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Regional Lymphadenitis Following Antileprosy Vaccine BCG with Killed *Mycobacterium leprae*[1]

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An effective antileprosy vaccine should protect against infection by *Mycobacterium leprae* or have therapeutic value against the disease. A number of potential vaccines based on either live BCG alone or with killed *M. leprae* (KML) or other killed mycobacteria such as ICRC, *Mycobacterium w.* and *M. vaccae* have been developed and have been claimed to be immunotherapeutic ([2, 4, 18, 20]). Some of them are currently being evaluated for their immunoprophylactic efficacy against leprosy ([6]). The combination vaccine BCG plus KML was also tested for immunoprophylactic efficacy, and it was seen that there were 18% fewer cases from the vaccine group BCG plus KML than from the BCG group ([3]). But, no difference was seen between the BCG plus KML group and the BCG group among the general population in the Karonga Prevention Trial ([11]). Apart from being effective, a good vaccine needs to be safe with minimal side effects so that it is acceptable to the population using it. In Phase-II and extended Phase-II studies, three different population sets in Thiruthani taluk of Chengai-MGR District in Tamil Nadu, India, were tested during the period between August 1989 and October 1990 to ascertain the acceptability of the vaccines and the side effects, if any. In this communication, we are reporting on episodes of regional lymphadenitis in subjects who received BCG plus KML.

MATERIALS AND METHODS

Skin-test antigens and vaccines

The following biologicals were used in the study: a) BCG batch 308, June 1989 (viability count of 6.6×10^6 per ml) supplied by BCG Laboratory, Madras, India, as 0.1 mg per dose; b) armadillo-derived killed *M. leprae* Lot IV; c) Rees' *M. leprae* soluble skin test antigen (MLSA) Lot Wel-4-EF1 as 1 μg protein per dose and d) lepromin-A Lot J-15-4, 21 7 88 as 30–40 million bacilli per ml supplied by IMMLEP. The doses of the various vaccines/placebos per 0.1 ml used for the Phase-II study were: a) BCG 0.1 mg + 6×10^8 KML; b) BCG 0.1 mg + 5×10^7 KML; c) BCG 0.1 mg + 5×10^6 KML; d) BCG 0.1 mg, or e) normal saline. For the extended Phase-II study they were: a) BCG 0.05 mg + 6×10^8 KML; b) BCG 0.05 mg + 5×10^7 KML; c) BCG 0.01 mg + 5×10^7 KML, or d) normal saline.

Subjects and follow up

In the Phase-II study, a total of 997 healthy volunteers (free from clinical leprosy and free from other contraindications for vaccines) in the age group 1–70 years were vaccinated on randomization with one of the four vaccines in 0.1 ml or with the control preparation. The study population included 247 children in the 1–6-year age group and 250 children in the 7–12-year age group; the remaining 500 individuals were in the 13–70-year age group. Vaccine was administered intradermally into the left deltoid region.

Further, the vaccinees were skin tested with Rees' MLSA on the upper third of the dorsum of the left forearm and with lepromin-A on the midvolar aspect of the right

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TABLE 1. *Phase-II suppurative regional lymphadenitis (left axillary).*[a]

Age Group	Vaccine arms							
	BCG 0.1 mg + 6×10^8 KML		BCG 0.1 mg + 5×10^7 KML		BCG 0.1 mg + 5×10^6 KML		BCG 0.1 mg	
	Vaccinees	Adenitis	Vaccinees	Adenitis	Vaccinees	Adenitis	Vaccinees	Adenitis
1–6	50	0	50	—	48	—	49	—
7–12	50	1 (2%)	50	—	50	—	50	—
13–70	100	6[b] (6%)	100	3 (3%)	100	3 (3%)	100	—
All (Total)	200	7 (3.5%)	200	3 (1.5%)	198	3	199	—

[a] Control arm: 0.1 ml normal saline in 200 individuals (50, 50, 100 in the three age groups).
[b] One case left supraclavicular.

forearm at the time of vaccination and at 12 weeks post-vaccination. Readings were taken for induration to ascertain the sensitization potential of the vaccines. The vaccine site was examined at 3, 8, 12 and 15 weeks post-vaccination. Details have been published earlier ([7, 9]).

