Cross Reactivity of Mycobacterium leprae and BCG

A Report on Further Studies

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In previous studies (1, 2, 8) we were able to show cross reactivity between Mycobacterium leprae and other mycobacteria. We used an experimental guinea pig system and a purified, enzyme-digested suspension of M. leprae. Considering delayed hypersensitivity only, cross reactivity existed among suspensions of mycobacteria (whole bacilli), including BCG, and M. leprae, as well as between the latter and BCC cell-wall fraction. Protoplasmic fraction of BCG and M. leprae cross-reacted only to a small degree. Even this was attributed to contamination of protoplasmic fractions with cell-wall material.

We concluded that the relationship between Hansen's bacillus and BCG was similar to that reported by Larson et al (5, 6, 7) and Ribi et al (8, 9) when cell walls and protoplasts from distantly related mycobacteria were tested in reciprocally sensitized animals. The question remained open as to whether or not cross reactivity between M. leprae and BCG protoplasm was indeed non-existent. Further, it could be argued that a purified suspension of M. leprae, processed as described (2, 8) might be considered as equivalent to a cell-wall suspension, rather than to one of unaltered bacilli. Thus, sensitizing animals to it would produce only cross reactivity to cell walls.

The following experiments were designed to shed light on these and related points.

MATERIALS AND METHODS

Crude (whole) lepromin was prepared as recommended by Hanks et al (4). It contained 157 x 10^6 bacilli per milliliter as determined by the method of Shepard et al (11). Lyophilized BCG cell-wall and protoplasmic fractions were kindly provided by Dr. Edgar Ribi, Hamilton, Montana. They were obtained by disrupting bacilli and separating the fractions by differential ultracentrifugation and were reported to be free of reciprocal contamination (15). To check this further, lyophilized materials were suspended or dissolved in water and droplets were stained by Gram, Ziehl-Neelsen and Wright-Giemsa methods. No acid-fast particles or other bacteria were seen in protoplasmic fractions by light microscopy. Acid-fast structures were abundant in cell-wall suspensions.

Droplets were also examined using a Hitachi HS-7S electron microscope. They were dried in 100-mesh parlodion-coated grids, and examined without further treatment, or after carbon coating, employing a Hitachi HUS-3B vacuum evaporator. No structures were observed in protoplasmic fractions. Broken mycobacteria were easily discerned in the cell-wall preparations.

Heat-stable freeze-dried glutamate BCG vaccine (Japan BCG Laboratory) was grown in Loewenstein-Jensen medium at 37°C. When abundant growth was obtained, bacteria were dislodged, washed, heat-killed and ground using a Tenbroeck apparatus. Wet weight was then determined. Processing was carried out as described previously (2, 8).

Albino guinea pigs of the Rockefeller strain were fed rabbit pellets and water ad libitum, supplemented twice weekly with fresh carrots and cabbage.

After the experiments were completed, representative experimental lesions were...
biopsied. Specimens were fixed in buffered formalin, processed routinely and stained by the hematoxylin-eosin and Fite-Faraco methods.

RESULTS

Preliminary experiments. These were done to determine appropriate concentrations of BCG fractions to be employed later. Six guinea pigs (500 gm mean weight) were sensitized with four milligrams, wet weight, of heat-killed BCG, emulsified in incomplete Freund's adjuvant (Difco). The emulsion was injected into the four foot pads and the neck of each animal. Three weeks later, each animal was injected intradermally with the following: 100 µg, 10 µg or 1 µg of cell-wall suspension or 500 µg, 50 µg or 5 µg of protoplasmic fraction. All fractions were dissolved or suspended in sterile phosphate-buffered saline, pH 7.2, and each was given in a volume of 0.1 ml. Six untreated guinea pigs were similarly injected, as controls, at the same time and in the same fashion.

Diameters of induration were measured at one, two, four, and eight days after injection. Mean values obtained are shown in Figures 1 and 2. Based on these, and on results obtained previously (3), it was decided to use 100 µg of cell-wall fraction, and 500 µg and 50 µg of protoplasmic fraction respectively in the experiments described below.

Definitive experiments. Nineteen guinea pigs (475 gm mean weight) were alternately injected with lepromin emulsified (1:1 v/v) in incomplete Freund's adjuvant or intradermally with lepromin alone. Animals were injected on five different occasions during a period of five months. Each guinea pig received a total of 1.1 ml of lepromin (approximately 173 x 10⁶ bacilli). At the same times and intervals, 18 guinea pigs (mean weight 468 gm) received phosphate-buffered saline, pH 7.2 with 0.5% of phenol, and 0.05% of Tween-80 (PBSTP) alone, or emulsified in incomplete Freund's adjuvant. These animals formed the "negative control group."

Thirteen guinea pigs (mean weight 456 gm) received 3 mg wet weight of heat-killed BCG, emulsified in incomplete adjuvant, five weeks before testing. They re-
Fig. 3. Reactions to 500 μg of BCG protoplasm. Definitive experiments. Values shown for the different groups are the means of diameters of induration (in mm).

Note lack of significant differences between lepromin-sensitized and negative control groups.

