STUDY ON ENVIRONMENTAL MYCOBACTERIA OBTAINED FROM SOUTH INDIAN BCG TRIAL AREA.

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Mycobacteria in the environment

Non Tuberculous Mycobacteria (NTM) are widely distributed in our environment and man is being constantly exposed to these organisms by various means(l). This immunologically important contact may be involved in the modulation of immunity to tuberculosis. Prior sensitization with NTM has beep considered as one of the explanations for the failure of BCG to provide protection against tuberculosis in the South Indian trial. Tuberculosis surveys using PPD-B have shown that in this area, prevalence of sensitization reaches 90%. in persons by age 14(2). Identification of NTM isolates from sputum samples in this area has shown *M.avium-intracellulare* and *M.scrofulaceum* to be among the important species(3). However. the actual distribution profile of the various NTM species in the environment of this area is not known.

In order to study this, in the first phase, standardisation experiments were carried out to choose an optimum method for the isolation of mycobacteria from soil and water samples(4). In the second phase, utilising the ideal procedure thus evolved. about 150 samples each of soil and water and 75 samples of dust collected from the South Indian BCG trial area on each of two occasions. once during the summer and once after the monsoon, were processed and the mycobacteria isolated were identified. Soil and water samples from a control area in Britain with a very low level of sensitization have also been included. NTM isolates obtained from sputum samples at the same time from subjects residing in the same area were also included for the purpose of comparison(5).

The results of the study indicated that the isolates belonging to *M.avium* complex (MAC) were predominant in water. dust and sputum samples and isolates belonging to the *M.fortuitum* complex were predominant in soil samples. There were no changes in profile between January and June: however, yield from environmental samples was lower in June.

Since organisms belonging to MAC include *M.avium, M.intracellulare* and an unnamed third specks. and *M.fortuitum* complex consists of *M.fortuitum, M.peregrinum, M. chelonae, M.abscessus,* the unnamed third biovar of *M.fortuitum* and the MCLO. a representative number of the isolates of both these groups were subjected to drug and heavy metal susceptibility studies to see as recorded by others whether susceptibility to these agents would be of help in comparing environmental and sputum isolates(6). However, using this procedure, we were unable to distinguish between isolates from the environment and from sputum.

Molecular characterisation of MAC

Phenotypically identified MAC isolates from different sources were further chracterised using plasmid profiles(7), identification by 3 specific probes, namely, DT1, DT6(8) and LiPA(9,10), analysis of lipids using gas chromatography-mass spectrometry (GC-MS) and analysis of mycolic acid pattern using thin layer chromatography(11). The results are shown in the Table.

i. Plasmid profile

Plasmid DNA analysis of a total of 62 MAC isolates, 13 each from water and dust, 16 from soil and 18 from sputum showed that the maximum number of plasmid-carrying strains were among the non-pigmented strains from water while MAC strains from sputum yielded few plasmids. These results are in contrast to those reported for plasmids from MAC strains in earlier studies where they have been reported to be preferentially found in clinical and aerosol isolates.

ii. Mycolic acid profile:

a. GC-MS : A total of 32 MAC isolates (8 each from water and dust and 10 each from soil and sputum) were also characterised for their fatty acid composition using W-MS. Among the dust isolates, 6 were identified as MAC, and 1 each as *M.phlei* and *M.fortuitum.* For soil, 6 were identified as MAC. Of the remaining 4 strains, 3 were identified as *M.fortuitum* and 1 as *M.xenopi.* For sputum, 6 were identified as MAC, 1 each as *M.fortuitum* and *M.xenopi* and 2 as *M.phlei.* For water, 4 were identified as MAC, 1 each as *M.gordonae* and *M.xenopi,* and 2 as *M.simiae.*

b. TLC : A total of 30 isolates (8 from water, 9 from dust, 11 from soil and 12 from sputum) were analysed for their mycolic acid pattern using TLC. All the water and dust isolates tested produced a pattern typical of MAC (type H; d-keto, w-carboxymycolate). Six isolates from soil and 8 from sputum also produced a type H pattern. Two isolates from soil and 3 from sputum produced a type D pattern while 3 isolates from soil and 1 from sputum produced a type E pattern.

