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Immune response & modulation of immune response induced in the guinea-pigs by *Mycobacterium avium* complex (MAC) & *M. fortuitum* complex isolates from different sources in the south Indian BCG trial area

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A total of 139 guineapigs were used to study the immune response and its modulation induced by *Mycobacterium avium* complex (MAC) and *M. fortuitum* complex strains obtained from different sources in the south Indian BCG trial area. The guineapigs were divided into groups and some were directly sensitised/immunised with different MAC strains, *M. fortuitum* complex strain or BCG and others were sensitised with MAC or *M. fortuitum* complex and then immunised with BCG. The resulting delayed type hypersensitivity (DTH) response in the different groups of guineapigs was studied by skin tests using PPD-RT23 and PPD-B, and protective response was studied by challenging the guineapigs with a south Indian low virulent strain of *M. tuberculosis* and enumerating the bacilli in spleen at different points of time. The 3 strains of MAC induced similar low levels of DTH to PPD-RT23 but much higher and varying levels of DTH to PPD-B. MAC strains from soil and sputum induced different levels of immune modulation during subsequent immunisation with BCG on the DTH response to PPD-RT23 and PPD-B. At 2 wk after challenge, 23.8, 81 and 90.5 per cent protection was induced by the standard strain, soil isolate and sputum isolate of MAC, respectively, while 33.3 per cent protection was induced by the *M. fortuitum* complex strain compared to the protection induced by BCG alone. Prior exposure to MAC or *M. fortuitum* complex did not have any modulatory effect on the protective immunity due to BCG at this time point. However, at 6 wk after challenge, while the guineapigs immunised with BCG were protected, modulation of the protective response resulting from BCG was observed in the guineapigs sensitised with MAC and *M. fortuitum* from soil.

Key words Immune response - immunomodulation - *Mycobacterium avium* complex - *M. fortuitum* complex - non-tuberculous mycobacteria - south Indian isolates

The nontuberculous mycobacteria (NTM) are ubiquitous in the environment[1]. Exposure of man to these organisms results in an immunologically effective contact as revealed by skin-test response to mycobacterial antigens[2-4]. As far back as 1962, Edwards and others[5] had suggested that mycobateria in the environment may have a bearing on the low-grade sensitivity often observed in the human population and that infection with such mycobacteria may have considerable influence on the course of subsequent infection with virulent tubercle bacilli. Such an immunomodulation has also been hypothesised to be one of the reasons for the failure of BCG to protect against adult-type pulmonary tuberculosis in the south Indian BCG trial[6]

In the south Indian BCG trial, the study population had a high level of scnsitisation to PPD-B[7]. Further, nearly 20 per cent of the NTM isolated from

sputum samples of subjects residing in this area belonged to the *M. avium* complex[8]. In a study recently reported by us[9], organisms of the *M. avium* complex (MAC) and *M. fortuitum* complex were found to be the predominant isolates from soil, water. house-dust and sputum samples in the south Indian BCG trial area. Subsequent to this, we carried out the present investigation in the guineapig model to study the extent of immune response and immunomodulation induced by MAC isolates obtained from sputum and environment and a soil isolate of the *M. fortuitum* complex in terms of delayed type hypersensitivity (DTH) response to mycobacterial skin-test antigens, PPD-RT23 and PPD-B, and protective response against challenge infection by south Indian low virulent (SILV) strain[10] of *M. tuberculosis.*

Material & Methods

The investigation was carried out in three experiments; the immune response resulting from exposure to MAC organisms was studied in the first experiment. immunomodulation by MAC organisms was studied in the second experiment. and the immune response and immunomodulation resulting from exposure to *M. fortuitum* complex organisms were studied in the third experiment.

Mycobacterial strains : The following strains were used in the experiments: *(i)* TMC 1403- standard strain of *M. intracellulare* (referred to as MACsd in text, tables and figures). *(ii)* MAC from soil (MACso)-strain A55/4 isolated from soil sample collected in the south Indian BCG trial area. *(iii)* MAC from sputum (MACsp)- strain TS11515 isolated from sputum sample of a symptomatic subject with no *M. tuberculosis* isolation residing in the south Indian BCG trial area. *(iv) M. fortuitum* - strain A33/1a isolated from soil sample collected in the south Indian BCG trial area. *(v)* BCG- a sub-culture on LJ from a fresh ampoule of BCG (BCG Laboratory, Madras). *(vi) M. tuberculosis* south Indian low virulent strain (SILV)- strain N9431 isolated from a pulmonary tuberculosis patient from the south Indian BCG trial area. established as a low virulent strain in guineapigs and maintained on LJ. This strain was passaged in guineapigs before each experiment.

Guineapigs : A total of 139 random bred guineapigs of the M strain, in the weight range 450-600g, maintained at the Tuberculosis Research Centre (TRC), Madras animal house, were used in the experiments.

Immunisation and challenge : In the 1 st experiment. there were 5 groups of 8 animals each which were immunised/sensitised with BCG or the different strains of MAC and then challenged with SILV as shown in Table I. In the 2nd experiment. there were 5 groups of 15 animals each which were sensitised with different strains of MAC prior to immunisation with BCG and challenge with SILV as shown in Table II. In the 3rd experiment, there were 4 groups of 6 animals each which were immunised/sensitised with BCG or *M. fortuitum* alone or sensitised with *M. fortuitum* prior to immunisation with BCG, followed by challenge with SILV as shown in Table III.

