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A NEW IMMUNOGENIC STRAIN: *MYCOBACTERIUM MARINUM* (SATO) AGAINST *M. ULCERANS* CHALLENGE IN MICE AND RAT EXPERIMENTS

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NECROTISING skin ulcers[1] of man caused by *Mycobacterium ulcerans* infection, originally limited to Congolese region has now attained global importance. This disease entity in human beings may still be counted as a newly recognized disease since many physicians are unfamiliar with it. Increasing incidence involving widespread areas of the world indicates that it is more common than had been originally thought. Although this disease has not been reported from this subcontinent the atmosphere for the growth of pathogen is conducive. The treatment and prophylaxis of the disease have not yet been perfected. BCG vaccination response is limited[2] in preventing the disease. It is of interest to search for some immunogenic strain against this infection for vaccine production either alone or in combination with BCG. The purpose of this communication is to report such an immunogenic strain *M. marinum* (SATO) which has been found to be effective in protecting *M. ulcerans* infection in rat and mice in several experiments.

In a general screening programme against this infection, several species of atypical mycobacteria were tested (table 1) on 1 mg dose of moist weight (approx. 10^9 bacilli) subcutaneously (s/c) and challenged with 1.5 mg moist weight (approx. 10^{13} bacilli) in 0.03 ml of *M. ulcerance* in the left foot pad s/c after 21 days of vaccination. The mycobacterial strains were homogenized in 0.05% tween saline (few drops) and suspended in a solution of disodium hydrogen phosphate of pH 8.7, and given in approriate quantities. The thickness of both the feet (inoculated and uninoculated) were measured by Vernier Calipers and the difference in thickness was considered as the degree of inflammation due to *M. ulcerans* infection which formed the index of foot pad inflammation. The degree of protection (as envisaged by decreased inflammatory response) afforded by the strain of *M. marinum* was appreciable and statistically significant (table 1). Other strains could not afford any protection and were therefore not studied.

TABLE 2

Protective response of M. marinum against intravenous challenge in mice. (5mg dose/mouse)

Mycobacterial strain	No. of mice/ group	Mean survival Time in days	*P.* value
M. marinum	9	72.5 ± 10.09	0.001
Control	10	35.1 ± 5.9	-

TABLE 1

Protective response of M. Marinum in comparison to other species of mycobacteria in rat foot-pad

Mycobacterial strain	Index of foot pad inflammation in mm ± S.E.			Decrease in foot pad inflammation (%)
	15th day	88th day	176th day	
B.C.G.	0.8 ± 0.16	0.6 ± 0.17	0.5 ± 0.09	38
M. tuberculosis strain H37Ra	0.9 ± 0.17	1.2 ± 0.2	0.8 ± 0.11	11
M. marinum	0.7 ± 0.13	0.2 ± 0.35	0.1 ± 0.04	80
Gause	1.1 ± 0.23	0.8 ± 0.13	0.8 ± 0.16	27
Bostrom	1.3 ± 0.07	1.2 ± 0.33	0.8 ± 0.27	38
Kirschberg	0.7 ± 0.21	0.9 ± 0.39	0.7 ± 0.15	0
Tubingen 71	1.2 ± 0.09	0.5 ± 0.09	0.7 ± 0.12	42
M. lepraemurium M.57	1.1 ± 0.16	1.0 ± 0.53	0.6 ± 0.18	45
Control	0.8 ± 0.12	1.2 ± 0.27	0.6 ± 0.13	25

TABLE 3

Protective response of M. marinum against M. ulcerans infection on rat foot pad

Mycobacterial Strain	Index of foot pad inflammation in m.m. ± S. E.			Decrease in foot pad inflammation (%)
	21st day	97th day	174th day	
M. marinum	0.5 ± 0.07	0.2 ±	0.1 ±	86
Control	0.7 ± 0.05	0.8 ± 0.8	0.9 ± 0.06	% Increase 29

This strain was further tested in 5 mg doses for its potency against this infection through intravenous challenge in mice. This strain afforded considerable degree of protection even through this route of infection (table 2). There is significant difference in the mean survival time (MST) of the vaccinated over the control group. This increased MST is a direct measure of the degree of protection afforded by any particular vaccinating strain over other parameters of study. Histopathologically the degree of lesions in visceral organs was much less severe than the control. The livers of vaccinated group in particular had no appearance of lesions, when unvaccinated livers had complete necrosis and areas of fatty degeneration with much extensive lesions.

In a third successive experiment in rats, through foot pad model in 2 mg dose s/c vaccination, the efficacy of this strain was further proved, (table 3) which seems to have been increased and appears to be directly proportional to the increased dose response. This study has provided us reasonable assurance and sufficient clues, through which this strain may easily be kept as a candidate vaccinal strain against this infection. It may also form a counterpart with BCG as a prelude to the preparation of a combined vaccine to combat this ailment more effectively. Further work is warranted in this direction, which is under progress.

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SEED-BORNE INFECTION OF MAIZE BY *XANTHOMONAS MAYDIS* IN KARNATAKA

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AMONG the several diseases reported on maize, the bacterial leaf spot caused by *Xanthomonas maydis* reported by Rangaswamy *et al.*[1] is an important disease from India. Noble and Richardson[2] indicated that the evidence concerning the seed-borne nature of the organism is contradictory. Recently, seedlings raised from maize seeds (CV Ganga Safed-2) received from the Seed Testing Laboratory, Hebbal showed necrotic lesions of this disease.

Maize seeds of Ganga safed-2 stored at 24° ± 2° C were tested by blotter method and paper towel method. For blotter method the seeds were sown on three layers of moistened blotters placed in Petri dishes (10 seeds per dish) and incubated at 24° ± 2° C under alternating cycles of 12 hr near day light tubes 40 cm from the top and darkness. For the paper towel method 200 seeds were sown between two layers of moistened paper at the rate of 100 seeds per paper towelling and kept under similar germinating conditions.

The infected seedlings showed very slow growth rate of the coleoptile region. The brown to black lesions were seen on the tip of the coleoptile extending downwards; ultimately the entire coleoptile region turned black (figure 1). A cut made at the infected part, was examined in a drop of water under the microscope and bacterial streaming was observed Paper towel method showed a higher percentage of seed infection than the blotter method.

Xanthomonas was readily isolated from the necrotic lesions of the coleoptile region. On nutrient agar medium, the bacterium produced smooth, buty-