iii. DNA probe:

a. LiPA: A total of 39 MAC strains (7 from dust, 9 from water, 11 from soil and 12 from sputum) were tested by the LiPA probe. A total of 16 isolates (3 from water, 5 from sputum and 8 from soil) were identified as Mycobacterium sp. other than MAC. Of the remaining 23 strains, none were identified as *M.avium* or *M.intracellulare,* 1 but identified as *M.scrofulaceum* and 22 as MAC intermediates. Two of the isolates, one each from soil and sputum were identified as M.malmoense by LiPA and both these isolates were identified as *M.fortuitum* by GC-MS.

b. DT1 and DT2 PCR: A total of 42 isolates were tested by 2 probes for the specific identification of *M.intracellulare* (DT1) and *M.avium* (DT6). Of these, 16 strains tested positive with DT1 *(M.intracellulare)* and none with DT6. No strain was identified as *M.avium* using these probes.

iv. 16S rRNA and PCR-REA

MAC strains that gave discrepant results. as they were DT1 positive but gave negative results by the *M.intracellulare* AccuProbe assay were subjected to a detailed molecular analysis. These included PCR-restriction enzyme analysis (PRA) of the hsp65 gene and 16S rRNA gene sequencing. The results confirmed the reported heterogeneity of *M.intracellulare* as only 32% gave PRA result compatible with published *M.intracellulare* profiles while the rest of the isolates were grouped in four previously unpublished profiles. 16S rRNA gene sequencing showed that only 42% were related to *M.intracellulare*, the rest being related to others. In conclusion, by this attempt a significant number of MAC isolates from South Indian BCG trial area which were not identified by the AccuProbe assay, (DT1 positive & DT6 negative) were characterised by PRA or 16S rRNA sequencingt(12).

Thus. these results emphasis the heterogencity of environmental mycobacteria, particularly of the MAC organisms. However, a tendency for greater agreement of the results of the various tests for isolates from water and dust was observed while there was greater discordance for the results of these tests for isolates from soil and sputum.

Immuno modulation studies in animal model

Studies on the modulation of immune response to BCG resulting from prior exposure to NTM in guinea pig models showed that oral exposure to MAI did not interfere with subsequent immune response to BCG(13). Prior exposure to NTM by subcutaneous and intradermal routes also did not interfere with the immune response to BCG in the early course of challenge infection but it appeared that immuno modulation could be taking place at the later stages of the infection(14). Some differences were observed in the immune response and modulation of immune response induced by the different strains of MAC. And also at 6 weeks after challenge. modulation of protective response resulting from BCG was observed in the animals sensitized with either MAC from soil or *M.fortuitum* from soil (15).

Thus. the results of this study suggest that certain modulation of the protective immunity due to BCG was probably taking place in animals exposed to NTM first. In populations of endemic areas of tuberculosis with high prevalence of NTM, prior exposure to NTM may have similar modulating effect over the immunity due to BCG in the later course of infection. This may explain, atleast partly, the varying efficacy of BCG seen in the different vaccination trials (16).

TABLE

Detailed characterisation using DNA probes (DT1, DT6 and LiPA), GC-MS, TLC and plasmid profiles of phenotypically identified *M.avium* complex (MAC) isolates obtained from different sources in the South Indian BCG trial area.