In the extended Phase-II study, in one group of villages, 860 healthy volunteers in the age group 1–70 years were vaccinated similarly with one of the three combination vaccines or placebo, skin tested with Rees' MLSA and lepromin-A at 12 weeks post-vaccination, and readings were taken as in the Phase-II study. In another village, 437 healthy subjects in the age group 13–70 years were vaccinated with BCG 0.1 mg in 0.1 ml. Details have been published earlier ([9]).

Follow up

All of the vaccinees were kept under surveillance to monitor for post-vaccination side effects, if any. In the Phase-II study, the study villages were visited once a week by a medical officer and vaccine-related complaints were recorded and followed up subsequently as detailed earlier ([7, 9]).

In the extended Phase-II study, the study subjects were visited on a weekly basis and specific enquiries were made for vaccine-related side effects, particularly regional lymph node enlargement. Clinical findings were recorded every week until healing took place.

Laboratory methods

In the Phase-II study, blood samples were collected from all vaccinees, and the serum was tested for phenolic glycolipid-I (PGL-I) antibody level by an ELISA and anti-35-kDa protein antibody by the serum antibody competition test (SACT).

In the extended Phase-II study, lymph node biopsies were obtained under local anesthesia and were divided into two parts. One part was processed for culture for acid-fast bacilli (AFB) and also for non-AFB organisms. The other part was fixed in 10% formalin and routinely processed. Paraffin-embedded sections were stained with a) hematoxylin and eosin and b) by the Fite-Farraco method for AFB. Further, the tissues were stained with anti-BCG antibody (DAKO Corporation, Copenhagen, Denmark) using the indirect immunoperoxidase method) ([12]).

RESULTS

Phase-II

Out of 997 vaccinees, 13 individuals developed signs of regional suppurative lymphadenitis. All of these individuals were followed up clinically. Of these 13, 7 were in the group that received BCG 0.1 mg + 6×10^8 KML, 3 each in the two groups that received BCG 0.1 mg + 5×10^7 KML and BCG 0.1 mg + 5×10^6 KML, respectively. One subject in the group BCG 0.1 mg + 6×10^8 KML was in the age group 7–12 years; the remaining 12 were in the age group 13–70 years (Table 1).

The course of events leading to suppurative adenitis in all of the individuals was similar as judged by their histories and observations. Regional lymph node (axillary or supraclavicular) enlargement was first noticed by these vaccinees around week 2, and it progressed to suppuration by week 8. Of these 13 cases, 7 were treated with incision and drainage; in the remaining 6 sub-

TABLE 2. *Antibody levels according to BCG scar status.*[a]

Anti-PGL-I antibody titer (OD value)	BCG scar		No BCG scar	
	No.	%	No.	%
0.00 – 0.03	272 (4)	89.5	561 (7)	83.2
0.04 – 0.07	25 (1)	8.2	71	10.5
0.08 – 0.11	5	1.6	30 (1)	4.5
0.12 – 0.15	2	0.7	10	1.5
0.16 +	0	—	2	0.3
Total	304 (5)	100.0	674[b] (8)	100.0

[a] Numbers with regional suppurative adenitis are shown in parentheses.
[b] $p > 0.5$.

jects the abscess opened and drained by itself. Except for cleaning and dressing with sterile dry gauze, no treatment was needed and recovery was uneventful.

