induces lepromin positivity in monkeys.[14] The vaccine generated sufficient delayed-type hypersensitivity responses in mice against *M. leprae*.[15] Various subunit components of *M. habana*, namely 65 kDa protein[16] and 23 kDa protein,[17] have been isolated and their putative role in immunity against *M. leprae* described. Thus, *M. habana* might prove to be useful as a potential vaccine against leprosy.

This study evaluated the efficacy (change in lepromin reactivity), safety and tolerance of *M. habana* vaccine in humans.

Materials and methods

The entire preclinical data[18] on *M. habana* vaccine was submitted to the Drugs Controller of India and permission to undertake this clinical study was obtained. The study was also cleared by the Institutional Ethics Committee and informed written consent was obtained from all the subjects before inclusion into the study. Total duration of study was 18 weeks.

SUBJECTS AND STUDY DESIGN

Thirty-one lepromatous leprosy (LL) cases and 36 household contacts (HC) were vaccinated. Another group of 10 household contacts were included as controls; they were repeatedly lepromin tested but received no vaccine. Subjects under 12 years, pregnant women, those with major illnesses, and those who were taking immune modulatory drugs, were excluded from the study.

Patients with typical clinical features of lepromatous leprosy, who were lepromin negative, had numerous bacilli in their slit skin smears/skin biopsies and had typical features of lepromatous leprosy on histopathology were included in the study, irrespective of their duration of treatment.

Individuals from families having at least one index case of lepromatous leprosy and who had been residing with the index case for at least 5 years were randomly included as household contacts for vaccination and as controls. They were included irrespective of their lepromin status.

FOLLOW-UP AND EVALUATION OF LL CASES AND HOUSEHOLD CONTACTS

After the initial pre-vaccination history and clinical examination (general and systemic) and initial lepromin test, the subject was vaccinated (day 0), and closely followed for changes in their clinical status thereafter. Changes at the site of vaccination were noted as size of induration, ulceration, pain, itching, discharge and regional lymphadenopathy. LL cases were

assessed for their subjective feeling of well being and systemic reactions like pyrexia and erythema nodosum leprosum (ENL). Change in lepromin reactivity was read at +6 and +15 weeks post-vaccination, i.e. 3 weeks after each lepromin injection. Safety profile investigations (Table 3) were done before vaccination and subsequently at +3 and + 15 weeks after vaccination.

During the trial, standard WHO multidrug therapy for multibacillary leprosy was continued and any other concomitant medication was avoided as far as possible.

LL cases were evaluated clinically using a body chart, the body being divided into head and neck, both upper limbs, both lower limbs, chest and abdomen and back and buttocks. Patients were clinically examined for impairment of pain (pinprick), touch (wisp of cotton, tip of finger), temperature (test tubes with warm and cold water), vibration (128 Hz tuning fork) and reflexes in the distribution of the major nerves viz. great auricular, ulnar, median, lateral popliteal and posterior tibial nerves. Sensation was graded as normal, impaired (percent impairment from normal reference point) and absence of a particular modality. Corneal and conjunctival reflexes were tested. A detailed ophthalmic evaluation was performed. Motor system was examined for nutrition, power (Standard Medical Research Council Scale, grade 0–5), reflexes and tone. Electromyography, nerve conduction velocity ($n = 12$) and slit lamp examination were done wherever possible (few cases only).

Each household contact was thoroughly evaluated for evidence of leprosy and only those who were clinically free of the disease were included for vaccination or as controls. Evaluation included a history for skin lesions, anaesthetic patches or other sensory symptoms and detailed examination for skin lesions, thickened nerves and sensory system examination.

VACCINE

Single dose vaccination was done with *M. habana*, a whole cell killed vaccine provided by Central Drug Research Institute, Lucknow. Each millilitre of vaccine contained 15 mg of wet weight of bacilli (1·5 mg = $6{\cdot}27 \times 10^8$ AFB = 63·3 μg of protein). A single vaccine dose was injected intradermally into left upper deltoid region using calibrated tuberculin syringes. Local changes at vaccination site, systemic reactions and changes in lepromin reactivity were noted.

Contacts were randomly allocated to receive 1·5 (group I, $n = 11$), 2.0 (group II, $n = 13$) and 2·5 (group III, $n = 12$) mg vaccine, respectively, while lepromatous leprosy cases received 1·5 mg vaccine (group IV, $n = 31$). Controls (group V, $n = 10$) received consecutive doses of lepromin but no vaccine.