For sensitisation and immunisation with MAC. *M. fortuitum* and BCG. 1 mg moist weight of the organisms [10^7-10^9] colony forming units (cfu)] in 0.1 ml sterile distilled water was injected intradermally in the inner side of the thigh.

For challenge with SILV, 1 mg moist weight of organism (10^7cfu) in 0.5ml sterile distilled water was injected intramuscularly in the thigh.

Skin tests : One week before challenging with SILV, all the animals were simultaneously skin-tested with 5 IU of PPD-RT23 (BCG Laboratory, Guindy, Madras) and 50 TU of PPD-B (BCG Laboratory, Guindy, Madras) as standardised in preliminary experiments, by intradermal injection of 0.1 ml of each antigen on the shaved dorsal flanks and were read at 24 h[11].

Bacterial enumeration in spleen (BE-spleen) : Following challenge with SILV, 4 guineapigs from each group were sacrificed using chloroform vapour at 2 and 6 wk in experiment 1, 5 guineapigs from each group at 2, 6 and 12 wk in experiment 2 and 3 guineapigs from each group at 2 and 6 wk in experiment 3. Spleens were collected aseptically and homogenised in 5 ml of sterile distilled water using a motorised Teflon grinder. From this homogenate and 5 serial 10-fold dilutions, 10 µl were inoculated into 2 slopes of Lownstein-Jensen (LJ) medium each. The slopes were read after 4 wk of incubation at 37°C. The $\log_{10}$ cfu of SILV in spleen for each animal was calculated based on the number of colonies obtained on LJ slopes.

Table I. Design of experiment 1 : Immune response induced by MAC organisms

Time (wk)	Group 1 n=8 Control	Group 2 n=8 BCG-SILV	Group 3 n=8 MACsd-SILV	Group 4 n=8 MACso-SILV	Group 5 n=8 MACsp-SILV
0		BCG	MACsd	MACso	MACsp
5	ST	ST	ST	ST	ST
6	SILV	SILV	SILV	SILV	SILV

Bacterial enumeration in spleen was done in each group at 2 and 6 wk after SILV challenge

BCG, Bacille Calmette Guerin.

MACsd, Standard strain of *M. intracellulare*, TMC 1403.

MACso, *M. avium* strain isolated from soil.

MACsp, *M. avium* strain isolated from sputum.

ST, Skin-test with PPD-RT23 and PPD-8.

SILV, South Indian low virulent strain of *M. tuberculosis*

Table II. Design of experiment 2 : immunomodulation by MAC organisms

Time (wk)	Group 1 n=15 Control	Group 2 n=15 BCG-SILV	Group 3 n=15 MACsd-BCG-SILV	Group 4 n=15 MACso-BCG-SILV	Group 5 n=15 MACsp-BCG-SILV
0			MACsd	MACso	MACsp
6		BCG	BCG	BCG	BCG
11	ST	ST	ST	ST	ST
12	SILV	SILV	SILV	SILV	SILV

Bacterial enumeration in spleen was done in each group at 2, 6 and 12 wk after SILV challenge

MACsd, Standard strain of *M. intracellualre*, TMC 1403.

MACso, *M. avium* strain isolated from soil.

MACsp, *M. avium* strain isolated from sputum,

BCG, Bacille Calmette Guerin.

ST, Skin-test with PPD-RT23 and PPD-R.

SILV, South Indian low virulent strain of *M. tuberculosis*

Statistical analysis : The results arc expressed as mean ± SD. Chi square tests for proportions. t-test for comparing the mean values of two groups and ANOVA for comparing more than two groups were used to test the significance of the data. The multiple comparison tests were also carried out along with ANOVA to categorise the group means. Log transformations were used to stabilise the variations in the data before performing statistical analysis. The SPSS/PC+ software package was used to perform the statistical analysis.

Results

DTH response induced by MAC : The results of the skin tests done using PPD-RT23 and PPD-B to study DTH induced by MAC organisms are given in Fig. 1. The DTH to PPD-RT23 and PPD-R in animals in the control group was negligible with the mean reaction being 0.4±0.9mm and 0.4±0.6mm for PPD-RT23 and PPD-B, respectively.

The skin test response to PPD-RT23 in the animals immunised with BCG (mean 7.0±2.0mm) was

