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Review Article

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A Unique Vaccine with Multiple Applications

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Introduction

The immune system consists of molecules and cells (and their products) to keep the Body free of afflictions. Clinical problems arise as and when there is failure or inadequate immunological response. How can the immune system be fortified against an undesirable infection? This is done by making of a vaccine against that infection. This article describes the development of a Vaccine against Leprosy. Most of us (>90%) are immune to this disease. The few, who become victims to leprosy, manifest pitiable symptoms. Interestingly this vaccine has found multiple applications. It is a potent invigorator of immune responses.

Nature of Immune Deficit in Leprosy

Those who develop leprosy are unable to react immunologically to some key antigens of M. leprae [1]. This bacillus, M. leprae, the causative micro-organism of Leprosy, was discovered by Armauer Hansen in Norway about 2 centuries back. He was unable to culture these mycobacteria in any one of the many media that he employed. Indeed Mycobacterium leprae requires a host cell, the macrophage, to grow [2]. T lymphocytes of healthy individuals generate the signal for macrophages to prevent the proliferation of phagocytosed M. leprae. Table 1 gives supporting data on the role of T cells in this process.

Table 1: Mycobacterial multiplication in cultivated macrophages derived from peripheral blood monocytes of Leprosy patients [3]

Patient No.	Clinical Status	CPM ^{3}H-thymidine incorporated per 5×10^5 phagocytic cells	
		Macrophages + Lymphocytes + M. leprae	Macrophages + M. leprae
1	LL	36,458	45,628
2	LL	53,929	59,596
3	LL	52,354	83,476
4	TT	6,332	54,969
5	TT	32	78,447
6	TT	381	26,260

LL: Lepromatous multibacillary Leprosy; **TT:** Tuberculoid pausibacillary form of Leprosy

In this experiment, monocytes- derived- macrophages from either Tuberculoid (TT) leprosy patients or from those suffering from Lepromatous leprosy (LL) form of the disease were infected with M. leprae derived from patients. Radioactive thymidine (^{3}H- thymidine) was used as a precursor for synthesis of DNA, an indicator of

multiplication of M. leprae. It was incorporated into DNA by M. leprae engulfed in macrophages derived from either Lepromatous (LL) or Tuberculoid (TT) leprosy patients. However, if T cells derived from TT patients were also included in culture, the incorporation of 3H- thymidine by M. leprae was restricted, whereas T lymphocytes derived from LL patients lacked this ability.

This key experiment shows the role of 'competent' lymphocytes to restrict the multiplication of M. leprae. The multibacillary lepromatous leprosy patients (LL) lack the ability of reacting immunologically to M. leprae antigens. Could the reactivity be restored by immunization with alternate mycobacteria?

Search for a cross-reactive Mycobacteria

We collected 17 cultivable atypical mycobacteria from various sources. Their potential to react with T cells of LL patients, was investigated by a series of studies reported in the entire October 1978 issue of the Journal "Leprosy in India" and in other Journals [4,5]. A few mycobacteria of potential ability were short-listed. Lepromin like preparations were made from these mycobacteria and tested in BL, LL multibacillary patients, who are otherwise consistently lepromin negative. Lepromin is a homogenate of M. leprae. A basic characteristic of BL, LL patients is their inability to mount this Delayed Hypersensitivity Cell Mediated Immunity to M. leprae. They are Lepromin negative. We tested the ability of these short-listed mycobacteria to convert a M. leprae lepromin negative patient to lepromin positivity status [6,7]. In these studies we involved competent leprologists in more than one region of India to overcome the contribution of environmental factors in the results obtained.

These studies led to the selection of a strain coded as Mw, a cultivable non-pathogenic mycobacteria with ability to render a M. leprae lepromin negative patient to lepromin positivity status. Figure 1 is an electron micrograph of Mw.

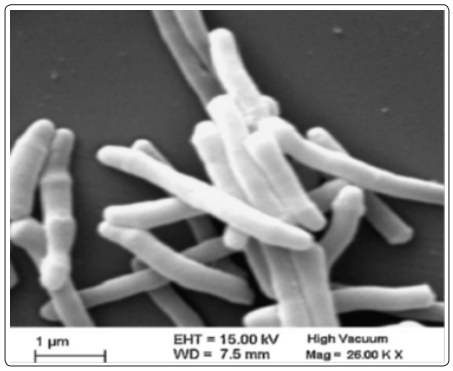

Figure 1: Electron micrograph of Mycobacterium Mw, now named as Mycobacterium indicus pranii (MIP)