LEPROMIN

The lepromin (Mitsuda) test was performed using 0.1 ml of lepromin A, a suspension of killed *M. leprae*, obtained from GWL Hansen's Disease Centre, Carville, Louisiana, 70721, USA. Lepromin (0·1 ml) was injected intradermally at −3, +3 and +12 weeks (day 0 = day of vaccination) on the ventral surface of the right forearm at variable distances from each other and local response in the form of maximum diameter of nodule was read 3 weeks later. A response of 3 mm or greater was taken as a positive response.

BACTERIOLOGICAL INDICES (BI) AND HISTOPATHOLOGY

Slit skin smears were taken from six sites (two each from eyebrows, ear lobes and skin lesions). Slides were stained by the Ziehl–Neelsen method and BI was calculated according

to Ridley logarithmic scale. Skin punch biopsies were obtained for all LL cases from skin lesions.

Results

Lepromatous leprosy cases with disease duration ranging from 6 months to 3 years and were on standard WHO regimens for multibacillary leprosy at the time of vaccination. Patients had characteristic skin lesions at the time of vaccination, ranging from asymmetrical to bilaterally symmetrical shiny or erythematous macules, nodules and plaques with normal or impaired sensation and normal hair growth. Nerve thickening was seen in 60% of the cases. Impaired sensation (loss/impairment of temperature, pain, sweating and touch with relative preservation of vibration and reflexes) in the distribution of ulnar, median and lateral popliteal nerves was noted in 20% of cases. No patient had facial nerve palsy, median nerve palsy, claw hand, foot drop, glove and stocking anesthesia or deformities. Disability grading (WHO) was grade 0 in 80% and grade 1 in the above-mentioned 20% of cases. Slit skin smears were performed in 24 of the 31 lepromatous leprosy patients, and revealed a median bacteriological index of 5·1 (range = 4·8–6·0). Skin biopsy and histopathological examination were performed in all 31 LL cases, which showed the characteristic histology of lepromatous leprosy in all cases.

The age and sex distribution among cases and contacts are shown in Table 1. The age of contacts was significantly lower as compared to cases. The mean lepromin reaction in patients with lepromatous leprosy and various groups of household contacts is shown in Table 2.

LEPROMIN CONVERSION AND AUGMENTED LEPROMIN REACTIVITY

Single dose vaccination induced lepromin conversion in 100% of lepromatous leprosy cases and lepromin negative household contacts (one contact in group I, 2 contacts in group II, 1 contact in group III) and augmentation of lepromin reactivity in 100% of lepromin positive household contacts and was stable for the 15-week duration of follow-up. Post-lepromin scar formation was observed in 65% lepromatous leprosy cases and 72–77% of household contacts (Table 2). The maximum increment in lepromin reactivity occurred in the initial 6 weeks after vaccination, subsequent to which the response plateaued; but the increments in lepromin reactivity continued up to 15 weeks post-vaccination, when the follow-up was

Table 1. Age and sex distribution of lepromatous leprosy cases and contacts (mean ± SD)

Characteristic	Contacts ($n = 36$)	Cases ($n = 31$)
Age	I 24.2 ± 8.1***	38.4 ± 14.1
	II 29.2 ± 14.2*	
	III 30 ± 10.1*	
Sex	Male = 58.3% ($n = 21$)	Male = 64.5% ($n = 20$)
	Female = 41.6% ($n = 15$)	Female = 35.4% ($n = 11$)

*$P < 0.05$.
**$P < 0.01$.
*** $P < 0.001$.

Table 2. Effect of *M. habana* vaccination on lepromin (Mitsuda) reaction

Group	Clinical	No. of subjects	Dose of vaccine (mg)	Lepromin (Mitsuda) test (mean ± SD, mm)			Post-lepromin scar (15 weeks)*
				Initial	+6 weeks	+15 weeks	
I	HC	11	1·5	7·0 ± 2·7	11·7 ± 2·5*	13·7 ± 1·9*	72·2%
II	HC	13	2·0	5·3 ± 2·8	10·6 ± 4·1*	11·9 ± 3·7*	76·9%
III	HC	12	2·5	5·4 ± 1·9	8·8 ± 2·4*	9·3 ± 3·4*	75%
IV	LL	31	1·5	1·2 ± 1·4	7·3 ± 3·3*	8·5 ± 4·4*	64·5%
V	Controls	10	—	5·3 ± 1·8	5·5 ± 1·8	5·5 ± 1·8	–

HC = healthy household contact.
LL = lepromatous leprosy cases.
Controls underwent consecutive lepromin testing but did not receive the vaccine.
[a]Percentage of patients with post-lepromin scar.
*$P < 0·05$.

