

MYCOBACTERIUM PHLEI: A NOVEL IMMUNOGENIC STRAIN AGAINST *M. TUBERCULOSIS* INFECTION IN EXPERIMENTAL ANIMALS

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THE view that BCG affords protection against tuberculosis has received a set-back following the results of a controlled trial conducted in South India¹. Even in the past the role of BCG had been a subject of controversy²⁻⁴. Therefore, renewed interest continues in searching for other immunogenic strains which have considerably increased during recent years. Some potential strains having immunogenic property have been identified⁵⁻⁸. We describe here a strain of a typical non-pathogenic mycobacterium *M. phlei* (TMC 1523) which has generated Listeria type response and has also afforded protection against *M. tuberculosis* H₃₇ Rv in mice and guinea pigs.

Mycobacteria produce two types of response when they are exposed to a homologous challenge after sensitization⁹. The 10th to 11th day response (swelling of foot-pad after challenge) is Listeria type and the 28th day response is said to be of Koch type. Mycobacteria producing Listeria type response have shown correlation with antigenicity in mycobacteria^{10,11}.

The strains of mycobacteria for this study were obtained from Trudeau Mycobacterial Culture Collection Centre (TMC), Trudeau Institute Inc, Saranac Lake, New York. The cultures were maintained on Löwenstein Jensen Medium by six monthly passage. The mycobacteria were initially tested in

albino Swiss mice for Listeria or Koch type response following Rook's technique and the strain, which produced Listeria type response, was selected for further studies. Out of several strains of mycobacteria tested *M. phlei* (TMC 1523) produced the best Listeria type response. The strain was tested in mice for its immunogenic potency against the live challenge of *M. tuberculosis* H₃₇ Rv (TMC 201). The animals (15 in number) were vaccinated with 1 mg wet weight (approx. 2.4×10^8 colony-forming unit, cfu) of *M. phlei* at 4 different sites on the back subcutaneously and challenged with 1.9×10^8 cfu (1 mg wet weight/mouse) of *M. tuberculosis* H₃₇ Rv intravenously 21 days after vaccination.

Several parameters like weekly body weight, general appearance, necropsy score, presence of acid fast bacilli in impression smears from visceral organs and survival time of animals were studied to evaluate the immunogenic potency of this strain. The degree of protection was mainly assessed on the basis of survival of the animals after challenge for 30, 60 and 90 days (S-30, S-60, S-90).

The statistical analysis of the survival data in mice suggests that sufficient degree of protection has been provided by *M. phlei* against virulent challenge with *M. tuberculosis* in mice (table 1). Other parameters also concurred with the protective effect. The control animals deteriorated in general appearance and lost body weight sooner than the experimental group after challenge. The visceral organs smear showed less number of bacilli in the vaccinated than in the control group.

M. phlei was further tested in guinea pigs which is known to be more susceptible to human tubercle bacillus infection. These guinea pigs (10 animals/group) were vaccinated with live culture of *M. phlei*, s/c (1 mg wet weight/animal) and challenged intracardially to obtain the survival data. The data

Table 1 Protective values of vaccination with *M. phlei* against *M. tuberculosis* H₃₇ Rv challenge in mice

Vaccinating strain	No. of mice		Per cent	No. of mice		Per cent	No. of mice		Per cent	Mean survival time	P
	D-30	S-30		D-60	S-60		D-90	S-90			
<i>M. phlei</i>	4	11	73.33	4	11	73.33	5	10	67.0	74.6 ± 10.0	< 0.001
BCG (Phipps)	7	8	53.33	7	8	53.33	7	8	53.33	58.78 ± 10.0	< 0.001
Unvaccinated control	11	4	26.60	15	0	—	0	0	0.0	16.7 ± 2.0	—

D = died animals; S = surviving animals; P value by students *t* test.

Table 2 Protective values of vaccination with *M. phlei* against *M. tuberculosis* H₃₇ Rv challenge in Guinea pigs

Vaccinating strains	No. of mice		No. of Guinea pigs			No. of Guinea pigs			Mean survival time	P	
	Guinea pigs	Per cent	Guinea pigs	Per cent	Guinea pigs	Per cent	Guinea pigs	Per cent			
	D-30	S-30	S-30	D-60	S-60	S-60	D-90	S-90	S-90		
<i>M. phlei</i>	3	7	70	3	7	70	3	7	70	69.7 ± 8.94	< 0.001
BCG (Phipps)	4	6	60	5	5	50	5	5	50	52.9 ± 12.74	< 0.02
Unvaccinated control	10	0	0	-	-	-	-	0	0	9.4 ± 0.97	-

D = died animals; S = surviving animals; P value by students t test.