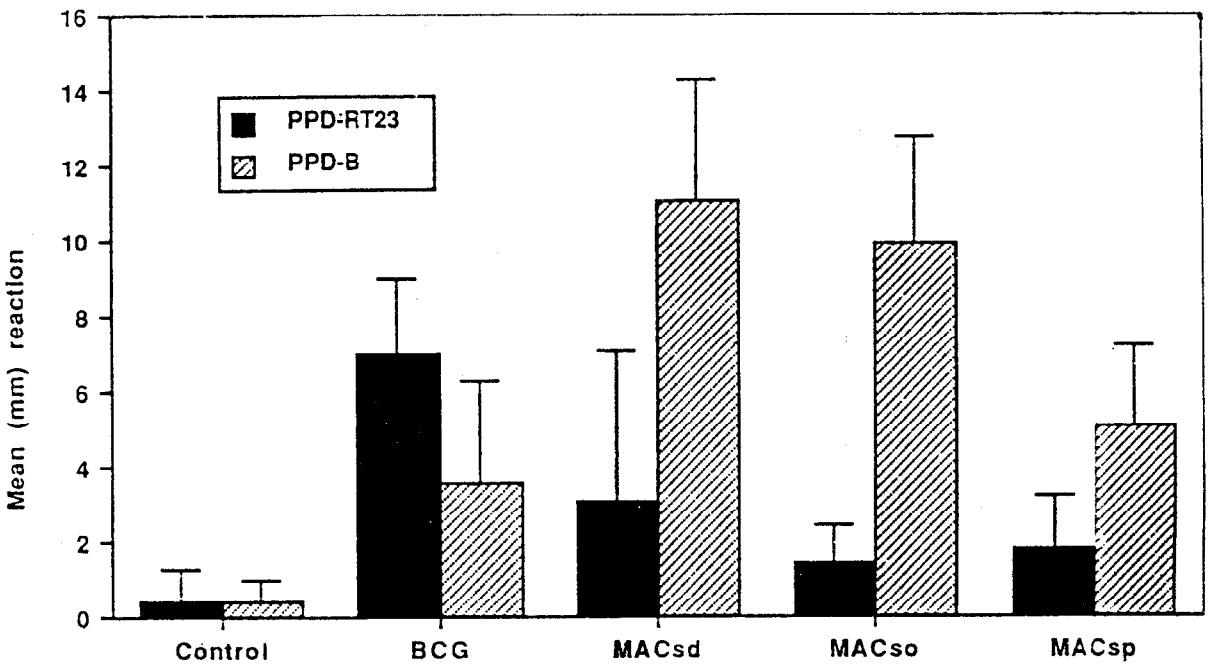

Fig. 1. Mean (mm) reaction to PPD-RT33 and PPD-B in the guineapigs exposed to different strains of MAC or BCG. 1. Control : Control animals, 2. BCG : Bacille Calmette Guerin, 3. MACsd : Standard strain of *M. intracellulare,* TMC 1403. 4. MACso : *M. avium* complex strain isolated from soil. 5. MACsp : *M. avium* complex strain isolated from sputum.

Table III. Design of experiment 3 : immune response and immunomodulation resulting from exposure to *M. fortuitum* complex

Time (wk)	Group 1 n=6 Control	Group 2 n=6 BCG-SILV	Group 3 n=6 *M. fortuitum* SILV	Group 4 n=6 *M. fortuitum*-BCG-SILV
0				*M. fortuitum*
6		BCG	*M. fortuitum*	BCG
11	ST	ST	ST	ST
12	SILV	SILV	SILV	SILV

Bacterial enumeration in spleen was done in each group at 2 and 6 wk alter SILV challenge

BCG, Bacille Calmette Guerin,

ST, Skin-test with PPD-RT23 and PPD-B,

SILV, South Indian low virulent strain of *M. tuberculosis*

significantly higher (P<0.03) compared to the responses observed in the animals immunised with any of the three MAC strains which were not significantly different from each other. Compared to that in the control group, the response to PPD-RT23 was significantly higher in the animals immunised with BCG (P<0.0001) or with MACsp (mean 1.8± 1.4mm; P=0.04) but not in the animals immunised with MACsd (mean 3.1±4.0mm) or with MACso (mean 1.4±1.0mm). Thus, the DTH response to PPD-RT23 was maximum in the animals immunised with BCG followed by the response in the animals immunised with MACsd, MACsp, MACso and the control animals in the order of decreasing reactivity.

The skin test response to PPD-B was also significantly higher (P<0.01) in the sensitised animals (means 3.6±2.7mm, 11.1±3.2mm, 9.9±2.9mm and 5.1±2.1mm in the animals sensitised with BCG, MACsd, MACso and MACsp, respectively) as compared to that observed in the control animals. The skin test response to PPD-B in the animals immunised with MACsd or MACso was similar and was significantly higher than the response in the animals immunised with BCG or with MACsp (P<0.001). Thus. the DTH response to PPD-B was maximum in the animals immunised with MACsd followed by the

response in the animals immunised with MACso, MACsp, BCG and the control animals in the order of decreasing reactivity.

Modulation of DTH response induced by MAC : The results of the skin tests done using PPD-RT23 and PPD-B to study modulation of DTH are given in Fig. 2. The DTH in animals in the control group was again negligible with the mean reaction being 0.2±0.8mm and 0.4±1.1 mm for PPD-RT23 and PPD-B, respectively. Compared to this group, significant levels of DTH ($P<0.01$) to both PPD-RT23 and PPD-B were seen in the animals immunised with BCG alone (means 8.6±4.2mm for PPD-RT23 and 4.3±3.1mm for PPD-B) or exposed to different strains of MAC prior to immunisation with BCG (means 9.5±2.6mm, 5.9±4.2mm, 9.6±3.0mm for PPD-RT23, and 6.6±3.3mm, 3.0±2.7mm and 6.0±3.6mm for PPD-B in animals sensitised with MACsd, MACso and MACsp, respectively).

However, among the sensitised and/or immunised guineapigs while the skin-test response in animals sensitised with MACsd or MACsp before immunisation with BCG was similar to that in the animals immunised with BCG without prior sensitisation for both PPD-RT23 and PPD-B, a significantly ($P<0.05$) lower skin test response to both PPD-RT23 and PPD-B was seen in animals pre sensitised with MACso.