Previous BCG scar status and serology (antibodies against PGL-I and 35-kDa protein of *M. leprae*) did not reveal any helpful clues to identify the group at risk. It was interesting to note that the anti-PGL-I antibody and the anti-35-kDa protein antibody levels were extremely low in this population (Table 2). Out of 978 serum samples tested by ELISA, 833 (85.2%) showed anti-PGL-I antibody levels in the range of 0.00 to 0.03 OD units, 96 (9.8%) samples in the range of 0.04 to 0.07 OD units, and 49 (5.0%) in the range of 0.08 to 0.16 OD units. Among the 13 individuals with suppurative adenitis, 1 individual (14 years, BCG scar negative group) showed an antibody level of 0.09 OD units; 1 individual (38 years, BCG scar positive group), 0.04 OD units; all of the other 11 individuals, 0.00 OD units. Similarly, only 4 individuals (3 in BCG scar positive group and 1 in the BCG scar negative group) out of 978 samples tested were positive by SACT, and all of these four belonged to the 13–70-year age group. All 13 individuals with suppurative adenitis were negative by SACT. Suppurative adenitis was observed in 5 out of 304 vaccinees in the BCG scar positive group and in 8 out of 674 BCG scar negative group ($p > 0.5$). It was found that suppurative adenitis had a direct relationship to the age of the subjects and the dose of the antigens. Among 13 individuals with suppurative adenitis, 12 were from the 13–70-year age group; 6 of these 12 individuals were from the vaccinees who received the highest dose, BCG 0.1 mg + 6×10^8 KML. None of the individuals in the 1–6-year age group, even with highest dose, developed suppurative adenitis.

Extended Phase-II

BCG 0.1 mg was given to 437 subjects in the age group 13–70 years and two of them had an enlarged left axillary lymph node by week 2 after vaccination. By week 8, the lymph nodes were not palpable. No case of suppurative adenitis was observed in this group.

With the combination vaccine BCG plus KML, we observed four cases of suppurative regional lymphadenitis. Of the 200 individuals who were vaccinated with BCG 0.05 mg + 6×10^8 KML, eight developed transient regional lymphadenitis which did not require any treatment and subsided uneventfully by week 15. Two vaccinees developed suppurative adenitis; one male (age 9) had a left supraclavicular lymph node involvement and another male (age 13) presented with a left axillary lymph node involvement. The onset and sequence of events leading to suppuration were similar to the observations in the Phase-II study. Lymph node specimens from both of these cases subjected for culture were negative for both AFB and non-AFB organisms. Histologically, AFB and mycobacterial antigens could not be demonstrated in either case. However, epithelioid cells, fibroblasts, plasma cells and few poorly formed giant cells were seen. In addition, the supraclavicular lymph node showed small areas of necrosis with minimal karyorrhexis.

Two other cases were seen with the lower dose of combination vaccines. One male

TABLE 3. *Extended Phase-II study: regional lymphadenitis.*

Age group	Vaccine arms											
	BCG 0.05 + 6×10^8 KML			BCG 0.05 + 5×10^7 KML			BCG 0.01 + 5×10^7 KML			BCG 0.1 mg		
	V[a]	E[b]	S[c]	V	E	S	V	E	S	V	E	S
1–6	50	—	—	50	—	—	50	—	—	—	—	—
7–12	50	3	1(2%)	50	—	—	50	1	1(2%)	—	—	—
13 +	100	7	1(1%)	100	1	1(1%)	100	—	—	437	2	—
All (Total)	200	10	2(1%)	200	1	1(0.5%)	200	1	1(0.5%)	437	2	—

[a] V = Vaccinees.
[b] E = Enlarged lymph node.
[c] S = Suppurative adenitis.

(age 40) from the vaccine group BCG 0.05 mg + 5×10^7 KML and one male child (age 12) from the BCG 0.01 mg + 5×10^7 KML group developed left axillary suppurative adenitis (Table 3). The onset of lymph node enlargement was seen by week 16 and week 18, respectively, and suppuration by weeks 18 and 20.

Histologically, these two cases resembled the adenitis seen in the patients given BCG 0.05 mg + 6×10^8 KML. However, in one of them (12-year-old male given BCG 0.01 mg + 5×10^7 KML) a minimal amount of mycobacterial antigen could be demonstrated. Culture from the biopsy material was negative for both AFB and non-AFB organisms. It was seen that 8 out of 12 individuals with lymph node enlargement were from the 13–70-year age group receiving combination vaccines and 2 among this group developed suppurative adenitis.

Post-lepromin test regional adenitis

In addition to the above, two subjects developed regional adenitis following lepromin testing. Laboratory investigations were not performed on them. Their brief case histories are given below.