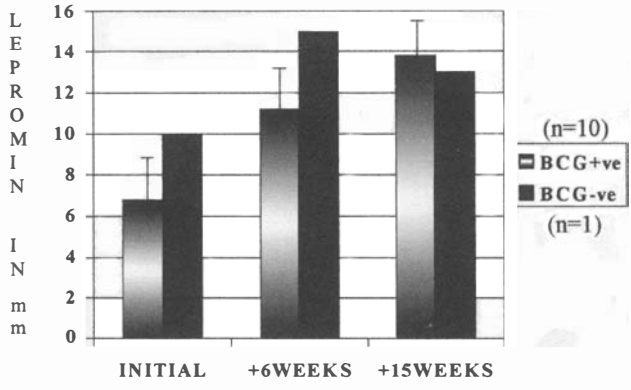
terminated. Among the contacts of lepromatous leprosy cases, comparable degree of augmentation in lepromin reactivity post-vaccination was observed with 1.5 mg and 2.0 mg vaccine, while a lesser degree of augmentation was seen with 2.5 mg vaccine. In 15% of cases, the size of the first lepromin test increased up to 15 weeks post-vaccination. Overall, post-vaccination, those without BCG vaccination scars showed higher mean values of lepromin augmentation (Figure 1A–D). There was no relation between lepromin reactivity and the gender of the subject.

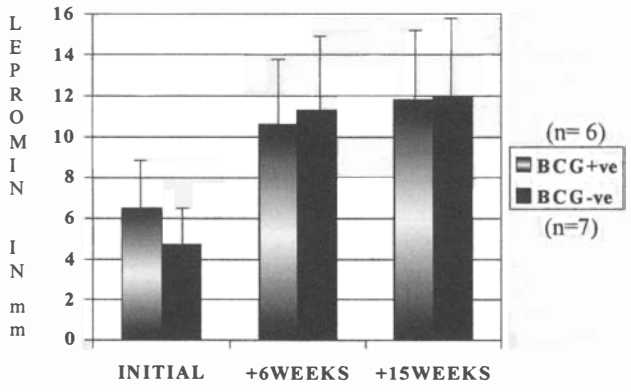
The control group of 10 household contacts of leprosy cases, of either sex, were given three lepromin injections consecutively (as per the schedule for those who received vaccination) but no vaccine. Initially, eight patients were lepromin positive (mean = 6 mm) and two were lepromin negative (<3 mm). The lepromin negative and six of the lepromin positive controls did not show any change in their consecutive lepromin reactivities. Two lepromin positive controls showed an increase of 1·0 mm in their subsequent lepromin reactivities (Table 2).

LOCAL SITE CHANGES

The administration of the vaccine did not produce any acute local reaction. The vaccine site remained quiescent for 7–8 days. Mild to moderate pain and tenderness started at the local site by 7–8 days post-vaccination in almost all cases and persisted for about 6 weeks. Induration started by days 10–12 and progressively increased till about day 16 and persisted thereafter for 7–8 weeks. The indurated area ulcerated by day 15–18 post-vaccination and started healing by 8–9 weeks (Figures 2, 3). Healing was usually complete by week 15 post-vaccination (Figure 4). An indurated nodule was formed in 100% of cases. Mean diameter of nodule among contacts was 18·5 mm (range = 15–23 mm) and among LL cases was 15·4 mm (range = 8–20 mm). However, there was no correlation between the degree of induration and lepromin reactivity.

Ulcers were formed in 100% of the cases and were punched out, with erythematous base and healthy looking granulation tissue. Mild serous or serosanguinous discharge was present. The mean diameter of ulcer among contacts was 7·5 mm (range = 4–10 mm) and among LL cases was 6·5 mm (range = 4·5–9·0 mm). The ulcer required no treatment in any case except local cleaning. The degree of ulceration and discomfort produced by vaccination was accepted by majority of subjects.