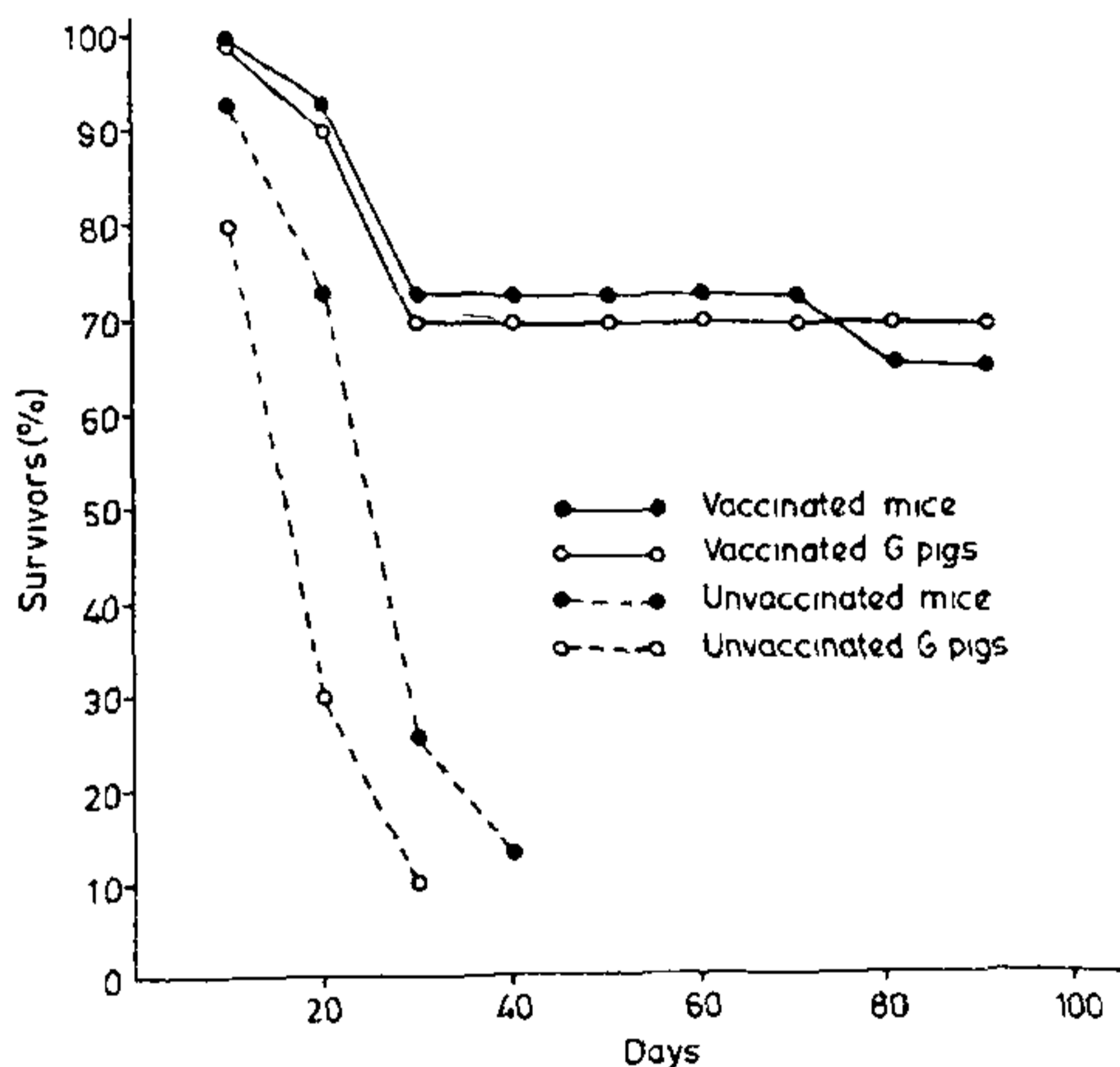


Figure 1. Protective values of *M. phlei* on vaccination against *M. tuberculosis* H₃₇ Rv challenge in mice and G. pigs.

presented in table 2 shows the percentage of survivors in the vaccinated group on days 30, 60 and 90 to be significantly higher. Percentage survivors in both the animal species are graphically represented in figure 1. It is obvious that the test strain affords sufficient degree of protection against human tubercle bacillus both in mice as well as in Guinea pigs.

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TOMATO SHOE STRING DISEASE INCITED BY NECROTIC STRAIN OF POTATO VIRUS Y IN HIMACHAL PRADESH — A NEW RECORD

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TOMATO (*Lycopersicon esculentum* Mill) is one of the most important cash crops grown in Solan,