A female (age 38) who was vaccinated with BCG 0.1 mg + 6×10^8 KML in the Phase-II study had suppurative axillary lymphadenitis at 8 weeks post-vaccination. She was skin tested at 12 weeks post-vaccination with Rees' antigen and lepromin-A. A week later she developed fever and supratrochlear lymph node enlargement on the side of the lepromin test. This adenitis gradually subsided in 3 weeks.

In a 13-year-old female, who received BCG 0.01 mg + 5×10^7 KML, the vaccination lesion healed uneventfully without any regional lymph node enlargement, but she developed lymph node enlargement on the ipsilateral supratrochlear region 4 weeks after retesting with lepromin-A. The lymph node suppurated and drained itself within about 3 weeks, subsequently healing and leaving a healthy scar.

DISCUSSION

The occurrence of local lymphadenopathy following immunization is a well-known phenomenon both in experimental animals and in man. Clinical enlargement of the local draining lymph node with BCG vaccination alone is reported to occur in 0.1% to 0.3% of the vaccinated individuals ([1]). Convit, *et al.* ([2, 3]) observed suppurative adenitis in an extremely small number of individuals—only 4 in a total of about 30,000 vaccinees in Venezuela. In the Karonga prevention trial, reference was made to glandular abscesses and exceedingly large ulcers by the authors ([15]).

In our experience, in the Phase-II study, suppurative adenitis was observed mainly in the subjects belonging to the 13–70-year age group. This occurred in 6 out of 100 individuals receiving BCG 0.1 mg + 6×10^8 KML and 3 out of 100 in those receiving either BCG 0.1 mg + 5×10^7 KML or BCG 0.1 mg + 5×10^6 KML.

In the younger age groups, 1 out of 50 vaccinees (7–12 years) who received the highest dose (BCG 0.1 mg + 6×10^8 KML) developed suppurative adenitis, but other vaccinees in the 1–6-year age group and all of those in the 1–6- and 7–12-year age groups who received BCG 0.1 mg + 5×10^7

KML and BCG 0.1 mg + 5×10^6 KML, respectively, remained free from complication.

In the extended Phase-II study, the occurrence of suppurative adenitis could be reduced to 1% by lowering the BCG dose alone to half, i.e., in the vaccine arm BCG 0.05 mg + 6×10^8 KML. However, further lowering of the BCG and KML dose did not result in totally overcoming the occurrence of suppurative adenitis (Table 3). It was interesting to note that in individuals receiving a lowered dose, BCG 0.05 + 5×10^7 KML and BCG 0.01 mg + 5×10^7 KML, lymph node enlargement was seen by weeks 16 and 18 post-vaccination and suppuration by about weeks 18 and 20, while with BCG 0.05 mg + 6×10^8 KML the suppuration occurred in two individuals by week 8.

Since the mycobacteria could not be grown in culture, it was clear that the adenitis was not due to progressive infection caused by BCG. Similarly, the biopsied material did not show the presence of non-acid-fast organisms, thus ruling out the possibility of bacterial contamination of the vaccine or superadded infection. The staining of tissues with antimycobacterial antibody using the immunoperoxidase technique is quite sensitive, and this method has been utilized to detect the presence of mycobacterial antigen in both human ([12]) and animal ([14]) tissues. Further, we used anti-BCG antibody since this has been shown to detect antigens which are common to both BCG and *M. leprae* ([10]). The absence of AFB in all of the cases and the minimal presence of mycobacterial antigen in only one case in the tissue indicated that the biopsies were probably performed in the resolving stage. Uneventful healing and subsidence of the lymph node swelling without any specific intervention measures would seem to support such a view.

Narayanan and his colleagues chartered the course of regional lymph node involvement following BCG, *M. leprae,* or *M. kansasii* vaccination in the guinea pig ([13]). They found that while BCG and *M. kansasii* induced maximum granuloma in the draining lymph node in 2–3 weeks, *M. leprae* took nearly 5 weeks to induce a granuloma. These granulomas almost completely resolved in about 10 weeks. In human beings, the development of lymph node abscess following BCG is very uncommon and virtually restricted to the first year of life, with local lymph node softening ([16]). Among children, the occurrence of suppurative adenitis may be due to deeper vaccination, higher dose of BCG, or a highly potent BCG strain ([5, 19]). In adults, one incident of local and regional lymph node abscess following a large overdose about 40 times higher than the normal dose had been recorded ([21]).