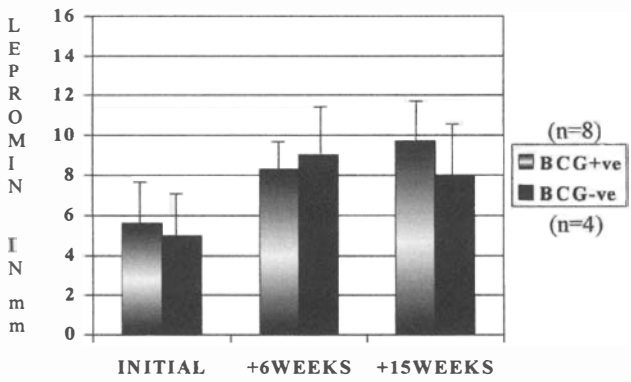


Figure 1. Lepromin reactivity (mean ± SD) in BCG scar positive and negative contacts and lepromatous leprosy (LL) cases, initially and after vaccination with *Mycobacterium habana* vaccine. (**A**) Eleven contacts, 1·5 mg vaccine; (**B**) 13 contacts, 2·0 mg vaccine; (**C**) 12 contacts, 2·5 mg vaccine; (**D**) 31 LL cases, 1·5 mg vaccine. Greater increase in lepromin reactivity was seen among BCG scar negative individuals (B & D).

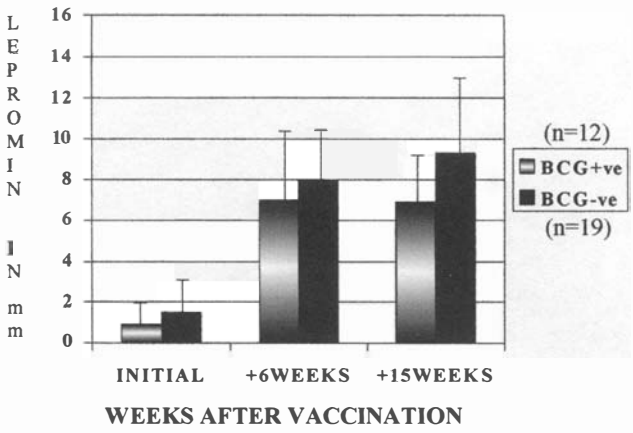


Figure 1. continued.

Regional lymphadenopathy, 0·5–1·5 cm non-tender, non-matted and non-suppurative, was observed in a small percentage of cases (n = 11/67), which resolved spontaneously.

SYSTEMIC REACTIONS

Some patients (n = 42/67; 19/31 LL cases, 23/36 contacts) developed mild to moderate pyrexia around days 3–4, which resolved in 7–10 days with antipyretics.

Twenty-six percent (n = 8/31) of the lepromatous leprosy cases developed ENL at 6–9 weeks post-vaccination. Of these, 25% (n = 2/8) had experienced previous episodes of ENL (ranging from one to three episodes). Patients developed mild to moderate pyrexia, aches,

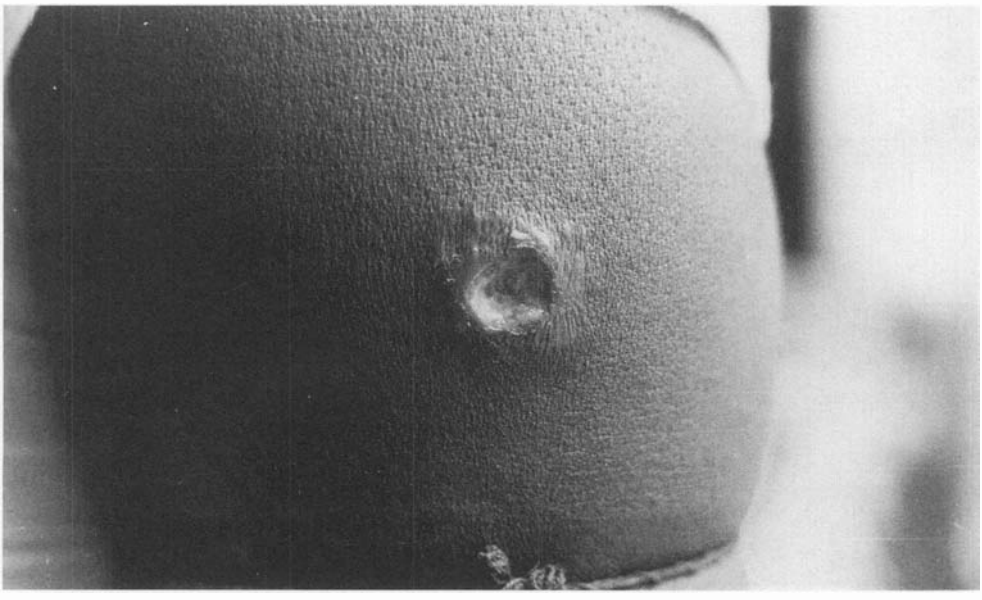


Figure 2. Vaccination site changes (induration and ulceration) 3 weeks after *M. habana* vaccination.

