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Comparative Study of Immunizing and Delayed Hypersensitivity Eliciting Antigens of *Mycobacterium leprae, M. tuberculosis, M. vaccae,* and *M. bovis* (BCG)[1]

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The immune responses evoked by mycobacteria, either humoral or cell-mediated, are not species-specific since most of the antigens of the genus are shared among its species ([7]). The skin-test antigens, tuberculins and lepromins, also lack adequate specificity for the same reason. Many attempts have, therefore, been made to characterize and to isolate the mycobacterial antigens responsible for the elicitation of delayed-type hypersensitivity (DTH) reactions.

From *Mycobacterium tuberculosis,* an array of DTH eliciting proteins with molecular weights of 150 kD ([8]), 45–48 kD ([6]), 35–42 kD ([30]), 28.5–35 kD ([5]), 28–30 kD ([10]), 22 kD ([28]), 20 kD ([30]), 5–10 kD ([46]), 9.7 kD ([17]), 5.8 kD ([25]), and 4–5 kD ([41]) have been isolated. Similarly from *M. leprae,* low-molecular-weight protein fractions ([16, 38]), low-molecular-weight anionic proteins ([1, 32]), and a high-molecular-weight cell-wall antigen ([29]) have been found to be capable of eliciting DTH responses.

The studies cited indicate that in both *M. tuberculosis* and *M. leprae* there is a possibility of more than one antigen taking part in cell-mediated immunity. We, therefore, undertook a study to broadly define the antigens of *M. leprae* and *M. tuberculosis* responsible for the elicitation of DTH responses in presensitized mice and guinea pigs. In addition, antigen fractions from *M. vaccae,* which has been found to be antigenically related to *M. leprae* ([27, 39, 42]), and fractions of BCG, which is closely related to both *M. leprae* and *M. tuberculosis* ([3, 13, 43]), were also used for a comparative assessment of species specificity.

The preparation of lepromin and tuberculin (or PPD) involves heating. Also, there are reports indicating a better immunogenicity of certain heated mycobacteria compared to their native counterparts. Therefore, the relative efficacies of heated mycobacterial antigens as immunogens and as elicitins for DTH were also studied.

MATERIALS AND METHODS

Mycobacteria. *M. bovis* BCG (Glaxo vaccine strain), *M. tuberculosis* H37Ra, and *M. vaccae* R877R (kindly provided by Dr. J. L. Stanford, Middlesex Hospital Medical School, London) were grown in Sauton's medium for 4–5 weeks. The mycobacteria were collected and washed three times with sterile normal saline by centrifugation. The final pellets were lyophilized and stored at 4°C until used. Armadillo-derived *M. leprae,* purified according to the WHO protocol ([44]), was kindly provided by Dr. R. J. W. Rees (Clinical Research Centre, Harrow, England) at a concentration of 10^9 bacilli per ml in normal saline.

Animals. Randomly bred guinea pigs weighing 300–350 g (obtained from Central Drug Research Institute, Lucknow, India) and Swiss (Rockefeller) strain mice (6–8 weeks old) were used in this study. The mice were raised in the animal house of Central JALMA Institute for Leprosy, Agra, India.

Immunizations. The lyophilized myco-

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bacteria were suspended in normal saline (50 mg dry weight/ml), and the bacilli were freed from the clumps by exposure to ultrasonic vibrations ([9]) in pulses of 10 sec, amounting to a total period of 1 min. The suspensions were then centrifuged at 1000 × *g* × 10 min, and the supernatants, containing mostly free bacilli, were collected and the bacterial numbers were determined ([12]). Immunizations of experimental animals were done with these (native) preparations as well as their autoclaved (15 lbs pressure × 15 min) counterparts. The sediments were stored for the preparation of soluble antigens by sonication.

Suggestions by Shepard *et al.* ([35]), Mehra and Bloom ([21]), and Collins ([4]) were followed for immunization of mice and guinea pigs. Native mycobacteria (10^7, 0.1 ml suspension) were injected intradermally (i.d.) in the inguinal region of each mouse. Fifty mice were immunized with each mycobacterial species and kept in groups of 5 to 6. The guinea pigs were immunized in groups of three with native and autoclaved (15 lbs pressure × 15 min) mycobacteria. Two i.d. injections of 0.1 ml each (10^8 of *M. leprae* or 10^7 of other mycobacteria) were administered into the groin region of each guinea pig. Groups of unimmunized mice and guinea pigs served as controls. The DTH responses in all of the animals were tested after 4 weeks.

Test antigens. Mycobacteria were sonicated in an ice bath for 15 min in pulses of 5 sec at 50% power output (Model UR 200P sonicator, Tomy Seiko Co. Ltd., Tokyo, Japan). The sonicates were centrifuged at 50,000 × *g* × 10 min, and the supernatants after protein estimation ([20]) were stored in aliquots at −70°C. Soluble antigen of *M. leprae* (armadillo-derived) was kindly provided by Dr. R. J. W. Rees, Clinical Research Centre, Harrow, England. An aliquot of each of the soluble antigens was autoclaved (15 lbs × 15 min) and stored.