The present report is the first on the BCG and KML combination causing suppurative adenitis. However, in the present study, since none of the children in the 1–6-year age group developed suppurative adenitis and, moreover, in adults the course of events was also uneventful after drainage and did not require any therapy, the possibilities of deeper vaccination and a large overdose can be ruled out. The different time courses of lymphadenitis observed in the vaccinated individuals reported here could be related to a number of factors, such as the dose of the organisms, prior exposure of the individuals to mycobacteria and their innate ability to mount a hypersensitivity response. Earlier a high level of nonspecific sensitization to mycobacterial antigens had been demonstrated among the population of Chengalpattu District ([8]).

The precise mechanisms involved in the development of suppurative adenitis are not clear. Skin-test reaction to Rees' antigen and lepromin-A did not show any association with regional lymphadenitis ([7]). Although the levels of antibody against *M. leprae* in the serum were not elevated, one feature worthy of note in these cases was the large number of plasma cells observed in the biopsies from three subjects. The role of these cells remains speculative although antibodies have been implicated in the genesis of some forms of mycobacterial granuloma ([17]).

It is possible that high levels of specific and nonspecific mycobacterial sensitization are responsible for the occurrence of adenitis when the dose of the antigen is increased, especially in subjects belonging to the older age groups. None of the 636 subjects who received only BCG 0.1 mg developed suppurative adenitis. By merely reducing the BCG dose to half, the rate of suppurative adenitis was reduced dramatically to a minimal self-limiting process.

Adenitis appears to be more a function of total antigen load than any direct BCG effect. In view of the foregoing, it was decided that BCG 0.05 mg + 6×10^8 KML could be accepted for the large-scale vaccine trial in South India.

SUMMARY

Phase-II and extended Phase-II studies were conducted in three different sets of the population in Thiruthani taluk, Chengalpattu District, South India, involving BCG and killed *Mycobacterium leprae* (KML) combination vaccines to ascertain the acceptability of the vaccines. In the Phase-II study, 997 healthy volunteers were vaccinated on individual randomization with one of the vaccine arms: BCG 0.1 mg + 6×10^8 KML, BCG 0.1 mg + 5×10^7 KML, BCG 0.1 mg + 5×10^6 KML, BCG 0.1 mg or normal saline. Blood samples were taken and the serum was tested for antibody levels against phenolic glycolipid-I (PGL-I) and the 35-kDa protein of *M. leprae.* In this study, we observed regional suppurative adenitis in 6% (6 out of 100), 3% (3 out of 100), and 3% (3 out of 100) of the vaccinees in the BCG 0.1 mg + 6×10^8 KML, BCG 0.1 mg + 5×10^7 KML, and BCG 0.1 mg + 5×10^6 KML vaccine arms, respectively, in the 13–70-year age group. Earlier BCG scar status, skin-test reactions to lepromin-A, Rees' MLSA, and serum antibody levels against PGL-I and the 35-kDa protein did not help to identify the group at risk of developing suppurative adenitis. Suppurative adenitis appears to have a direct relationship between the age of the subject and the dose of the vaccine. In order to overcome the problem of regional suppurative adenitis and to know the mechanism involved, an extended Phase-II study was conducted in similar groups of the population by reducing the BCG and KML doses, i.e., with BCG 0.05 mg + 6×10^8 KML, BCG 0.05 mg + 5×10^7 KML, and BCG 0.01 mg + 5×10^7 KML. Biopsy specimens were collected from lymph nodes of the suppurative adenitis cases and were subjected for culture and histopathological examination. The observations showed that regional suppurative adenitis could be reduced to 1% in the BCG 0.05 + 6×10^8 KML group, 0.5% in the BCG 0.05 + 5×10^7 KML group, and 0.5% in the BCG 0.01 + 5×10^7 KML group. This phenomenon of suppurative adenitis appears to be related to the total dose of mycobacterial antigens. Suppurative adenitis was seen by weeks 18 and 20 post-vaccination in the latter two lower doses; whereas it was seen by week 8 in the higher dose of the combination vaccines. No case of suppurative adenitis was observed in the BCG 0.1 mg group. Culture and histopathology ruled out the possibilities of progressive BCG infection and superadded infection. Considering the above results, BCG 0.05 mg + 6×10^8 KML was accepted for a large-scale vaccine trial in South India.