DTH response and modulation of DTH response induced by M. fortuitum complex : The results of the skin tests done using PPD-RT23 and PPD-B to study the DTH induced by *M. fortuitum* complex are given in Fig 3. The DTH in animals in the control group was negligible at 48 h (1.7±2.8 and 0.8+1.2mm, respectively, for PPD-RT23 and PPD-B). Compared to the control animals. significantly higher DTH responses to PPD-RT23 was observed in all the other groups ($P=0.05$). On the other hand, the DTH response to PPD-B in the animals sensitised with *M. fortuitum* prior to immunisation with BCG (5.7±3.6mm) was significantly higher than that in the control animals ($P=0.03$) and in the animals

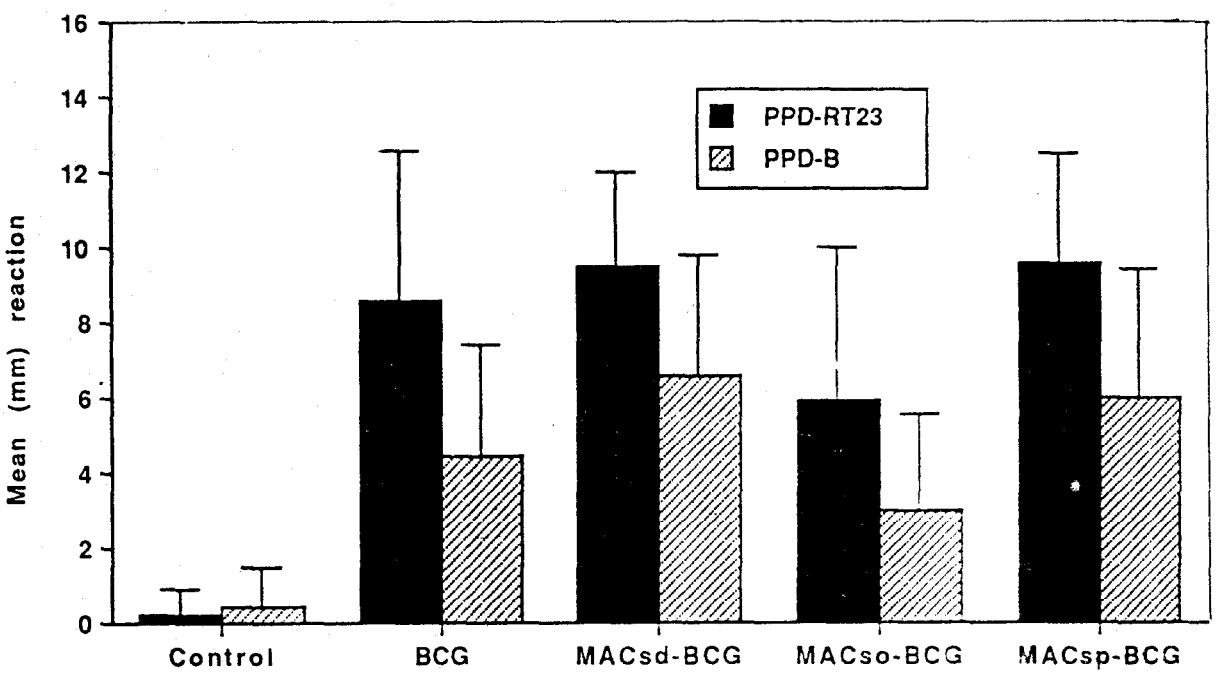

Fig. 2. Mean (mm) reaction to PPD-RT23 and PPD-H in the guineapigs exposed to different strains of MAC or BCG. 1. Control : Control animals, 2. BCG : Bacille Calmette Guerin, 3. MACsd-BCG : Standard strain of *M. intracellulare,* TMC 1403 followed by BCG, 4. MACso-BCG : *M. avium* complex strain isolated from soil followed by BCG, 5. MACsp-BCG : *M. avium* complex strain isolated from sputum followed by BCG.

