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Effect of oral exposure of *Mycobacterium avium intracellular* on the protective immunity induced by BCG

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Abstract. The relative protective efficacy of oral administration of mycobacteria as compared to the conventional intradermal route of vaccination has been assessed in guinea pigs. Skin test reactivity to partially purified protein derivative and protective immunity to challenge with virulent *Mycobacterium tuberculosis* were used as parameters of protective immunity.

Oral immunisation of guinea pigs either with BCG or with *Mycobacterium avium intracellulare* induces skin test reactivity and protective immunity comparable to that induced by intradermal route of vaccination. Oral exposure of *Mycobacterium avium intracellulare* prior to oral or intradermal dose of BCG did not interfere with the protective immunity induced by BCG in guinea pigs challenged with *Mycobacterium tuberculosis* $H_{37}Rv$.

Keywords. Protective immunity; BCG; oral immunisation, *Mycobacterium tuberculosis* $H_{37}Rv$; *Mycobacterium avium intracellulare.*

Introduction

The immune response is modulated by the nature, quantity and duration of the immunogen challenge. The oral route is one path by which the immune system comes into contact with an invading organism or its antigens. With recent advances in the immunological concepts there is a growing interest in the effects of oral exposure to antigens (Kagnoff, 1978).

It has long been held that oral intake of mycobacterial antigen can be a means of conferring protective immunity against tuberculosis. Indeed as early as in 1957 the oral administration of BCG to man was shown to confer delayed type hypersensi tivity (DTH) (De Assis, 1957). The justification for oral BCG administration was the fact that infection by tubercle bacilli commonly took place by ingestion. Also, it was known that the incompletely differentiated intestinal mucous membrane, present immediately after birth, was permeable to microbial transit towards the lymphatic and blood circulation. Further, autopsy studies proved that BCG passed through the intestinal membrane (Sol Roy Rosenthal, 1980). However, the value of

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Abbreviations used: DTH, Delayed type hypersensivity; NTM, non tuberculous mycobacteria; MAI, *Mycobacterium avium intracellular;* PPD, partially purified protein derivative; LJ medium, Lowenstein Jensen medium; MRI, mean root index; cfu, colony units; VC, viable count.

oral BCG vaccination in protection against disease was never adequately established in animals or in man, having been superseded by intradermal vaccinaton which can yield high protection (leading article in Tubercle, 1960). Nevertheless, with the failure of intradermal BCG to confer more than a slight protection in some recent clinical trials there is renewed interest in oral BCG (Tuberculosis Prevention Trial, 1980). This interest arises from two different points of view: (i) either oral BCG might be superior to intradermal BCG in conferring protective immunity since it mimicks the natural route of infection and (ii) in some regions of the world, natural oral exposure to environmental mycobacteria might play the role of natural vaccination or even deviate the immune responses thus preventing the protective effect of BCG vaccination.

The latter possibility that prior infection with various non tuberculous mycobateria (NTM) might in some way cross protect against subsequent infection by *Mycobacterium tuberculosis* was examined by Youmans *et al.* (1961). Palmer and Long (1966) tested this hypothesis and found that infection with NTM could provide as much as 50% protective effect as that produced by BCG.

Rook *et al.* (1981) postulated that two types of cell mediated responses could result in persons exposed to certain NTM prior to BCG vaccination; 'the Koch type' and the 'Listeria type' reactions. The former results in blocking the generation of acquired immunity to BCG vaccine whereas the latter enhances the protection. Orme and Collins (1983), using *M. kansasii* in mice found no interference by *M. kansasii* on BCG vaccination. But they speculated that oral immunisation (exposure) with *M. avium intracellulare* (MAI) complex might induce tolerance which might interfere with the immune response to subsequent BCG immunisation.

Among all the atypical mycobacteria isolated and identified so far from the BCG trial area in South India, MAI is the most common (Paramasivan *et al.,* 1985). Hence, in our present study, we have examined whether oral exposure to MAI influences the protective immunity offered by BCG.

Materials and methods

Animals

Random bred male guinea pigs maintained at the Tuberculosis Research Centre and weighing 300-400 g were used.