RESUMEN

Para establecer la aceptabilidad del BCG y de *Mycobacterium leprae* muerto por calor (MLMC) como vacunas contra la lepra, se realizaron estudios de fase II y de fase II ampliada en tres grupos de la población de Thiruthani taluk, Distrito de Chengalpattu, al sur de la India. En el estudio de fase II, se vacunaron 997 voluntarios sanos con uno de los siguientes esquemas de vacunación: 0.1 mg de BCG + 6×10^8 MLMC, 0.1 mg BCG + 5×10^7 MLMC, 0.1 mg de BCG + 5×10^6 MLMC, 0.1 mg de BCG, o solución salina fisiológica. Se tomaron muestras de sangre y el suero se utilizó para buscar anticuerpos contra el glicolípido fenólico-I (PGL-I) y el antígeno de 35 kDa de *M. leprae.* En el grupo de 13 a 70 años de edad, observamos adenitis supurativa regional en 6%, 3%, y 3% de los vacunados con BCG + 6×10^8 MLMC, BCG + 5×10^7 MLMC, y BCG + 5×10^6 MLMC, respectivamente. La presencia de cicatríz por BCG, las reacciones en piel a la lepromina A, el MLSA de Rees, y los niveles de anticuerpos anti-PGL-I y anti-35 kD, no ayudaron a identificar el grupo en riesgo de desarrollar adenitis supurativa. La adenitis supurativa parece tener una relación directa entre la edad del sujeto y la dosis de vacuna. Para resolver el problema de la adenitis supurativa regional y para conocer los mecanismos involucrados, se realizó un estudio de fase II ampliada en grupos similares de la población, utilizando dosis reducidas de vacunas BCG y MLMC (0.05 mg de BCG + 6×10^8 MLMC, 0.05 mg de BCG + 5×10^7 MLMC, y 0.01 mg de BCG + 5×10^7 MLMC). Se tomaron biopsias de ganglios linfáticos de los casos con adenitis supurativa y se usaron para cultivo y para examen histopatológico. Las observaciones mostraron que la adenitis supurativa regional pudo reducirse al 1% en el grupo de 0.05 mg de BCG + 6×10^8 MLMC, al 0.5% en el grupo de 0.05 mg de BCG + 5×10^7 MLMC, y al 0.5% en el grupo de 0.01 mg de BCG + 5×10^7 MLMC. Este fenómeno de adenitis supurativa parece estar relacionado con la dosis total de los antígenos micobacterianos. La adenitis supurativa se observó entre las semanas 18 y 20 post-vacunación con las 2 dosis más bajas, mientras que ocurrió hacia la semana 8 con las dosis más altas

de vacuna combinada. En el grupo vacunado con 0.1 mg de BCG no se observó adenitis supurativa. Los cultivos y la histopatología descartaron las posibilidades de una infección progresiva por BCG o de una infección asociada. Considerando los resultados anteriores, se aceptó la combinación de 0.05 mg de BCG + 6 $\times$ 10^8 MLMC como la vacuna más apropiada para ensayos de campo de amplia cobertura en el sur de la India.