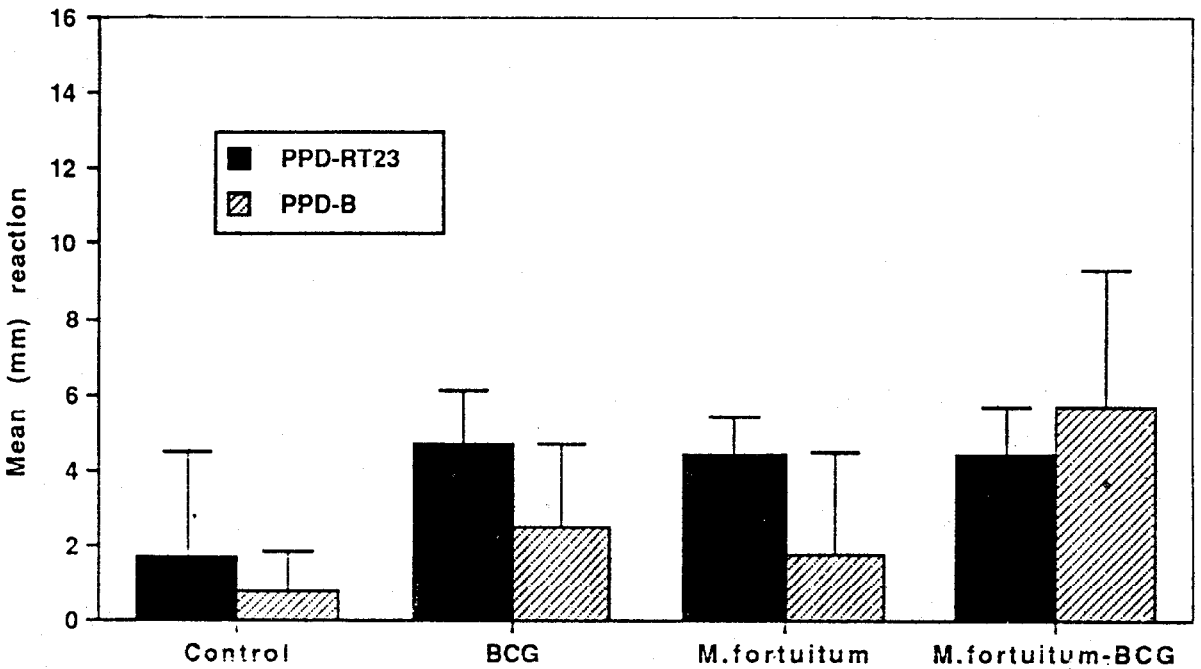

Fig. 3. Mean (mm) reaction to PPD-RT23 and PPD-R in the guineapigs exposed to BCG, *M. fortuitum* or *M. fortuitum* followed by BCG.
1. Control : Control animals, 2. BCG : Bacille Calmette Guerin, 3. *M. fortuitum*- BCG : *M. fortuitum* followed by BCG.

immuniscd with *M. fortuitum* alone (1.8±2.7mm) (P=0.03) but not significantly different from that in the animals immunised with BCG (2.8±2.2mm).

Protective response induced by MAC : The results of BE-spleen in the experiment carried out to estimate protective response against challenge infection with SILV in the guineapigs exposed to different strains of MAC or BCG arc given in Fig. 4.

At 2 wk after challenge, the $\log_{10}$ cfu of SILV in the spleen was high (4.8±0.6) in the control animals. Compared to this group, the count was significantly lower (P=0.002) in the animals immunised with BCG (2.7±0.5) indicating that BCG elicited a good protective response. Considering this reduction of 2.1 $\log_{10}$ cfu in the animals immunised with BCG as 100 per cent protection. 23.8 per cent (4.3±0.4), 81 per cent (3.1±2.1) and 90.5 per cent (2.9±2.0) protection was seen in the animals exposed to MACsd, MACso and MACsp, respectively. However, the counts in the animals immunised with the different strains of MAC were not significantly different from that in the control animals.

At 6 wk after challenge, SILV could not be detected in the spleen of any of the animals immunised with BCG. In all the other groups. detectable numbers of SILV were present at least in some of the animals in each group. The mean counts in the animals exposed to MACsp (1.9±2.2) and MACso (0.9±1.7) were higher than that in the control animals (0.6±1.2) which was similar to the count in the animals exposed to MACsd (0.6±1.2). However, the mean counts in these groups were not significantly different from each other or from that in the control. In all animals other than those immunised with MACsp there was marked reduction (P<0.01) in the counts at 6 wk after challenge as compared to the counts at 2 wk after challenge.

Modulation of protective response induced by MAC : At 2 wk after challenge, the $\log_{10}$ cfu of SILV in spleen was high (5.3±0.9) in the control animals and compared to this group, the count was significantly (P<0.01) lower in the animals immunised with BCG without prior sensitisation (2.0±1.9; Fig 5) indicating that BCG elicited a good protective response. Considering this as 100 per cent protection, 97 per