Experimental design

The experimental schedule is outlined in table 1.

BCG administration

Freeze-dried BCG vaccine, (Central BCG Laboratory, Guindy, Madras) was used for both intradermal and oral vaccination. Two mg of BCG is equivalent to 2.0×10^7 colony forming units.

For intradermal vaccination a dose of 0.075 mg BCG in 0.1 ml was injected intradermally in the shaved right flank. BCG was orally administered with a specially devised L-shaped canula attached to a syringe.

Table 1. Experimental design.

Day '0'	3rd Week	24, 48 and 72 h after skin test	9th Week	15th and 17th Week
Vaccination	Skin test with PPD (3 days before) challenge with $H_{37}Rv$	Skin test reading	Sacrifice, scoring and VC culture	Count colonies

Skin test reactivity to partially purified protein derivative

Ten μg of partially purified protein derivative (PPD) (Weybridge Laboratories, England) in 0·1 ml was injected intradermally on the left flank 3 days before challenge infection. The skin test reaction was assessed after 24, 48 and 72 h and the diameter of the area of induration measured in millimetres.

MAI

One of the MAI strains isolated from the BCG trial area in South India during 1981 was used.

M. tuberculosis strain $H_{37}Rv$

The $H_{37}Rv$ strain used was the strain maintained in Tuberculosis Research Centre, Madras. It has been periodically passaged in guinea pigs and is subcultured on Lowenstein Jensen medium (LJ medium).

Assessment of protection after vaccination

Challenge with M. tuberculosis $H_{37}Rv$: All groups of animals were challenged with virulent tubercle bacilli 3 weeks after vaccination, by injecting subcutaneously 1 mg moist weight of the bacilli in 0.5 ml of distilled water in the right thigh of each animal.

Assessment of tubercular lesion: All surviving animals were sacrificed after 42 days and the extent of disease present in the various organs scored after randomization of the animals. Any animal dying earlier was examined by autopsy and the extent of disease scored. The scoring system of Mitchison *et al.* (1960) was adopted. Morphological scores based on the severity of the disease (No. of tubercles, areas of necrosis and caseation) for the spleen were 0, 10, 20, 30 and 40; for the liver: 0, 8, 15, 23 and 30; for the lungs: 0, 5, 10, 15 and 20; and, for the site of inoculation and its draining lymphnodes, values were from 0-10 depending on the extent of involvement of the regional glands. Thus, macroscopic scores ranged from a minimum of 0 to a maximum (of 100) score for all organs of severely diseased animals of 100.

The mean root index: The mean root index (MRI) was obtained by dividing the total score by the number of days of survival of the animals (irrespective of whether

they died or were killed) and taking its square root. The root index of disease thus served as a measure of the degree of protection afforded by vaccination, the smaller the root index, the greater the protection.

Colony counts on spleen homogenates: A portion of the spleen taken aseptically from the animal was weighed and homogenised in 5 ml distilled water. The number of colony forming units (cfu) was determined by inoculating LJ medium with serial dilutions of the homogenate. Colonies were counted 6 and 8 weeks after incubation. The viable count (VC) was calculated as follows:

$$VC = cfu \times \frac{\text{Total wt. of spleen}}{\text{Wt. of portion taken}} \times \frac{1}{\text{Vol. inoculated-LJ medium}} \times 5.$$

Results

Table 2 shows the result of various doses of oral BCG administration on DTH and protection against challenge with *M. tuberculosis* $H^{37}Rv$. The intradermally vaccinated animals developed skin test reactivity (mean induration of 12.7 mm) and showed a MRI of 0.78. The mean log viable count of bacilli in the spleen was 4.3. None of the animals receiving oral doses ranging from 0·075-6 mg developed skin test reactivity or protective immunity.

Table 2. Lack of effect of smaller doses of oral BCG immunisation on DTH and protection against challenge with $H_{37}Rv$.