Initially, the profiles of soluble mycobacterial antigens were studied by analytical polyacrylamide slab gel electrophoresis (PAGE), without sodium dodecyl sulfate (SDS), on 0.7-mm-thick slabs of 12.5% gel ([18]). Protein bands were stained either with PAGE blue 83 (BDH, Poole, England) or with silver stain ([45]). Afterwards, the soluble antigens of *M. tuberculosis, M. vaccae,* and BCG were fractionated into four fractions each using preparatory PAGE. Electrophoresis was performed without SDS in 2.7-mm-thick slabs of 12.5% gel. Sterile distilled water was used for the preparation of buffers and solutions. One ml of antigen (containing 2 mg protein) was put into 10-cm-long sample slots prepared in the stacking gel. Electrophoresis was run overnight at 80 V in a Pharmacia (Uppsala, Sweden) L/S apparatus. Afterwards, the stacking gel area beside and below the bromophenol blue dye front was removed. From the remaining gel, a 1-cm-wide marginal strip was cut out along the length of the gel for reference protein staining and the rest of the gel was cut at right angles to the direction of electrophoresis into four equal parts. Each part was separately minced with a surgical knife and suspended in tubes containing 15 ml sterile normal saline. For elution, the tubes were kept at 4°C for 48 hr; after collecting the elutes by filtration, the gel pieces of each fraction were resuspended in 10 ml of fresh saline for further overnight elution. The latter elutes were pooled with earlier ones, and all of the fractions were lyophilized and reconstituted in 2 ml distilled water. This was followed by dialysis of the fractions against normal saline for 72 hr with two changes of saline. The protein concentration of each fraction was estimated ([20]). The molecular weights were determined by running in parallel the marker proteins (Pharmacia, Uppsala, Sweden) and plotting a standard curve (relative mobilities vs molecular weights). Finally, the fractions were stored in sterile vials after filtering through millipore membranes (0.22 μ pore size). A control gel fraction was also prepared as above, except that normal saline was used instead of antigen for PAGE.

The *M. leprae* protein antigens MY1 (12 kD) and MY2 (35 kD) ([14, 15]) were affinity purified from soluble antigen of armadillo-derived *M. leprae* using the monoclonal antibodies ML06 and ML04, respectively. The molecules MY1 and MY2 and the soluble antigen devoid of both the molecules (MLSE) were kindly provided by Dr. J. Ivanyi, Medical Research Council Unit for Tuberculosis and Related Infections, Hammersmith Hospital, London.

Tests for DTH. Mice and guinea pigs were tested for DTH responses with unauto-

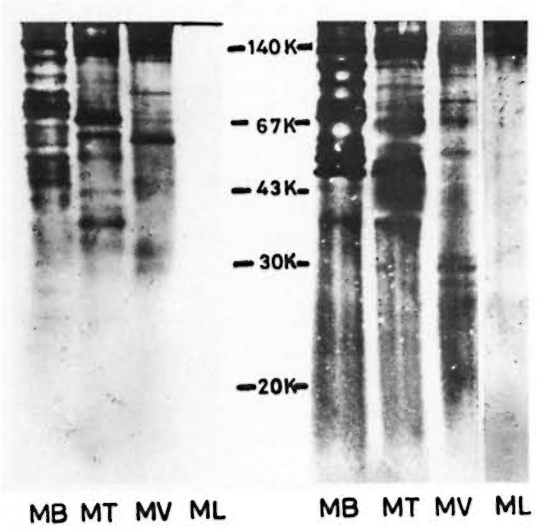

FIG. 1. Polyacrylamide slab gel electrophoresis (PAGE) of the soluble antigens of *M. bovis* (MB), *M. tuberculosis* H37Ra (MT), *M. vaccae* (MV), and *M. leprae* (ML). Lanes on the left were stained with PAGE blue 83, and their replicates on the right were stained with silver stain. K = molecular weights of marker proteins in kilodaltons.

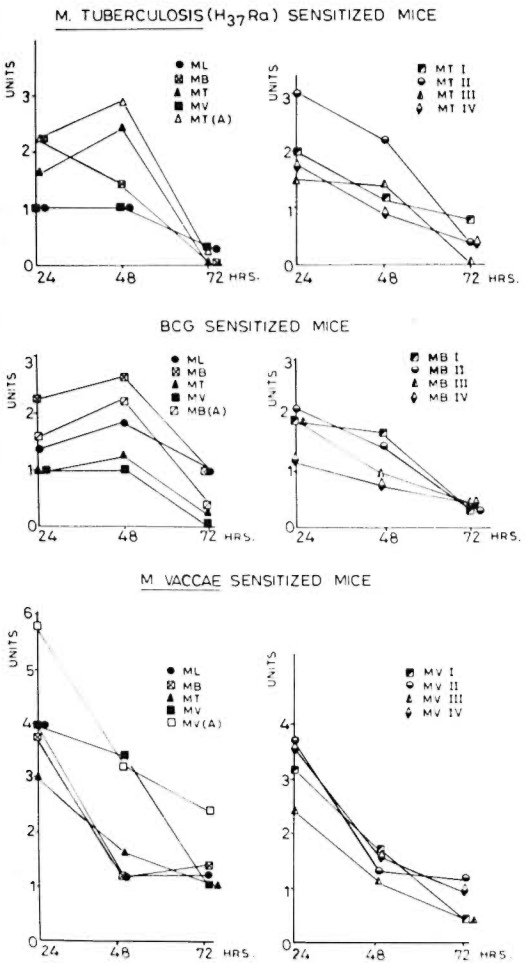

FIG. 2. Kinetics of foot pad DTH responses in mice immunized with unautoclaved mycobacteria tested with the following soluble antigens: *M. leprae* (ML), *M. bovis* BCG (MB), *M. tuberculosis* H37Ra (MT), and *M. vaccae* (MV). (A) = Autoclaved; I–IV = fractions of soluble mycobacterial antigens.