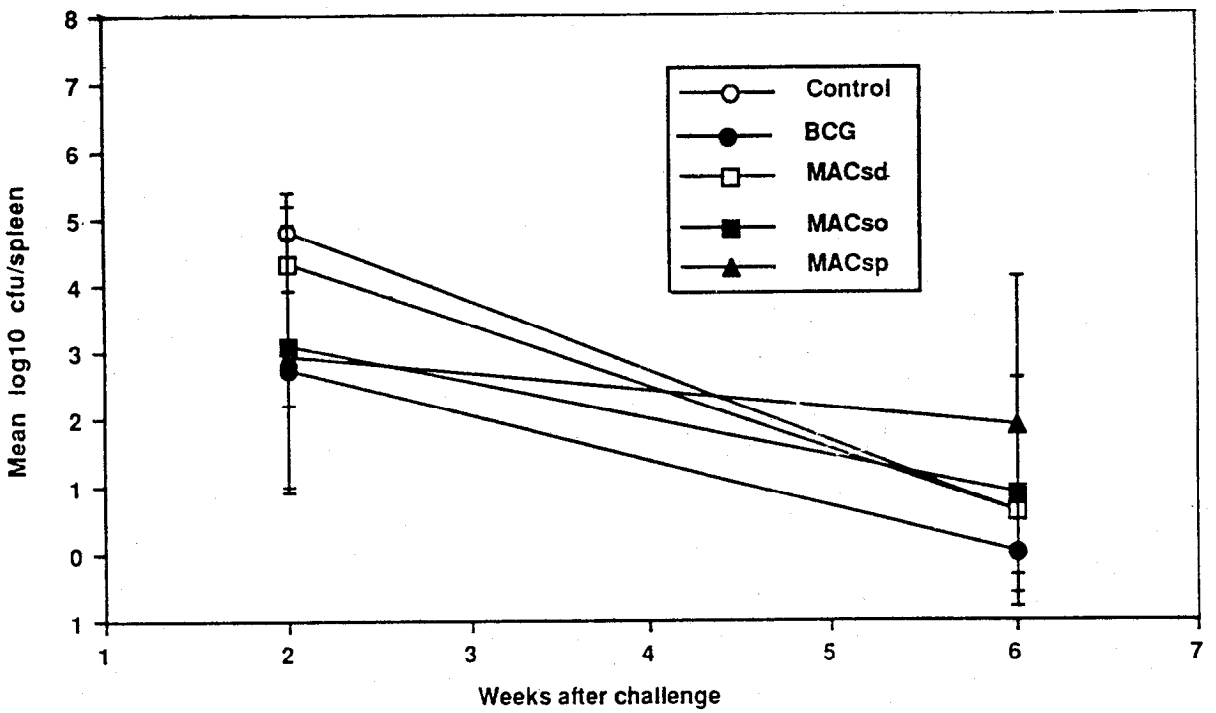

Fig. 4. Bacterial enumeration in spleen in the guineapigs exposed to different strains of MAC or BCG.
1. Control : Control animals, 2. BCG : Bacille Calmette Guerin, 3. MACsd : Standard strain of *M. intracellulare,* TMC 1403, 4. MACso : *M. avium* complex strain isolated from soil, 5. MACsp : *M. avium* complex strain isolated from sputum.

cent (2.1±0.3), 103 per cent (1.9±1.8) and 84.8 per cent (2.5±1.5) protection was seen in the animals immunised with BCG after prior sensitisation with MACsd, MACso and MACsp, respectively. The counts in the animals sensitised with MACsd, MACso or MACsp prior to immunisation with BCG were not significantly different from that observed in the animals immunised with BCG without prior sensitisation. indicating that prior exposure to MAC organisms did not have any modulatory effect on the protective response elicited by subsequent BCG immunisation during the early course of challenge infection in guineapigs studied.

At 6 wk after challenge, data for 1 animal each from groups 2, 3 and 4 and 2 animals from group 5 were not available because of death not due to tuberculosis. In the remaining animals. the challenge organisms could not be detected in the spleens of animals immunised with BCG without prior sensitisation or sensitised with MACsd or MACsp prior to immunisation with BCG. However, in 2 of 4 animals sensitised with MACso prior to immunisation with BCG, the challenge organisms were still present in the spleen, indicating a modulatory effect and the mean counts in these animals (1.6±1.9) was slightly higher but not significantly different from that observed in the control animals (0.5±1.1).

At 12 wk after challenge, data for 1 animal-each from groups 3 and 4 were not available because of death not due to tuberculosis. In the remaining animals. in all the groups including the controls, the challenge organisms could not be detected in the spleen.

Protective response and modulation of protective response induced by M. fortuitum complex : The results of BE-spleen in the experiment carried out to estimate protective response and its modulation against challenge infection with SILV in the guineapigs immunised with *M. fortuitum* or sensitised with *M. fortuitum* prior to immunisation with BCG are given in Fig. 6. Data for 1 animal from group 2 was not available because of death not due to tuberculosis.

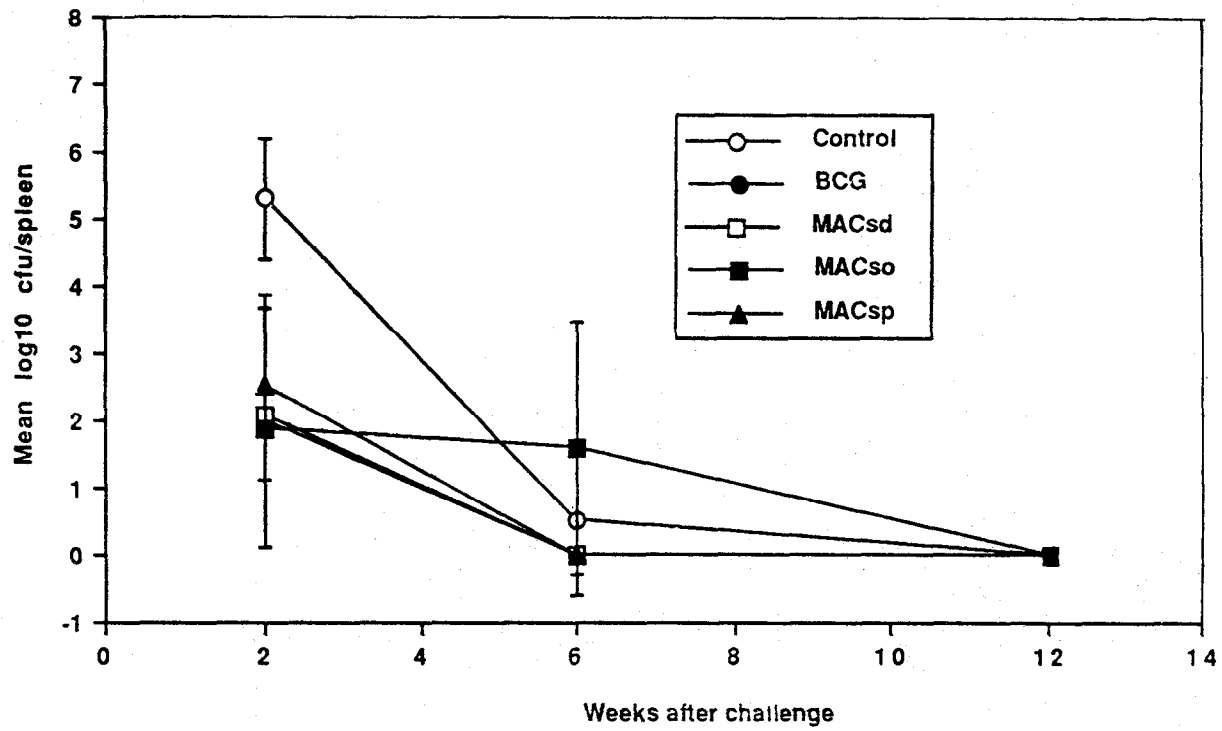

Fig. 5. Bacterial enumeration in spleen in the guineapigs exposed to different strains of MAC or BCG.
1. Control : Control animals, 2. BCG : Bacille Calmette Guerin, Not visible at 2, 6, and 12 wk., 3. MACsd-BCG : Standard strain of *M. intracellulare,* TMC 1403 followed by BCG, 4. MACso-BCG : *M. avium* complex strain isolated from soil followed by BCG, 5. MACsp-BCG : *M. avium* complex strain isolated from sputum followed by BCG.