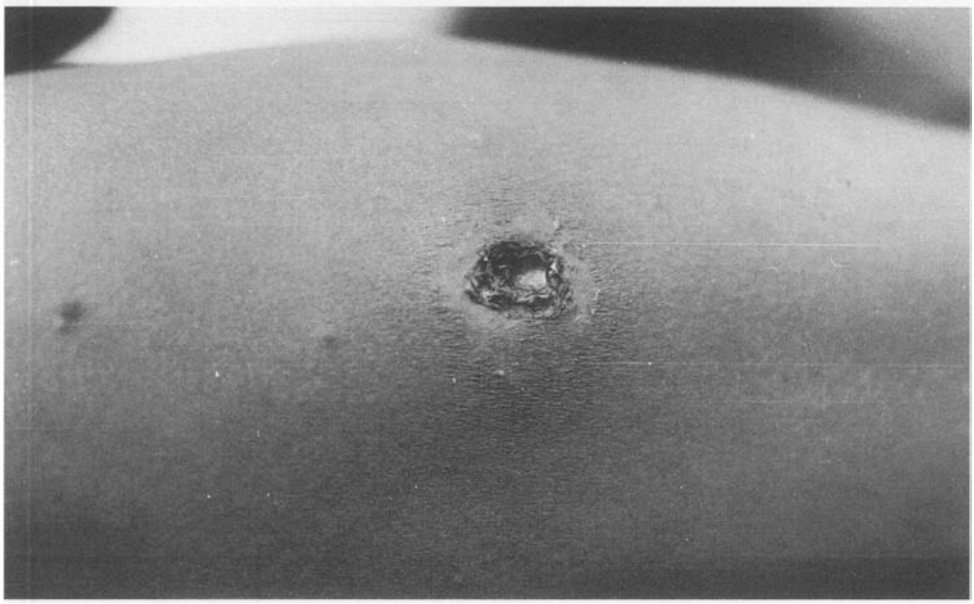


Figure 3. Vaccination site changes (induration and ulceration) 9 weeks after *M. habana* vaccination.

arthralgias (but no arthritis) and an increase in the size and number of cutaneous lesions in the form of tender, erythematous macules, nodules and plaques. The nerves became more tender on palpation, reflexes were exaggerated almost universally; however, there was no clinically manifest increase in or new development of hypoaesthesia or anaesthesia. There was no

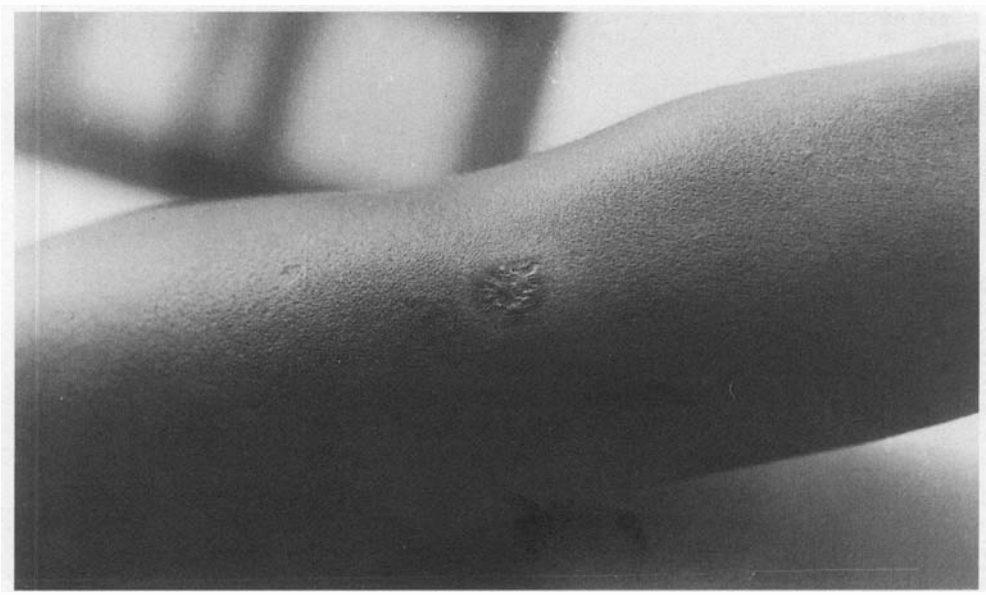


Figure 4. Vaccination site changes (healed scar) 15 weeks after *M. habana* vaccination.