claved and autoclaved soluble antigens of mycobacteria and their fractions. Working dilutions were 300 μg protein/ml of normal saline for mice and 100 μg/ml for guinea pigs. If necessary, all of the fractions (I to IV) of each soluble antigen were diluted individually to the same extent, so that the sum total of their protein concentrations became either 300 μg/ml or 100 μg/ml. MY1 and MY2 were used at a concentration of 10 μg protein/ml and MLSE at 100 μg protein/ml. The groups of animals were coded before DTH testing, and the tests were performed in a double-blind manner.

Forty μl of test antigen was injected subcutaneously (s.c.) in the right hindfoot pad of each mouse (5–6 mice in each group). Unimmunized mice, injected with test antigen or control PAGE elutes, served as controls. Thicknesses of left and right hindfoot pads were measured with a dial gauge (Model Pocotest A02T; Carobronze Ltd, London) at 24, 48, and 72 hr, and the foot pad enlargement was recorded for each mouse by subtracting the thickness of the left hindfoot pad from that of the right hindfoot pad.

The guinea pigs were tested in groups of three. One hundred μl of each test antigen was injected i.d. in the freshly shaven flanks (4–6 test antigens on each flank). Unimmunized guinea pigs, tested with antigens or control PAGE elute, served as controls. The indurations were recorded with a Vernier scale at 6, 24, 48, and 72 hr.

Expression of results. Mouse foot pad enlargements were recorded in units (1 U = 0.1 mm), and the mean diameter of guinea pig skin indurations were recorded in millimeters. The results were expressed as the mean of the foot pad enlargements or skin indurations in a group of animals ± the standard error of mean. Significance of differences were measured by applying the un-