Group[a]	Skin test induration in mm[b] Mean ± S.D.	MRI[c] ± S.D	Log VC spleen Mean ± S.D.
Control	0	1·26 ± 0·13	6·3 ± 0·45
0·075 mg (ID)	12·7 ± 4·0	0·78 ± 0·19	4·3 ± 0·60
0·075 mg (oral)	0	1·17 ± 0·12	6·0 ± 0·53
1.5 mg (oral)	0	1·19 ± 0·12	6·7 ± 0·43
3.0 mg (oral)	0	1·21 ± 0·12	6.1 ± 0·42
6.0 mg (oral)	0	1·11 ± 0·43	5·6 ± 0.15

[a] 6 animals per group.
[b] 10 µg PPD was injected interadermally and reading were taken at 24.48 and 72 h.
[C] Mean of the square root of total score divided by No.of survival days.

Since a single oral dose of 6 mg of BCG failed to sensitize or confer immunity the effect of a large single dose (30 mg) or 6 multiple doses (consecutive days) of 5 or 10 mg were tested and the results are summarised in table 3. Both groups of animals developed skin test reactivity and protective immunity.

Subsequently, the effect of oral immunisation with MAI was studied using a single dose of 30 mg. Table 4 shows that, the guinea pigs which were given either intradermal or oral BCG immunisation showed a positive skin test reaction and a lower MRI. Similar results were seen with animals exposed to oral MAI.

The influence of MAI on BCG induced protective immunity when given orally prior to BCG is shown in table 5. The animals which were exposed to MAI prior to

Table 3. DTH responsiveness and protection against challenge with *M. tuberculosis* $H_{37}Rv$ after larger doses of oral BCG.

Group[a]	Skin test induration (mm)	MRI ± S.D.	Log VC spleen Mean ± S.D.
Experiment No. 1			
Control	1·0 ± 1·0	1.0 ±0·03	5·74 ± 0·4
BCG			
0·07 mg (ID)	18·6 ± 2·0	0·71 ± 0·10	4·70 ± 0·4
5·0 mg '0' × 6 days	15·0 ± 0	0·65 ± 0·16	4·9 ±0·5
10·0 mg '0' × 6 days	16·0 ± 2·0	0·74 ± 0·15	4·6 ±0·9
30 mg oral Single dose	14·0 ± 2·0	0·76 ± 0·03	4·77 ± 0·4
Experiment No. 2			
Control	1·5 ± 1·0	1·42 ± 0·17	6·00 ± 0·3
BCG			
0·075 (ID)	13·7 ± 2·0	0·66 ± 0·23	4·90 ± 0·2
30 mg (oral)	11·3 ± 2·0	0·68 ± 0·10	4·40 ± 0·31

[a]6 animals in each group.

Table 4. DTH and protective immunity induced by MAI.

Group[a]	Skin test Mean ± S.D. (mm)	MRI ± S.D. (mm)
Control	2·4 ± 1·5	1·4 ±0·17
BCG (intradermal)	9·2 ± 2·2	0·96 ± 0·095
BCG (oral/30 mg)	10 ±1·6	0·98 ± 0·01
MAI (oral/30 mg)	7·8 ± 1·9	0·72 ± 0·29

[a] 5 animals each group

BCG developed skin test reactivity (induration of 17.3 mm) and showed a MRI of 0·52.

Table 6 shows the influence of MAI on BCG induced protective immunity when given orally 3 weeks prior to intradermal BCG. The control animals gave a MRI of 0·95 and a viability count of 17×10^3 In contrast, the group of animals which received BCG (orally alone) and the group which received MAI (orally) prior to intradermal BCG showed a lower MRI and lower viability count when compared to those of controls ($P < 0·05$).

Table 5. Influence of MAI on BCG induced protective immunity when given orally 3 weeks prior to oral BCG.

Group[a]	Skin test Mean ± S.D. (mm)	MRI ± S.D. (mm)
Control	1·3 ± 2·3	0·86 ± 0·07
BCG (oral/30 mg)	17·6 ± 0·57	0·50 ± 0·27
M. avium (oral/30 mg)	13.6 ± 2·3	0·47 ± 0·07
M. avium (3 weeks prior to BCG immunisation)	17·3 ± 2·0	0·52 ± 0·09

[a] 3 animals in each group.