Table 3. Mean values of safety profile investigations initially and after vaccination with *M. habana* vaccine in lepromatous leprosy cases (LL) and household contacts (HC) (mean ± SD)

Investigation	Group	Initial	+3 weeks	+15 weeks
Hb %	HC	11·8 ± 1·7	11·0 ± 1·7	12·2 ± 1·4
	LL	11·0 ± 1·8	12·5 ± 1·1*	11·7 ± 1·7
TLC (per mm^3)	HC	7431·4 ± 38·7	7758·1 ± 2018·3	7297 ± 894·2
	LL	7520 ± 1452	7170·6 ± 932·5	7384·2 ± 2018·3
Platelets (per mm^3)	HC	2·6 ± 0·4	2·7 ± 0·3	2·7 ± 0·2
	LL	2·6 ± 0·2	2·7 ± 0·4	2·7 ± 0·2
RBC (per mm^3)	HC	3·9 ± 0·4	3·7 ± 0·5	3·9 ± 0·4
	LL	3·4 ± 0·5	4·1 ± 0·3*	3·8 ± 0·5
ESR (mm 1st h)	HC	9·5 ± 6·3	10·4 ± 6·1	9·6 ± 4·5
	LL	9·6 ± 5·6	8·70 ± 6·9	10·0 ± 5·3
PCV (cc%)	HC	36·5 ± 5·5	33·6 ± 5·0	38·2 ± 5·6
	LL	34·8 ± 4·6	38·4 ± 3·6**	36·3 ± 4·5**
Blood urea (mmol/l)	HC	8·6 ± 2·8	9·8 ± 4·3	8·3 ± 1·4
	LL	8·7 ± 2·6	8·6 ± 3·1	9·4 ± 2·0
Serum creatinine (μmol/l)	HC	70·7 ± 8·8	70·7 ± 8·8	70·7 ± 8·8
	LL	79·5 ± 8·8	61·8 ± 8·8	79·5 ± 8·8
Random blood sugar (mmol/l)	HC	5·3 ± 2·7	5·2 ± 1·2	5·6 ± 1·4
	LL	5·0 ± 1·5	5·7 ± 3·0	5·1 ± 1·0
Serum bilirubin (μmol/l)	HC	13·6 ± 3·4	13·6 ± 3·4	13·6 ± 3·4
	LL	15·3 ± 8·5	13·6 ± 3·4	17·1 ± 13·6
SGOT (IU/l)	HC	24·1 ± 6·1	24·7 ± 8·4	25·9 ± 14·4
	LL	23·7 ± 4·9	25·6 ± 9·9	29·7 ± 8·9*
SGPT (IU/l)	HC	33·3 ± 21·8	30·7 ± 18·1	31·5 ± 20·4
	LL	35·9 ± 26·5	27·4 ± 12·1	40·2 ± 35·3
Serum alkaline phosphatase (IU/l)	HC	113 ± 63	99 ± 52	99 ± 46
	LL	97 ± 35	112 ± 46	116 ± 49*
Serum protein (g/l)	HC	82 ± 8	79 ± 7	82 ± 10
	LL	79 ± 6	76 ± 5*	77 ± 6*
Serum albumin (g/l)	HC	45 ± 4	44 ± 4	46 ± 3
	LL	44 ± 4	46 ± 3	42 ± 4
Serum uric acid (μmol/l)	HC	404·4 ± 118·9	422·3 ± 83·2	404·4 ± 95·1
	LL	386·6 ± 65·4	446·1 ± 190·3	422·3 ± 89·2
Serum cholesterol (mmol/l)	HC	3·8 ± 0·9	3·5 ± 0·9	3·6 ± 0·8
	LL	3·5 ± 0·8	4·2 ± 0·8	3·5 ± 0·4
Serum triglyceride (mmol/l)	HC	1·7 ± 0·8	1·5 ± 0·7	1·4 ± 0·5
	LL	1·9 ± 1·2	1·9 ± 0·8	1·7 ± 0·7
Serum HDL (mmol/l)	HC	0·9 ± 0·2	0·9 ± 0·1	0·9 ± 0·1
	LL	0·9 ± 0·1	0·9 ± 0·1	0·9 ± 0·1