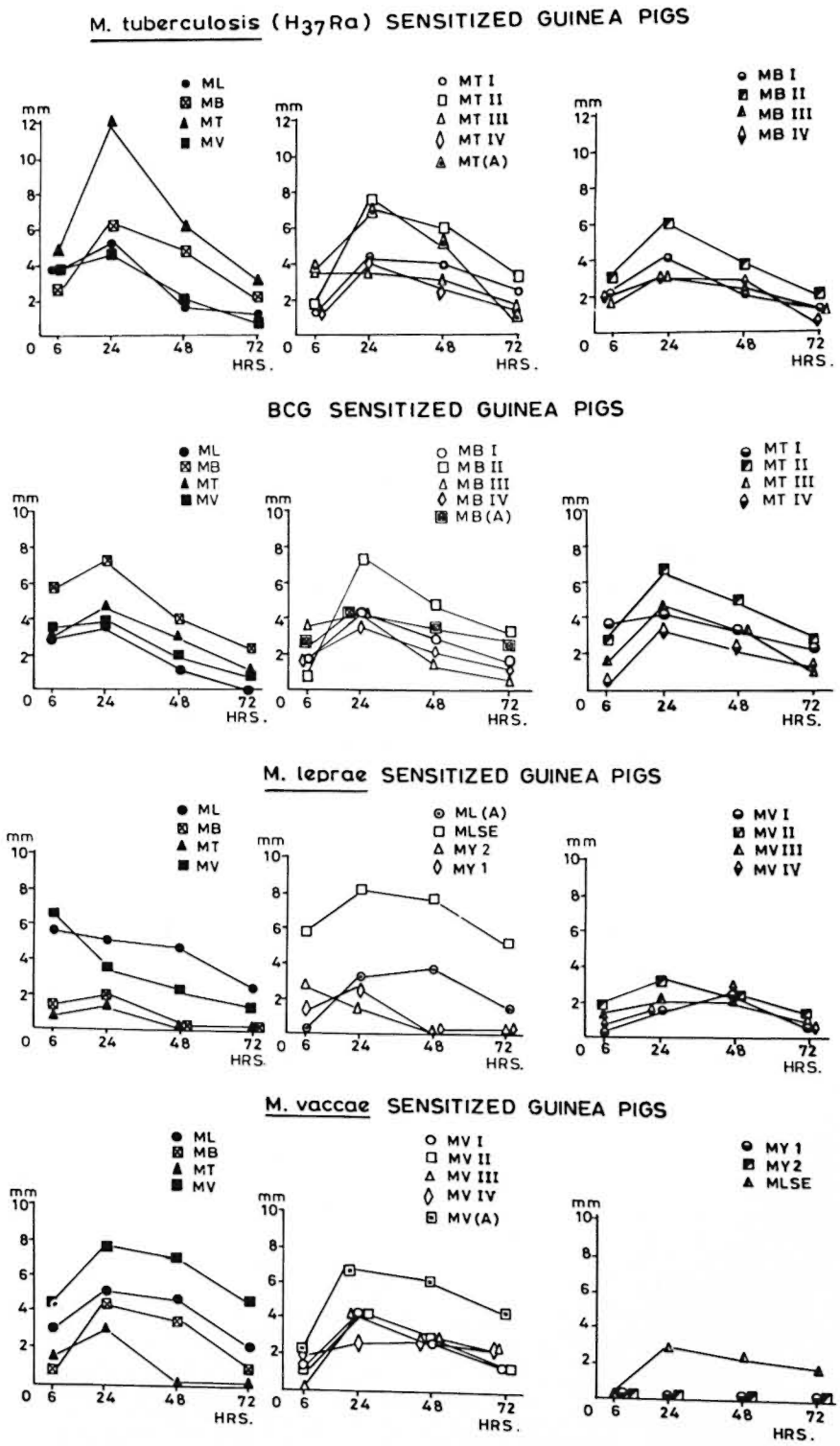

FIG. 3. Kinetics of DTH responses in guinea pigs immunized with unautoclaved mycobacteria. Test antigens are as in Figure 2. Those guinea pigs immunized with autoclaved mycobacteria also showed similar kinetics.

paired "t" test for mice and paired "t" test for guinea pigs.

RESULTS

Antigen fractions. Figure 1 shows the PAGE profiles of the unfractionated soluble antigens. Lanes on the left were stained with PAGE blue 83 and their replicates on the right were stained with silver stain. The bands in *M. leprae* soluble antigen could not be stained prominantly due to a lesser protein concentration in this sample (1 mg/ml) compared to the other three antigens (5 mg/ml). The Table shows protein concentrations and molecular weights of antigen fractions obtained by preparatory PAGE. The protein concentrations decreased from high to low molecular weight fractions in each of the three soluble antigens (*M. tuberculosis,* BCG, and *M. vaccae*).

Kinetics of DTH responses. The kinetics of the DTH responses of mice and guinea pigs are represented in Figures 2 and 3, respectively. The peak DTH responses (elicited by either soluble antigens or their fractions) were observed at 24–48 hr in the mice and at 24 hr in the guinea pigs. The maximum DTH responses of the control (unimmunized) mice (1 U) and guinea pigs (2 mm) are represented as base-line values in Figures 4 and 5, respectively.

Efficacies of autoclaved vs unautoclaved immunizing and test antigens. The comparative peak DTH (24 hr) responses in guinea pigs immunized with autoclaved or unautoclaved mycobacteria are shown in Figure 5. The DTH responses (to all test antigens) in guinea pigs immunized with autoclaved (A) and unautoclaved (U) *M. tuberculosis* or BCG were not significantly different from each other ($p > 0.05$). However, the responses in *M. leprae* (A) immunized guinea pigs were significantly higher ($p < 0.01$) than the corresponding values from guinea pigs immunized with *M. leprae* (U). In guinea pigs immunized with *M. vaccae* (A) also, some of the DTH reactions were markedly larger (but not statistically significant) than those of *M. vaccae* (U) immunized guinea pigs.

The guinea pig DTH reactions elicited by autoclaved soluble antigens of *M. tuberculosis,* BCG, and *M. leprae* were significantly smaller ($p < 0.05$) than the corresponding reactions elicited by unheated soluble antigens (Fig. 5). However, in mice, these differences were not statistically significant (Fig. 4).

Antigenic relatedness between mycobacteria. The differences in DTH responses to soluble antigens of *M. tuberculosis* and BCG were not statistically significant ($p > 0.05$) in the groups of mice immunized with *M. tuberculosis* and BCG (Fig. 4) or in the group of guinea pigs immunized with BCG (Fig. 5). However, this difference was significant ($p < 0.05$) in guinea pigs immunized with *M. tuberculosis.* The soluble antigen of *M. vaccae* did not produce a DTH response in both the groups of mice (i.e., those immunized with *M. tuberculosis* and BCG), whereas *M. leprae* elicited a marginal reaction (1.8 ± 0.6 U) in BCG-immunized mice only (Fig. 4). Taken together, these results indicate an antigenic proximity of *M. tuberculosis* with BCG.

DTH tests in mice and guinea pigs immunized with either *M. leprae* or *M. vaccae* (Figs. 4 and 5) reflected antigenic nearness of these two species of mycobacteria. The differences in the DTH responses to *M. leprae* and *M. vaccae* soluble antigens in these groups of animals were not significant ($p > 0.05$). On the other hand, in guinea pigs immunized with *M. leprae* or *M. vaccae,* the DTH responses elicited by the respective soluble antigens were significantly higher ($p < 0.05$) than the responses elicited by the soluble antigens of BCG or *M. tuberculosis.*

Potency and specificity of antigen fractions. Potencies of DTH-eliciting soluble antigen fractions of *M. tuberculosis,* BCG, *M. vaccae,* and *M. leprae* were compared by simultaneous tests in mice and guinea pigs immunized with the respective mycobacteria. The species specificities of these fractions were determined by testing them in guinea pigs immunized with the antigenically nearest mycobacterium, judged on the basis of DTH tests in mice (Fig. 4). Thus, antigen fractions of *M. tuberculosis* were tested in BCG-immunized guinea pigs (and vice versa) and the antigen fractions of *M. leprae* were tested in *M. vaccae*-immunized guinea pigs (and vice versa).