At 2 wk after challenge, the $\log_{10}$ cfu of SILV in the spleen was high (3.9±0.3) in the control animals. Compared to this group, the counts were lower in the animals immunised with BCG (1.8±1.5), or with *M. fortuitum* (3.2±0.8) and in the animals sensitised with *M. fortuitum* prior to immunisation with BCG (1.9±2.0) but the differences were not significant. Thus. considering the reduction of 2.1 $\log_{10}$ cfu in the animals immunised with BCG as 100 per cent protection, 33.3 per cent protection was seen in the animals immunised with *M. fortuitum* and 95.2 per cent protection in the animals immunised with BCG after prior sensitisation with *M. fortuitum.*

At 6 wk after challenge, while SILV could not be detected in the spleen of the animals immunised with BCG, viable SILV could be detected in 1 of 3 animals in the group either immunised with *M. fortuitum* or sensitised with *M. fortuitum* prior to immunisation with BCG and the mean counts in these animals (0.9±1.6) was similar to that in the control animals (0.9±1.6).

Discussion

The results of the present study showed that in guineapigs immunised with MAC, the skin test response to PPD-B was greater than that to PPD-RT23 which would be expected since the DTH response to homologous antigens is greater than that to heterologous antigens[3,4].

Some differences were observed in the DTH response induced by the different strains of MAC tested. The level of DTH response to PPD-B. the homologous antigen, was significantly lower in the animals immunised with MAC from sputum as compared to that in animals immunised with either the standard strain, TMC 1403 or MAC from soil. MAC obtained from soil and sputum samples also induced different levels of immune modulation on the DTH response. The immune modulation by prior sensitisation with MAC of the DTH resulting from immunisation with BCG in terms of reduced response to PPD-RT23 and PPD-B was highest in the animals which had been sensitised with MAC from

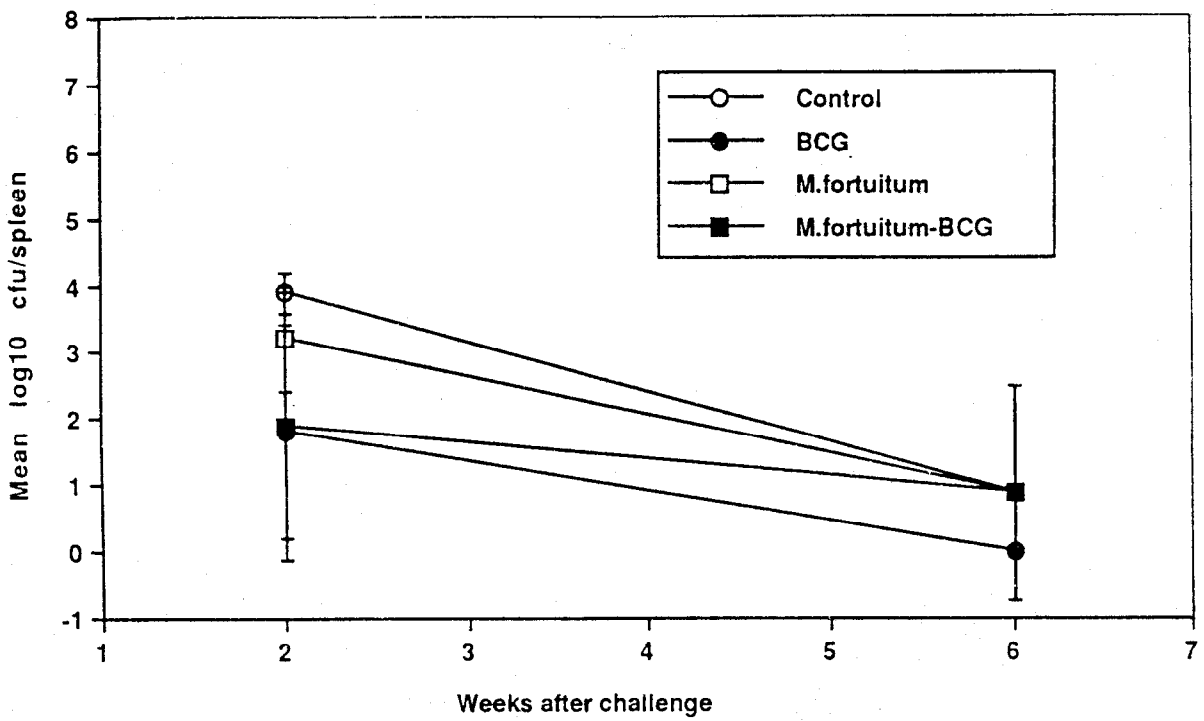

Fig. 6. Bacterial enumeration in spleen in the guineapigs exposed BCG, *M. fortuitum* or *M. fortuitum* followed by BCG. 1. Control : Control animals, 2. BCG : Bacille Calmette Guerin, 3. *M. fotuitum*- BCG *: M. fortuitum followed* by BCG.

soil. It has been shown that prior sensitisation of guineapigs with *M. simiae* leads to a reduction in the DTH response to PPD-S[12] resulting from subsequent vaccination with BCG, which was termed as 'immune modulation', while there is no apparent effect on the protective response[11]. Brown et al[13] have also shown that depending on the timing of exposure of mice *in vivo* to *M. vaccae* before BCG vaccination. oral administration of this species of environmental mycobacteria can either enhance. mask or interfere with the expression of sensitisation with BCG.