*$P < 0·05$.
**$P < 0·01$.
***$P < 0·001$.
Hb, haemoglobin; TLC, total leukocyte count; RBC, red blood cell count; ESR, erythrocyte sedimentation rate; PCV, packed cell volume.

weakness or paralysis of muscle groups and no muscle wasting. These episodes were not associated with any organomegaly, cardiac or pulmonary abnormalities. There was no rhinitis, epistaxis or epididymo-orchitis. There was no swelling, discharge, or pain in the eye. Corneal reflexes were intact in all the patients. The episodes of ENL were easily controlled with antipyretics and non-steroidal anti-inflammatory drugs, requiring corticosteroids in only two cases. Duration of ENL was 3–4 weeks except in two cases where it lasted for 7–8 weeks. Overall, the systemic side effects were easily controlled and were not accompanied clinically by organ, nerve, or ocular damage.

Two patients, both of whom had ENL, developed concomitant clinical jaundice 4 weeks post-vaccination. In both cases, patients had been taking MDT for more than 6 months. The jaundice was insidious in onset, associated with a prodrome of nausea, vomiting and malaise 1 week prior to clinical detection. Both the patients had 3–4 cm tender, soft hepatomegaly with no splenomegaly. Serum bilirubin (patient 1, 3·5 mg%; patient 2, 4·5 mg%), SGOT (patient 1, 150 IU/l; patient 2, 210 IU/l) and SGPT (patient 1, 450 IU/l; patient 2, 600 IU/l) were elevated with marginally raised serum alkaline phosphatase (patient 1, 140 IU/l; patient 2, 160 IU/l). Ultrasound revealed hepatomegaly with a mild decrease in echogenicity and contracted gallbladder in both the cases. Rifampicin, dapsone and clofazimine were stopped on account of possible hepatotoxicity. Viral markers for HBsAg and anti-HCV were negative. Both patients were conservatively managed and recovered completely by the end of 3 weeks after clinical detection, after which drug therapy was re-started, without subsequent re-appearance of jaundice till the end of follow-up.

There was complete absence of nerve palsies both among cases and contacts, post-vaccination and no new sensory or motor loss was detected in these patients.

One lepromatous patient presented with bilateral pitting pedal oedema 3 weeks after vaccination, non-commensurate with his serum protein and albumin status, which had resolved completely by the end of the study.

Two patients presented 4–6 weeks post-vaccination with papulonodular eruptions all over the body. No features suggestive of ENL were present. The rashes subsided on their own at final follow-up.

INVESTIGATIONS

There was an increase in the mean values of Hb%, RBC count and PCV in household contacts and of PCV in lepromatous patients, post-vaccination. Alterations in the liver function tests were also observed in patients of lepromatous leprosy (Table 3), especially in the two who developed clinical jaundice and ENL. Slit lamp examination could not be performed due to constraint of resources. Other patients who developed ENL had no proteinuria and the liver and renal function tests were unremarkable.

Discussion

Leprosy has a very long incubation period, and years of observation will therefore be required to measure the efficacy of a vaccine in terms of its capacity to lower incidence rates. As a first step, it is essential to show that a 'candidate' vaccine is able to induce *persistent* changes in immunity as evidenced by changes in the Mitsuda lepromin reaction. Available clinical, laboratory and experimental evidence clearly show that the late lepromin (Mitsuda) reaction

is closely linked to the immune status of the host against *M. leprae*. Early work by Dharmendra and Chatterjee[8] has shown that lepromin-negative individuals in endemic areas run a very high risk of contracting the multibacillary forms of the disease.

One hundred percent lepromin conversion or augmentation, which was observed in the present study with a single dose of vaccine, has been rarely reported with administration of other vaccines.[1–5,19] In the present study, the maximum change in lepromin reactivity was observed in the first 6 weeks post-vaccination, after which the degree of rise, although continuing for 15 weeks, plateaued off. Further follow-up will be required to ascertain whether this gradual rise continues, stabilizes or steadily declines. The maximum increment in lepromin reactivity with other vaccines has been variably described from 1 month onwards; most have described peak values at 8–9 months post-vaccination with stability for 3–5 years of follow-up.