In guinea pigs (Fig. 5), DTH reactions to fraction II (16–24 kD) of *M. tuberculosis*

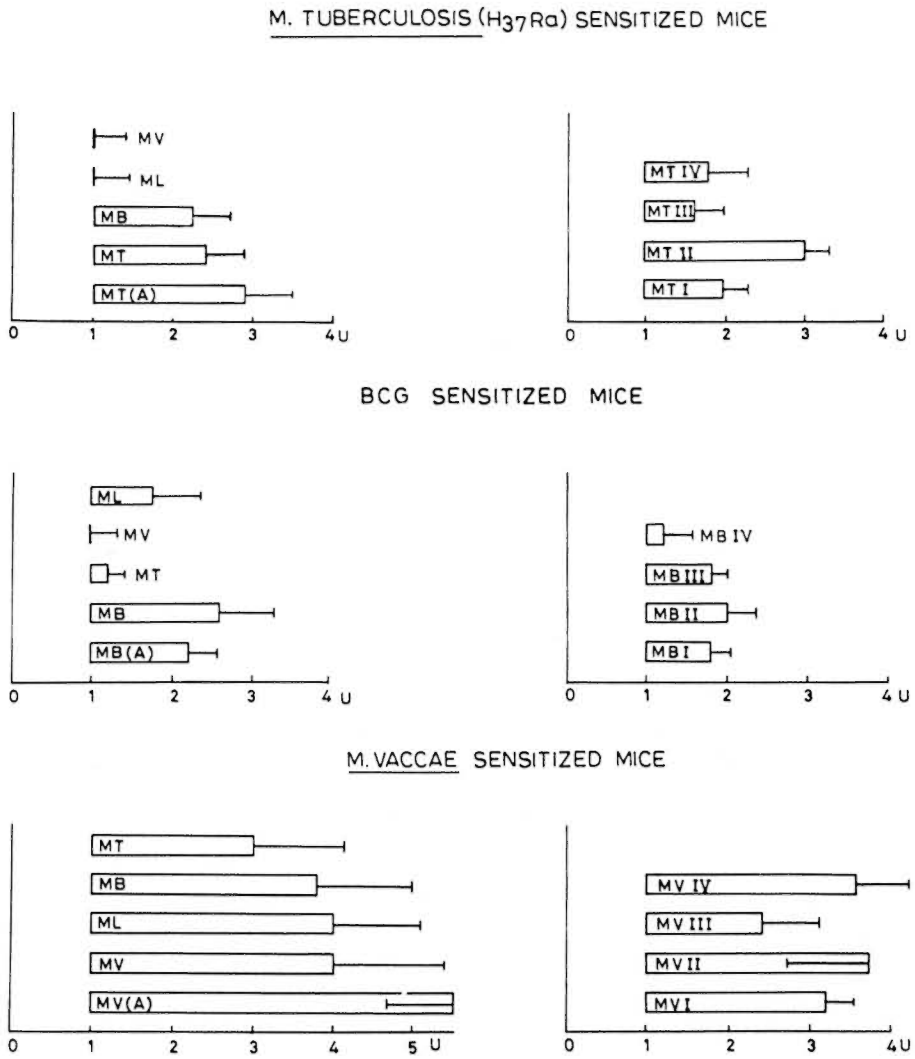

FIG. 4. Comparison of peak DTH responses in mice immunized with unautoclaved mycobacteria. Test antigens are as in Figure 2. Base-line value (1 U) is maximum DTH response in control mice.

and that (20–27 kD) of BCG were significantly larger ($p < 0.05$) than those elicited by the corresponding fractions I, III, or IV. Although fraction II constituted only about 15% of the total soluble proteins of *M. tuberculosis* or BCG (The Table), the reactions elicited by it were equal to (in the case of BCG) or nearer to (in the case of *M. tuberculosis*) those elicited by the respective whole soluble antigens. In mice (Fig. 4), the reactions elicited by these antigen fractions were not statistically different from each other. None of the fractionated antigens of *M. tuberculosis* or BCG elicited species-specific DTH reactions (Fig. 5).

All four fractions of *M. vaccae* mounted good DTH responses in mice and guinea pigs immunized with *M. vaccae*, and the differences in reaction sizes were statistically insignificant (Figs. 4 and 5). However, fraction II (15–24 kD) of *M. vaccae* elicited a significantly larger reaction ($p < 0.05$) in guinea pigs immunized with *M. vaccae* compared to that in guinea pigs immunized with *M. leprae*.