P values—Control *versus* BCG (oral) < 0·05;
Control *versus M. avium* < 0·05;
Control *versus M. avium*
3 weeks prior to BCG < 0·05.

Table 6. Influence of prior oral exposure of MAI on BCG induced protective immunity.

Group[a]	MRI ± S. D.[b]	Viable count[c]
Control	0·95 ± 0·12	$17 \times 10^3 \pm 5{\cdot}8 \times 10^3$
BCG (oral/30 mg)	0·65 ± 0·15	$7{\cdot}6 \times 10^3 \pm 3{\cdot}6 \times 10^3$
Oral MAI (3 weeks prior to ID BCG)	0·61 ± 0·2	$3{\cdot}9 \times 10^3 \pm 1{\cdot}78 \times 10^3$

[a] Consists of 5 animals in each group.

[b] *P* value is significant when MRI of oral BCG immunised animals and the animals which received oral MAI and intradermal BCG were compared with control group(< 0·05).

c P value is significant when viability count of oral BCG immunised animals and the animals which received oral MAI and intradermal BCG were compared with control group (< 0·05).

When the MRI and the VC of oral BCG immunised animals were compared with hose of the animals which received oral MAI and subsequent intradermal BCG there was no significant difference.

Discussion

High prevalence of atypical mycobacterial infection with resultant sensitization in the population of the BCG study area in Chingleput District, has been implicated as one of the mechanisms for the lack of protection by BCG against adult type of tuberculosis. The present study was carried out to examine the hypothesis, that oral exposure to MAI influences the protective immunity offered by BCG.

The initial experiments were set up to establish the protective immunity offered by oral BCG. Three weeks after oral immunisation with BCG, guinea pigs

developed marked skin test reactivity to PPD-S. A single oral dose of live BCG upto 6 mg weight did not induce skin test positivity whereas in separate experiments a dose of 30 mg was effective.

As there is no immunological test, either *in vivo* or *in vitro,* that correlates fully with protective immunity, an *in vivo* challenge with virulent *M. tuberculosis* H_{37} Rv was chosen as the means to examine protective immunity. It was seen that doses of BCG that did not confer delayed type hypersensitivity did not confer protection and protection was always seen when DTH was present. Nevertheless, there was no correlation between extent of delayed type hypersensitivity and degree of immunity as revealed by either lower viability count or lower disease indices.

Oral immunisation with MAI resulted in a mean skin induration of 7.8 mm as compared to 10 mm and 9.2 mm in animals given BCG orally or intradermally. This difference in skin test induration to PPD-S bears no relationship to MRI because MAI infected animals gave the lowest MRI as compared to those in animals given oral or intradermal BCG. Similar observations were obtained in groups of animals primed with MAI prior to BCG administration.

Rook *et al* (1981) proposed the hypothesis that contact with NTM might in some way jeopardise or interfere with the generation of acquired cell mediated immunity resulting from BCG immunisation and hence provide an explanation for the failure of the recent BCG trial in South India. Orme and Collins, (1983) concluded from their experiment that despite the presence of *M. kansasii* infection BCG vaccinated animals were fully resistant to subsequent aerosol challenge with virulent *M. tuberculosis.* Continuing in a similar line Orme and Collins (1984), using animals with pulmonary infection by *M. kansasii, M. Simiae, M. avium* and *M. scrofulaceum* showed that subsequent intravenous inoculation with 10^6 BCG had no discernible effect on the course of NTM infection within the lungs. However, all the BCG vaccinated group were fully resistant to subsequent acute aerogenic challenge with *M. tuberculosis* regardless of the presence of pulmonary NTM infection. Edwards *et al.* (1982) and more recently Smith *et al* (1985) reported that there was no interference due to MAI on the protective efficacy of BCG by using an aerosol challenge model in the guinea pigs. Collins (1983) nevertheless hypothesised that oral infection caused by MAI might induce immunological perturbation within the host capable of interfering with subsequent immune response.

Results of the present study using guinea pigs as model system, showed that oral exposure with MAI did not interfere with the protective immunity induced by BCG.

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