The present study has demonstrated that the maximum lepromin augmentation or conversion was obtained with the smallest dose of 1·5 mg vaccine. That 1·5 mg vaccine is probably the maximum tolerated dose and requires reduction is further suggested by the constellation of reduced responses with higher doses of 2·5 mg and higher incidence of ENL reaction.

The diminished responses seen with higher doses of vaccine can be explained by either suppression of immature B-cells by antigen excess or the suppression of cell mediated immunity by 'blocking' immune complexes.

The degree of lepromin conversion or augmentation in lepromin reactivity was consistently less among BCG scar positive individuals. This is contrary to other leprosy vaccine trials, which show a higher degree of augmentation among BCG vaccinated individuals.[1]

The phenomenon of augmentation in the nodule size of prevaccination lepromin after vaccination has also been reported by Deo *et al.*[2] and probably reflects continuing delayed reactivity at site of first lepromin test; the clearance of *M. leprae* is markedly retarded in lepromatous leprosy patients as shown by Convit *et al.*[20]

Sixty to 75% of all subjects in the present study developed post-lepromin scars, which have been proposed as indicators of stable changes in cell mediated immunity by Dharmendra[9] and Walters.[21]

A control group of unvaccinated, healthy household contacts who were repeatedly lepromin tested did not exhibit any change in subsequent lepromin reactivity, indicating that the conversion was not merely a consequence of the previous lepromin test and that lepromin did not act as a mini-vaccine. It has also been demonstrated that *M. leprae* bacilli (armadillo grown), at 4 times the concentration contained in lepromin, failed to induce lepromin conversion.[22] Household contacts were chosen as controls because they had a greater chance of reacting to lepromin as compared to LL cases who are anergic to lepromin (lepromin negative). Since repeated lepromin testing did not alter the lepromin reactivity of lepromin positive or lepromin negative household contacts, lepromin negative LL cases were not included as controls subsequently.

The local vaccination site changes observed with *M. habana* vaccination are similar to those observed with various other vaccines.[2,4,6] The large induration and ulceration occurring in a number of vaccinated subjects may, by itself, not be considered as an unacceptable side effect of the vaccine, given that most people have accepted both BCG and smallpox vaccines. None of the local site changes bore any correlation with lepromin conversion in the present study.

Zaheer *et al.* observed type I upgrading reactions in 45.2% of their vaccinated

lepromatous leprosy cases.[4] We did not observe type I reactions during our study, which could be due to the short duration of our study or because only a single dose of vaccine was given in our trial as opposed to the other vaccine trials.[4]

Twenty-six percent of lepromatous leprosy patients in the present study developed type 2 reactions in variable time duration of 6–9 weeks post-vaccination. Development of ENL after vaccination has been described by Deo *et al.* with ICRC vaccine, to occur 3–4 weeks after vaccination in 42·2% cases with high bacteriological index.[2] ENL after vaccination has also been described by Zaheer *et al.* with *Mycobacterium w* vaccine, to occur in 56·6% of lepromatous leprosy cases, while unvaccinated patients taken as controls developed ENL in 60·2% cases.[4]

Two cases of ENL in the present study developed jaundice; The jaundice could have been a result of concomitant viral hepatitis or due to ENL. Convit *et al.* have observed two cases of hepatitis with jaundice in borderline leprosy cases with reversal phenomenon post vaccination.[6]

Two lepromatous patients in the present study developed a papulonodular eruption 4–6 weeks after vaccination. This has been previously reported by Convit *et al.*,[6] who demonstrated tuberculoid histology of the papules. The rash was seen to subside on its own at subsequent follow-ups.

The changes in safety profile investigations did not reveal any abnormality that could directly be attributed to the vaccine except for changes in liver function tests in lepromatous leprosy cases which could also be due to concomitant multi drug therapy regimen or due to the nature of the disease itself.

Thus, *M. habana* vaccine appears to be useful in stimulating specific cell mediated immunity against *M. leprae* as evidenced by lepromin conversion or augmentation of reactivity observed in the present study. Further, the vaccine, by virtue of minimal, well tolerated side-effects and good acceptability among the trial population, may be deemed tolerable.

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