The affinity-purified molecules of *M. lep-*

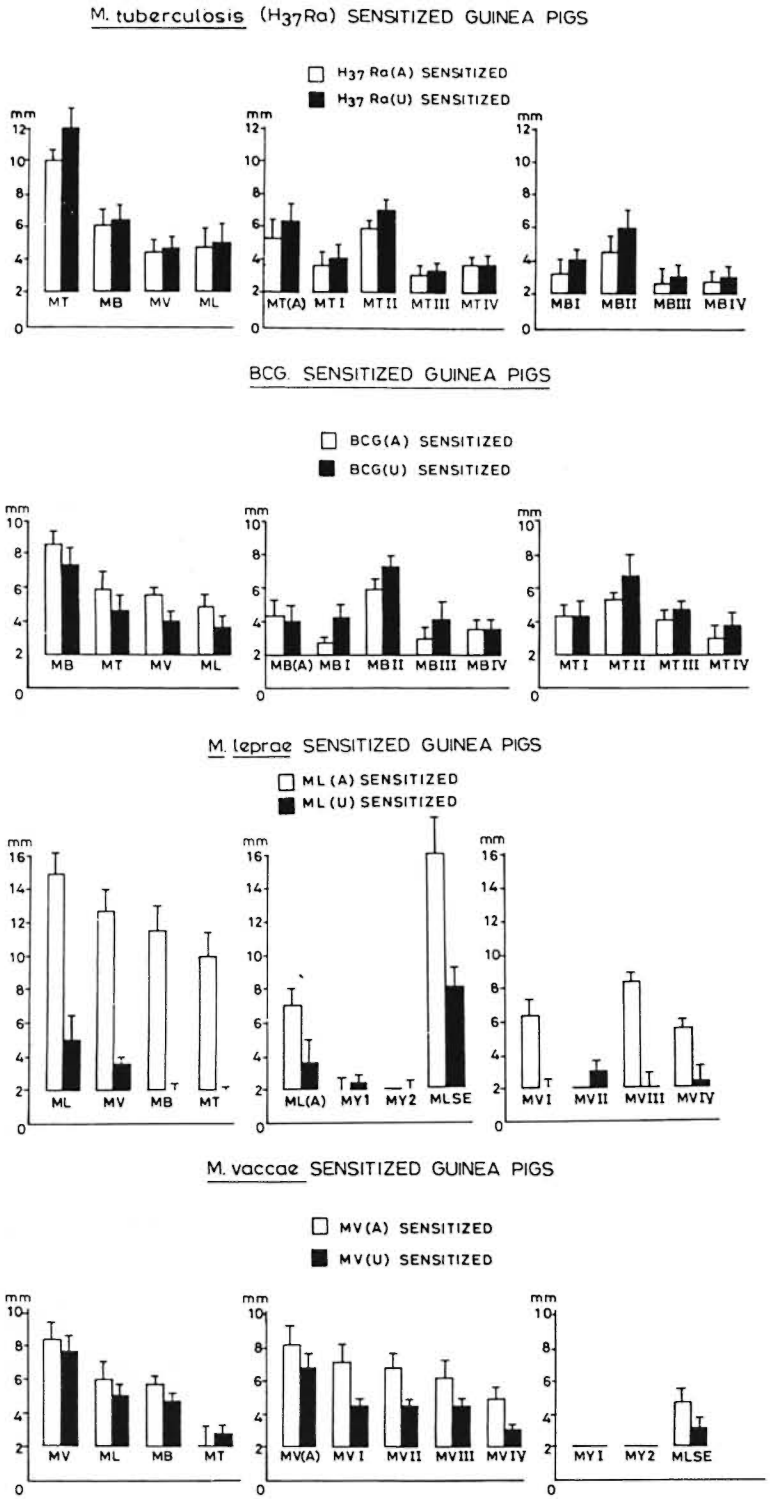

FIG. 5. Comparison of peak DTH responses in guinea pigs immunized with autoclaved (□) and unautoclaved (■) mycobacteria. Test antigens are as in Figure 2. Base-line value (2 mm) denotes maximum DTH response in control guinea pigs.

THE TABLE. *Protein concentration and molecular weights of antigen fractions obtained by preparatory PAGE.*

Antigen fractions	*M. tuberculosis* H37Ra		*M. vaccae*		*M. bovis* BCG	
	Protein (μg/ml)	Mol. wt. range (kD)	Protein (μg/ml)	Mol. wt. range (kD)	Protein (μg/ml)	Mol. wt. range (kD)
I	25	<16	15	<15	15	<20
II	65	16–24	50	15–24	60	20–27
III	115	24–45	115	24–43	180	27–55
IV	140	>45	130	>43	300	>55
Total protein	345		310		555	

[a] kD = kilodaltons.

rae, MY1 (12 kD) and MY2 (35 kD), did not elicit significant DTH reactions in guinea pigs (Fig. 5). However, MLSE (soluble antigen devoid of MY1 and MY2) elicited strong DTH reactions. Moreover, the reaction elicited by MLSE in guinea pigs immunized with unautoclaved *M. leprae* (8.0 ± 1.15 mm) was even stronger ($p < 0.05$) than the reaction elicited by the whole soluble antigen of *M. leprae* (5.0 ± 1.53 mm). MLSE also exhibited specificity for *M. leprae*. The reactions produced by it in *M. vaccae*-immunized guinea pigs (4.67 ± 0.67 mm and 3.0 ± 0.58 mm, respectively, in animals immunized with autoclaved and unautoclaved preparations) were significantly smaller ($p < 0.05$) than those in *M. leprae*-immunized guinea pigs (16.02 ± 2.3 mm and 8.0 ± 1.15 mm, respectively).