Every guinea pig received intradermal injections of BCG cell-wall and protoplasmic fraction as mentioned before. Diameters of induration were measured at four hours, and at 1, 2, 4, 5, 9, 15 and 32 days after injection. Results were statistically evaluated by the t-test. At the end of the experiment selected sites were biopsied.

Mean values obtained from readings are depicted in Figures 3, 4, 5, and 6. These show that BCG-sensitized animals reacted markedly to BCG protoplasm. There was no significant difference in reactions to this fraction between normal and lepromin-sensitized guinea pigs at any of the concentrations employed.

Ten micrograms of cell-wall material elicited reactions which were more marked in lepromin-sensitized guinea pigs than in negative controls. Differences were statistically significant at 1, 4, 5, 9 and 15 days after testing (values of P were less than 0.001, except at 15 days when 0.001 < P < 0.01).

One hundred micrograms of cell walls induced a noticeable inflammatory response even in non-sensitized animals and thus results were less demonstrative.

Histopathologic findings. Microscopic features correlated very well with gross appearances. Five hundred micrograms of BCG protoplasm induced in normal controls and lepromin-sensitized animals, a mild to moderate inflammatory infiltrate. This infiltrate was formed mainly by lymphocytes and histiocytes. It affected the dermis (particularly around hairs and blood vessels), and the dermal-subcutaneous junction. There was no appreciable difference between sections from animals of these two groups. Fifty micrograms of protoplasm induced a similar, but much milder response in animals from such groups.

In contrast, the inflammatory response was massive in BCG-sensitized guinea pigs. There were isolated or confluent macrophages (epithelioid cells) and typical giant cells. There was necrosis. The infiltrate affected the dermis and dermal-subcutaneous junction. These features were present using both concentrations of protoplasm, with a difference in degree only.

Cell-wall material produced a similar picture in BCG-sensitized and lepromin-sensitized animals. With both concentrations, there was a typical tuberculoid
infiltrate with giant cells and confluent macrophages (epithelioid cells) present in mid-dermis and at the junction of dermis and subcutaneous tissue.

In normal guinea pigs, one hundred micrograms of cell walls induced a moderate inflammatory response. In one instance there was a tuberculoïd focus. In others, there was only a rather diffuse infiltrate where macrophages predominated. Ten micrograms of this fraction induced in normal guinea pigs a mild infiltrate formed by lymphocytes and some macrophages.

Acid-fast stains were not demonstrative since acid-fast particles were scarcely or not at all, seen. No difference could be detected, by the use of these stains, between experimental groups.

It should be noted that in BCG-sensitized guinea pigs, responses to protoplasm were of greater intensity than reaction to cell walls, and had tuberculoïd features. This means that in exquisitely-sensitized animals, BCG protoplasm may induce tuberculoïd responses. It is not absolutely necessary to assume contamination with cell-wall material to account for this phenomenon.

DISCUSSION

Using purified BCG cell walls and protoplasm in intradermal testing, the existence of clear-cut antigenic relationships between BCG cell walls and Mycobacterium leprae were confirmed. No cross reactivity was detected between the latter and BCG protoplasm. These results clarified questions left open from previous work (1, 2, 3).

Findings such as these help in explaining the seeming paradox of BCG being able to convert a previously-negative Mitsuda reaction, while no relationship is apparent between a positive Mitsuda and Mantoux reactions to Mycobacterium tuberculosis tuberculin.

We submit that the Mitsuda reaction is a response to cells walls. Tuberculin reactions are equivalent to a response to protoplasm.
We suggest that *M. tuberculosis* and *M. leprae* are related, but distantly. Their cell walls cross react, but not their protoplasts or tuberculins. There may exist other mycobacteria more closely related to *M. leprae* whose protoplasts and tuberculins would cross react with the latter. The influence which infection with such organisms may have on naturally occurring immunity to *M. leprae* and on the additive effect on BCG vaccination is worth thinking about, and has been commented upon \(^{1,2,3}\). 

**SUMMARY**

Guinea pigs were sensitized with crude lepromin. Animals were tested with BCG cell wall and protoplasmic fractions. There was cross reactivity between *M. leprae* and BCG cell walls. No relationship was found between BCG protoplasm and *M. leprae*. These results confirm and clarify previous observations where an enzyme digested suspension of *M. leprae* was used as sensitizer.

Reported findings help in explaining the lack of correlation between Mitsuda and *M. tuberculosis* tubercul reactions. They also pose interesting questions concerning possible relationships between mycobacterial ecology, immunity to leprosy and effectiveness of BCG vaccination.
RéSUMÉ

En employant la lépromine brute comme préparation désensibilisante, nous avons confirmé qu'il y a une relation antigénique entre les parois cellulaires du BCG et Mycobacterium leprae. En revanche, nous ne pouvons pas détecter aucune réactivité croisée entre le protoplasme de BCG et M. leprae.

Ces résultats, avec des autres déjà publiés, aident à expliquer l'absence de relation entre les réactions de Mitsuda et Mantoux. Ils posent aussi des questions intéressantes concernant les relations possibles entre écologie mycobactérienne, immunité contre la lèpre et efficacité de la vaccination par le BCG.

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REFERENCES