RÉSUMÉ

On a réalisé des études de phase II et des études extensives de phase II avee des vaccins combinés BCG et *Mycobacterium leprae* tué (MLT) dans trois différents groupes de population au Thiruthani Taluk, dans le Distict de Chengalpattu dans le Sud de l'Inde, afin d'évaluer l'acceptabilité des vaccins. Dans l'étude de phase II, 997 volontaires en bonne santé ont été vaccinés en ayant reçu de manière aléatoire: BCG 0.1 mg + 6 $\times$ 10^8 MLT, BCG 0.1 mg + 5 $\times$ 10^7 MLT, BCG 0.1 mg + 5 $\times$ 10^6 MLT, BCG 0.1 mg ou du serum salin normal. On a pris des échantillons de sang et on a analysé le serum pour déterminer les taux d'anticorps contre le glycolipide phénolique I (PGL-I) et la protéine de 35-kDa de *M. leprae*. Dans cette étude, nous avons observé une adénite suppurative régionale chez respectivement 6% (6 sur 100), 3% (3 sur 100) et 3% (3 sur 100) des personnes du groupe d'âge 13–70 ans vaccinées avec le BCG 0.1 mg + 6 $\times$ 10^8 MLT, BCG 0.1 mg + 5 $\times$ 10^7 MLT, BCG 0.1 mg + 5 $\times$ 10^6 MLT. La présence d'une cicatrice de BCG, les réactions au test cutané à la lepromine A, au MLSA de Rees et les taux d'anticorps sériques vis-à-vis du PGL-I et de la protéine de 35 kDa n'ont pas aidé à identifier le groupe à risque de développer une adénite suppurative. Le développement d'une adénite suppurative apparaît être en relation directe avec l'âge du sujet et la dose vaccinale. Afin de surmonter le problème d'adénite suppurative régionale et de connaître le mécanisme impliqué, nous avons conduit une étude extensive de phase II dans des groupes similaires de populations en réduisant les doses de BCG et de MLT, c'est à dire avec du BCG 0.05 mg + 6 $\times$ 10^8 MLT, BCG 0.05 mg + 5 $\times$ 10^7 MLT, BCG 0.01 mg + 5 $\times$ 10^7 MLT. On a prélevé, pour culture et examen histopathologique, des biopsies des ganglions lymphatiques des cas présentant une adénite suppurative. Les observations ont montré que la fréquence de l'adénite suppurative régionale pouvait être réduite à 1% dans le groupe BCG 0.05 mg + 6 $\times$ 10^8 MLT, à 0.5% dans le groupe BCG 0.05 mg + 5 $\times$ 10^7 MLT, et à 0.5% dans le groupe BCG 0.01 mg + 5 $\times$ 10^7 MLT. Ce phénomène d'adénite suppurative apparaît associé à la dose totale d'antigène mycobactériens. L'adénite suppurative a été observée aux dix-huitième et vingtième semaines dans les deux derniers groupes aux doses plus faibles, alors qu'il a été observé à la huitième semaine avec la dose plus élevée de combinaison vaccinale. Aucun cas d'adénite suppurative n'a été observé dans le groupe ayant reçu le BCG 0.1 mg. La culture et l'histopathologie ont exclu la possibilité d'infection progressive par le BCG et d'infectian surajoutée. Considérant les résultats ci-dessus, le BCG 0.05 mg + 6 $\times$ 10^8 MLT a été accepté pour un essai de vaccination à grande échelle dans le Sud de l'Inde.

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REFERENCES

1. Bhushan, K. Normal and abnormal BCG reactions. NTI Newsletter **15** (1978) 65–69.
2. Convit, J., Aranzazu, N., Zuniga, M., Ulrich, M., Pinardi, M. E., Castellazzi, Z. and Alvarado. J. Immunotherapy and immunoprophylaxis. Lepr. Rev. **54** Special Issue (1983) 47s–60s.
3. Convit, J., Sampson, C., Zuniga, M., Smith, P. G., Plata, J., Silva, J., Molina, J., Pinardi, M. E., Bloom, B. R. and Salgado, S. Immunoprophylactic trial with combined *Mycobacterium leprae* BCG vaccine against leprosy: preliminary results. Lancet **339** (1992) 446–450.
4. Deo, M. G., Bapat, C. V., Bhale Rao, V., Chaturvedi, R. M., Chulawala, R. G. and Bhatki, W. S. Potential antileprosy vaccine from killed ICRC bacilli—a clinicopathological study. Indian J. Med. Res. **74** (1981) 164–171.
5. Griffith, A. H. Overdose of BCG vaccine. Tubercle **44** (1963) 247.
6. Gupte, M. D. Vaccines against leprosy. Indian J. Lepr. **63** (1991) 342–349.
7. Gupte, M. D., Anantharaman, D. S., Britto, L. J., Vallishayee, R. S., Nagaraju, B., Kannan, S. and Sengupta, U. Sensitization potential and reactogenicity of BCG with and without various doses of killed *M. leprae*. Int. J. Lepr. **60** (1992) 340–352.
8. Gupte, M. D., Anantharaman, D. S., Nagaraju, B., Kannan., S. and Vallishayee, R. S. Experiences with *Mycobacterium leprae* soluble antigens in a leprosy endemic population. Lepr. Rev. **61** (1990) 132–144.
9. Gupte, M. D., Vallishayee, R. S., Britto, L. J. and Anantharaman, D. S. Sensitization potential and reactogenicity of varying doses of BCG