DISCUSSION

The results of the present study are in conformity with earlier studies ([4, 21, 25, 34, 42]), indicating that a) intradermal or subcutaneous injections of intact mycobacteria suspended in normal saline is the most effective method for immunization, b) DTH reactions peak at 24–48 hr in mice and at 24 hr in guinea pigs, and c) certain mycobacteria, especially *M. leprae* and *M. vaccae*, may work as better immunogens in heat-killed forms.

We have observed that autoclaved mycobacteria were either equipotent (in the case of *M. tuberculosis* and BCG) or better (in the case of *M. leprae* and *M. vaccae*) immunogens for DTH compared to their unautoclaved counterparts. The DTH reactions elicited by autoclaved soluble antigens, except those of *M. vaccae*, were significantly smaller than the reactions elicited by corresponding unheated antigens in guinea pigs. However, in mice, the differences in reactions elicited by unautoclaved and autoclaved soluble antigens were not significant. Previously, we have reported the superiority of autoclaved over unautoclaved soluble antigen of *M. leprae* for eliciting skin DTH responses in leprosy patients ([31]). Heating has been found to eliminate the activity of a majority of mycobacterial antigens ([11, 31, 36]), the main reason being heat denaturation of proteins. However, the ability of heated mycobacterial antigens to still immunize for and elicit DTH responses indicates that heating can be an important first step toward the elimination of nonspecific (also, possibly immunosuppressive) antigenic determinants.

We did not come across the problem of lesser immunogenicity of *M. vaccae* which has been reported to occur in certain strains of mice ([42]). The antigenic proximity of *M. vaccae* and *M. leprae*, as observed by us, has also been suggested by a few earlier studies ([27, 39, 42]), based on which a trial of *M. vaccae* as a candidate vaccine for leprosy has been initiated ([40]). However, greater protection by the BCG vaccine against mouse foot pad infection with *M. leprae* has been reported ([33]). In the present study, BCG was found to be antigenically nearer to *M. tuberculosis* and, to a lesser extent, to *M. leprae*.

Preparatory PAGE is an established technique for the fractionation of mycobacterial antigens ([2, 19, 23]). Unlike many studies in the past, we have used fractionated antigens at concentrations which were equal to their concentrations in the corresponding whole

soluble antigen. This provided a fairer assessment of the potencies of the constituent antigens.

The 16–24 kD fraction of *M. tuberculosis* and the 20–27 kD fraction of BCG were found to contain most potent DTH eliciting antigens. The responses elicited by <16 kD antigen of *M. tuberculosis* and <20 kD antigen of BCG were second best. These findings correlated well with some of the previous studies, indicating a predominant role of comparatively lower molecular weight fractions of *M. tuberculosis* and BCG in the elicitation of DTH responses ([10, 17, 25, 28, 30, 41]). The quantum of DTH reactions were apparently independent of the protein concentrations of the respective test antigens. The most potent fractions (I and II) of both *M. tuberculosis* and BCG contained only about 15–20% of the total soluble proteins. None of the soluble antigen fractions of *M. tuberculosis* or BCG gave an indication of species specificity. Earlier studies have indicated an almost complete genetic and antigenic overlap of these two species of mycobacteria ([7, 13, 43]).

Although all four fractions of *M. vaccae* antigens took part in the elicitation of DTH, the 15–24 kD antigen (fraction II) might contain species-specific determinants since it produced a significantly smaller reaction in *M. leprae*-sensitized guinea pigs.

The 12 kD (MY1) and 35 kD (MY2) proteins of *M. leprae* elicited insignificant DTH reactions when used at concentrations which were one tenth of the whole soluble antigen. This was done on the basis of earlier reports ([14, 15]) that, individually, these molecules constitute <10% of the total soluble protein of *M. leprae*. However, we have reported ([37]) a very good antibody response in leprosy patients against an epitope of the 35 kD molecule. Certain mycobacterial antigens have been shown to preferentially induce either humoral or cell-mediated immune responses ([32, 36]). MLSE, on the other hand, elicited a reaction which was stronger than that produced even by the whole soluble antigen. Comparable results have been obtained by us in a similar study conducted with leprosy patients (unpublished data). MY1 and MY2 bear *M. leprae*-specific epitopes. Hence, the enhancement of DTH reactions after elimination of these molecules may indicate the immunosuppressive nature of at last one of the molecules. The suppressive nature of some *M. leprae* antigens has already been indicated by earlier studies ([22, 26]). The reaction produced by MLSE in *M. vaccae* sensitized guinea pigs was significantly weaker, indicating further that this fraction might contain antigens responsible for the elicitation of *M. leprae*-specific DTH.