CODES	Origin	DT1	DT6	GC-MS	TLC	LIPA	PLASMID
MAC66	NA	ND	ND	MAC	ND	ND	N
MAC57	DUST	ND	ND	ND	ND	ND	PL
MAC47	DUST	N	N	ND	H	ND	N
MAC61	DUST	ND	ND	MAC	ND	ND	N
MAC59	DUST	ND	ND	MAC	ND	ND	N
MAC60	DUST	ND	ND	M.phlei	ND	ND	N
MAC10	DUST	+	N	MAC	H	MIC4	PL
MAC11	DUST	+	N	MAC	H	MAC(MIC1.2)	N
MAC12	DUST	N	N	MAC	H	ND	N
MAC9	DUST	+	N	MAC	H	MIC4	PL
MAC21	DUST	+	N	ND	H	M.sp	PL
MAC31	DUST	+	N	ND	H	MAC(MIC1.2)	N
MAC8	DUST	+	N	ND	H	MIC4	PL
MAC36	DUST	N	N	ND	H	MAC(MCO11)	PL
MAC35	DUST	+	N	ND	H	MIC4*	N
MAC20	DUST	+	N	M.fortuitum	H	MIC4	NA
MAC46	SOIL	N	N	ND	D	M.sp	N
MAC40	SOIL	N	N	ND	ND	ND	N
MAC53	SOIL	ND	ND	M.xenopi	ND	ND	N
MAC32	SOIL	N	N	M.fortuitum	E?	M.sp	N
MAC3	SOIL	N	N	M.fortuitum	E	M.mal	PL
MAC22	SOIL	*	N	M.fortuitum	E?	M.sp	N
MAC1	SOIL	+	N	MAC	D	M.sp	N
MAC2	SOIL	N	N	MAC	H	MAC(MIC1.2)	N
MAC18	SOIL	N	N	MAC	H	MAC(MCO11)	PL
MAC52	SOIL	ND	ND	MAC	ND	ND	PL
MAC28	SOIL	+	N	MAC	H	M.sp	N
MAC51	SOIL	ND	ND	MAC	ND	ND	N
MAC48	SOIL	N	N	ND	H	M.sp	N
MAC45	SOIL	N	N	ND	H	M.sp	PL
MAC37	SOIL	N	N	ND	H	M.sp	PL
MAC65	SPUTUM	ND	ND	ND	ND	ND	N
MAC13	SPUTUM	N	N	M.fortuitum	D	M.malmoense	N
MAC24	SPUTUM	*	N	M.fortuitum	D?	M.malmoense	N
MAC67	SPUTUM	ND	ND	M.xenopi	ND	ND	N

CODES	Origin	DT1	DT6	GC-MS	TLC	LIPA	PLASMID
MAC62	SPUTUM	ND	ND	M.phlei	ND	ND	N
MAC64	SPUTUM	ND	ND	M.xenopi	ND	ND	N
MAC14	SPUTUM	N	N	ND	H	M.scrofulaceum	PL
MAC33	SPUTUM	N	N	MAC	H	M.scrofulaceum	PL
MAC17	SPUTUM	N	N	MAC	H	M.sp	N
MAC15	SPUTUM	N	N	ND	E?	M.sp	N
MAC16	SPUTUM	+	N	MAC	H	MAC(M.sp)	N
MAC19	SPUTUM	N	N	ND	ND	ND	N
MAC63	SPUTUM	ND	ND	MAC	ND	ND	N
MAC39	SPUTUM	RGM	N	ND	D?	M.sp	PL
MAC38	SPUTUM	+	N	ND	H	MAC	N
MAC49	SPUTUM	+	N	ND	D	M.sp	N
MAC29	SPUTUM	+	N	ND	H	MIC4	N
MAC27	SPUTUM	*	N	MAC	H	MAC(MIC1.1.b)	N
MAC43	SPUTUM	N	N	ND	H	MAC(MCO11)	N
MAC30	SPUTUM	*	N	MAC	H	MIC4*	N
MAC58	WATER	ND	ND	ND	ND	ND	N
MAC41	WATER	*	N	ND	ND	ND	N
MAC23	WATER	N	N	M.godonae	H	MIC4	PL
MAC55	WATER	ND	ND	M.simirae	ND	ND	PL
MAC56	WATER	ND	ND	M.simiae	ND	ND	PL
MAC54	WATER	ND	ND	M.xenopi	ND	ND	PL
MAC44	WATER	N	N	ND	H	MIC4	N
MAC25	WATER	N	N	MAC	H	MIC4?	N
MAC4	WATER	RGM	N	ND	H	M.sp	N
MAC50	WATER	*	N	ND	H	M.sp	N
MAC42	WATER	N	N	ND	H	M.sp	N
MAC7	WATER	+	N	MAC	H	MAC(M.sp)	PL
MAC5	WATER	+	N	ND	H	MIC4	N
MAC26	WATER	+	N	ND	H	MIC4	N
MAC6	WATER	N	N	MAC	H	MIC4*	PL
MAC34	WATER	+	N	MAC	H	M.sp	PL

NA - NOT AVAILABLE; + - POSITIVE; N - NEGATIVE; ND - NOT DONE; * - CONTAMINATED; RGM - RAPID GROWING MYCOBACTERIA; MAC - *M.avium* complex by either GC-MS (pattern typical for MAC) or LiPA *(M.avium, M.intracellulare* or MAC intermediates)

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