plus killed *Mycobacterium leprae;* an extended study. Int. J. Lepr. **61** (1993) 563–569.

10. Harboe, M., Mshana, R. N. Closs, O., Kronvall, G. and Axelsen, N. H. Crossreactions between mycobacteria-II. Crossed immunoelectrophoretic analysis of soluble antigens of BCG and comparison with other mycobacteria. Scand. J. Immunol. **9** (1979) 115–124.
11. Karonga Prevention Trial Group. Randomized controlled trial of single BCG, repeated BCG, or combined BCG and killed *Mycobacterium leprae* vaccine for prevention of leprosy and tuberculosis in Malawi. Lancet **348** (1996) 17–24.
12. Mshana, R. N., Belehu, A., Stoner, G. L., Harboe, M. and Haregewoin, A. Demonstration of mycobacterial antigens in leprosy tissues. Int. J. Lepr. **50** (1982)1–10.
13. Narayanan, R. B.; Badenoch-Jones, P. and Turk, J. L. Experimental mycobacterial granulomas in guinea pig lymph nodes: ultrastructural observations. J. Pathol. **134** (1981) 253–265.
14. Orell, J. M., Brett, S. J., Ivanyi, J., Coghill, G., Grant, A. and Beck, J. S. Measurement of the tissue distribution of immunoperoxidase staining with polyclonal anti-BCG serum in lung granulomata of mice infected with *Mycobacterium tuberculosis.* J Pathol. **164** (1991) 41–45.
15. Ponnighaus, J. M., Fine., P. E. M., Bliss, L., Gruer, P. J. K., Kapira-Mwamondwe, B., Msosa, E., Rees, R. J. W., Clayton, D., Pike, M. C., Sterne, J. A. C. and Oxborrow, S. M. The Karonga prevention trial: a leprosy and tuberculosis vaccine trial in northern Malawi. I. Methods of the vaccination phase. Lepr. Rev. **64** (1993), 338–355.
16. Simmonds, F. A. H. Delayed regional adenitis after BCG vaccination. Tubercle **45** (1964) 160.
17. Spector, W. G., Mariyanayagam, Y. and Ridley, M. J. The role of antibody in primary and reinfection BCG granulomas of rat skin. J. Pathol. **136** (1982) 41–57.
18. Talwar, G. P., Zaheer, S. A. and Mukherjee, R. Immunotherapeutic effects of a vaccine based on a saprophytic cultivable mycobacterium, *Mycobacterium. w.* in multibacillary patients. Vaccine **8** (1991) 121–129.
19. Ten Dam, H. G., Toman, K., Hitze, K. L. and Guld, J. Present knowledge of immunization against tuberculosis. Bull. WHO **54** (1976) 255–269.
20. Walia, R., Sarathchandra, K. G., Pandey, R. M., Parida, S. K., Zaheer, S. A., Kar, H. K., Mukherjee, A., Mukherjee, R. and Talwar, G. P. Field trials on the use of *Mycobacterium w.* vaccine in conjunction with multidrug therapy in leprosy patients for immunotherapeautic and immunoprophylaxis purposes. Lepr. Rev. **64** (1993) 302–311.
21. Watkins, S. M. Unusual complications of BCG vaccination. Br. Med. J. **1** (1971) 442.