In the experiment carried out to study the DTH induced by organisms of the *M. fortuitum* complex from soil, *M. fortuitum* induced the same level of DTH response to both PPD-RT23 and PPD-B as BCG, and appeared to boost the DTH response to PPD-B in the animals subsequently immunised with BCG. This may be due to the presence of antigens common to both *M. fortuitum* and BCG in PPD-B.

With regard to protective immune response induced by nontuberculous mycobacteria, in Palmer and Long's extensive study using more than 1000 guincapigs and survival time as a measure of protection. it was shown that *M. fortuitum* infection induced approximately 15 per cent. *M. avium* and *M. scrofulaceum* infections induced approximately 50 per cent while *M. kansasii* infection induced approximately 70 per cent of the protective efficacy of BCG[14]. In the present study, using a more precise estimation. *i.e.*, BE-spleen, the reduction of 2.1 $\log_{10}$ cfu of SILV in spleen at 2 wk after challenge in the animals immunised with BCG compared to that in the control animals as 100 per cent protection, 23.8. 81 and 90.5 per cent protection was seen in the animals exposed to MACsd, MACso and MACsp, respectively, indicating that the standard strain of MAC maintained in the laboratory induced a lower protective effect compared to the other strains. Similarly, 33.3 per cent protection was seen in the animals exposed to the *M. fortuitum* complex strain. Palmer and Long[14] had earlier reported that the protection induced by *M. fortuitum* was 15 per cent of that induced by BCG. At 6 wk after challenge, 100 per cent protection was seen in the animals immunised with BCG and the challenge organisms could not be detected in their spleens. On the other hand, the resistance to dissemination of infection to

the spleen in the animals immunised with different strains of MAC was not greater than that seen in the control animals and viable challenge organisms could still be detected in their spleens.

The guineapigs used in the present study seemed to have a high degree of natural resistance to mycobacterial infection as seen by the reduction in BE-spleen even in the control animals by 6 wk after challenge in the absence of any evidenced of prior exposure to mycobacterial antigens as evidenced by the negligible skin test response to PPD-RT23 and PPD-B in these animals. Mitchison *et al* [10] have already noted in the 1960s that the M-strain guineapigs might differ in their susceptibility to tuberculosis, and it is possible that this difference has become more marked over the years[15,16].

In the present study, prior exposure either to MAC or *M. fortuitum* was not seen to have any modulatory effect on the protective immunity due to BCG in the early course of challenge infection since the number of challenge organisms recovered from spleen at 2 wk after infection in animals sensitised with MAC or *M. fortuitum* prior to immunisation with BCG was comparable to that observed in the animals immunised with BCG alone. Other workers[17,18] have also observed similar absence of any modulation due to prior sensitisation with MAC on the protective effect of BCG. The hypothesis that oral immunisation with MAC might induce tolerance which might interfere with the immune response to subsequent BCG was examined in an earlier study" carried out at the TRC, Madras. It was found that oral exposure with MAC did not interfere with the protective immunity induced by BCG. However, at 6 wk after challenge in the present study. modulation of protective response resulting from BCG was observed in the animals sensitiscd with either MAC from soil or *M. fortuitum* from soil and viable challenge organisms were recovered from the spleens of these animals while in all the other animals, except the controls, which were directly challenged, the challenge organisms could not be detected in the spleen by this point of time.

Abrahams[20] first suggested that the initial mycobacterial infection may set the reaction pattern to subsequent mycobacterial infections. Later, Stanford and Rook[21] suggested that there may be two types of cell mediated responses to mycobacteria, one of them, the Listeria-type being protective and the other, the Koch-type leading to adverse reactions to subsequent mycobacterial infections. It may be possible to understand the basis for the observed differences in the immunogenicity of MAC strains by using techniques such as DNA fingerprinting, RNA typing, plasmid profile, isoenzyme pattern, *etc.*, to study these strains at the molecular level. In a comparison of drug susceptibility of MAC isolates from AIDS patients in British Columbia with those from animals, isolates from patients were more susceptible as compared to those from animals[22]. Further frequency of plasmids has been found to be much higher in clinical and aerosol isolates of *M. avium, M. intracellulare* and *M. scrofulaceum* as compared to those from soil. dust, sediment and water[23]. Such differences could also be occurring in the antigenic composition and immunogenicity of MAC isolates from different sources, and these differences might have been responsible for the differences in the immune response and immune modulation induced by the different strains in the present study.

Though many studies have been done using animal models to investigate the effect of nontuberculous mycobactcrial infections on the protective response induced by subsequent BCG vaccination[11,13-15,17-19,] they have mainly relied on standard strains and have not attempted a comparison between wild-type strains of the same species obtained from different sources. This was attempted in the present study for the first time to our knowledge. from an area where a large scale BCG trial was conducted. Future studies using these isolates may need to focus on the effect of multiple exposures and aerosol infections with these mycobacteria on subsequent protective response which would be more simulatory of the actual sequence of events taking place in man[24].

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