SUMMARY

A variety of mycobacterial antigens have been associated with the delayed-type hypersensitivity (DTH) reactions in human beings and animals. An attempt has been made in the present study to identify the major antigen fractions of *Mycobacterium leprae* and *M. tuberculosis* responsible for the elicitation of DTH and to evaluate their specificity by a comparison with the antigens of two closely related mycobacteria, BCG and *M. vaccae*. Guinea pigs and mice were sensitized with native or heat-killed *M. leprae, M. tuberculosis, M. vaccae,* and BCG. Four fractions each of the latter three mycobacteria, obtained by preparatory polyacrylamide slab gel electrophoresis, were used for elicitation of DTH. Three fractions of *M. leprae* (MY1, MY2, and MLSE) were obtained by affinity chromatography using monoclonal antibodies.

Heat-killed *M. leprae* and *M. vaccae* proved to be better sensitizers for DTH compared to their native counterparts, whereas there were no significant differences between the efficacies of autoclaved and unautoclaved *M. tuberculosis* or BCG. The best DTH responses were recorded with a 16–24 kD fraction of *M. tuberculosis,* a 20–27 kD fraction of BCG, and MLSE of *M. leprae*. The purified molecules of *M. leprae,* MY1 (12 kD) and MY2 (35 kD), did not elicit a DTH reaction. Although none of the fractions of *M. tuberculosis* or BCG exhibited species specificity, fraction II (15–24 kD) of *M. vaccae* and MLSE might be comprised of species-specific determinants.

RESUMEN

Se han asociado una variedad de antígenos micobacterianos con las reacciones de hipersensibilidad tipo tardio (HTT) en los humanos y en animales de experimentación. En el presente estudio se ha hecho un

intento para identificar las fracciones antigénicas del *Mycobacterium leprae* y del *M. tuberculosis* responsables de la inducción de la HTT. También se ha tratado de evaluar su especificidad por comparación con los antígenos de 2 micobacterias íntimamente relacionadas, BCG y *M. vaccae*. En el estudio se sensibilizaron cobayos y conejos con *M. leprae*, *M. vaccae* y BCG nativos o muertos por calor. Para inducir las reacciones de HTT se usaron 4 fracciones de cada una de las 3 últimas micobacterias obtenidas por electroforesis preparativa en gel de poliacrilamida. También se obtuvieron 3 fracciones del *M. leprae* (MY1, MY2 y MLSE) por cromatografía de afinidad usando anticuerpos monoclonales.

El *M. leprae* y el *M. vaccae* muertos por calor resultaron ser mejores inductores de HTT que las preparaciones nativas. Con el *M. tuberculosis* y el BCG no hubieron diferencias significativas entre las preparaciones nativas o las autoclaveadas. Las mejores respuestas de HTT se obtuvieron con una fracción 20–27 kD de l BCG y con la fracción MLSE del *M. leprae*. Las moléculas MY1 (12 kD) y MY2 (35 kD) no indujeron reacciones de HTT. Aunque ninguna de las fracciones del *M. tuberculosis* o del BCG exhibieron especificidad de especie, la fracción II (15–24 kD) del *M. vaccae* y la fracción MLSE del *M. leprae* podrían contener determinantes especificos de especie.

RÉSUMÉ

On a associé un grand nombre d'antigènes mycobactériens avec les réactions d'hypersensibilité de type retardé, tant chex l'homme que chez l'animal. On a dès lors essayé dans cette étude d'identifier les fractions antigéniques principales de *Mycobacterium leprae* et de *M. tuberculosis*, qui sont responsables de l'hypersensibilité de type retardé; on s'est attaché à évaluer leur spécificité en comparant les antigènes des ces deux mycobactéries à ceux de mycobactéries qui leur sont étroitement apparentées, le BCG et *M. vaccae*. Pour ce faire, on a sensibilisé des cobayes et des souris par *M. leprae*, *M. tuberculosis*, ou *M. vaccae*, soit à l'état naturel ou tués par la chaleur, ainsi que par le BCG. Quatre fractions, de chacune des trois mycobactéries citées, ont été utilisées pour induire cette hypersensibilité de type retardé. Ces fractions avaient été obtenues par électrophorèse sur un gel de polyacrylamide. Trois fractions de *M. leprae* (MY1, MY2, et MLSE) ont été obtenues par des techniques de chromatographie d'affinité, en utilisant des anticorps monoclonaux.

Il s'est révélé que *M. leprae* et *M. vaccae* tués par la chaleur constituaient des agents sensibilisants plus efficaces pour l'induction de l'hypersensibilité de type retardé, que leurs équivalents non dénaturés. Par contre, aucune différence significative n'a été trouvée suite au traitement par la chaleur pour *M. tuberculosis* autoclavé ou non autoclavé ou pour le BCG. Les réponses les meilleures d'hypersensibilité de type retardé ont été enregistrées avec une fraction de *M. tuberculosis*, de 16 à 24 kD, une fraction de BCG de 20 à 27 kD, et la fraction MLSE de *M. leprae*. Les molécules purifiées de *M. leprae*, MY1 (12 kD) et MY2 (35 kD), n'entraînaient pas de réaction d'hypersensibilité de type retardé. Quoiqu'aucune des fractions de *M. tuberculosis* ou de BCG n'a témoigné d'une spécificité d'espèce, la fraction II (15–24 kD) de *M. vaccae* et la fraction MLSE pourraient comprendre des déterminants spécifiques pour l'espèce.

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