Therapeutic and Immunoprophylactic Potential of Mw (MIP)

This was tested by giving Mw along with the standard muti-drug treatment to multibacillary leprosy patients. The inclusion of Mw expedited the clearance of M. leprae and shortened significantly the recovery of the patients [8,9]. With permission of the Drugs Controller General of India (DCGI) and Ethics Committees, a vaccine consisting of heat-killed Mw, was taken through Phase II, III and Field trials. Mw vaccine was indeed found to have good therapeutic properties [10]. Its immuno-prophylactic potential was established in contacts of leprosy patients [11]. The vaccine received the approval of the Drugs Controller General of India (DCGI) and has been passed onto a Company M/s Cadilla Pharma for making it available to Public.

Remarkable Therapeutic properties of the vaccine

Figure 2 shows a few patients treated with the usual drugs and the vaccine [12]. What is remarkable is the recovery of the patients to a form as if they never had leprosy. There were no blemishes and deformities observed in leprosy patients treated with Mw vaccine, which persist if they received only the conventional multi-drugs treatment.

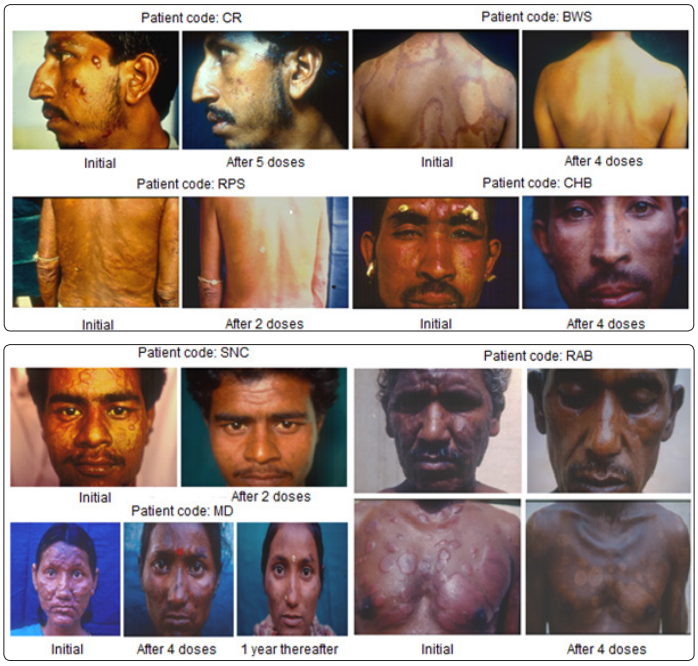

Figure 2: Some representative cases of LL/BL multibacillary patients treated with MDT plus Mw (*Mycobacterium indicus pranii*)

What is further amazing is their conversion to lepromin positivity status (Figure 3), enhancing their ability to resist becoming again a patient of leprosy [13].

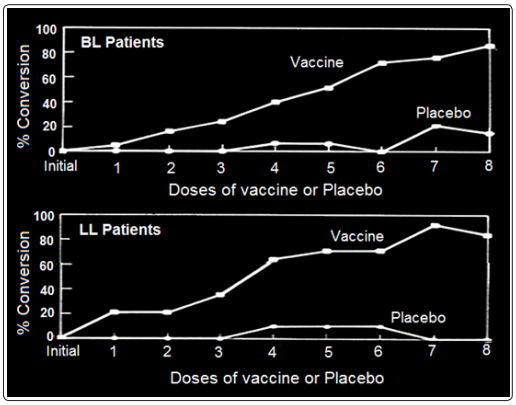

Figure 3: Conversion of lepromin status of BL/LL patients treated with MDT ± Mw (MIP)

Gene sequencing of Mycobacterium w

The gene sequence of Mw has been determined [14,15]. Mw is considered ancestor of both M. leprae and M. tuberculosis. It is named as Mycobacterium indicus pranii (MIP) [16]. Pran is my

familiar name, and NII is the National Institute of Immunology, of which I was the Founder Director and from where the clinical and field trials were conducted on this mycobacteria.

Additional Properties of the MIP vaccine

Mycobacterium indicus pranii shares antigens not only with M. leprae, but also with M. tuberculosis. In fact prevention of tuberculosis in guinea pigs after inoculation of Tuberculosis $H_{37}R_v$ is the biological test carried out on each batch of MIP grown by the company manufacturing the vaccine. Furthermore in contrast to BCG, MIP has no genetic restrictions and prevents tuberculosis in all strains of mice (Figure 4) [13].

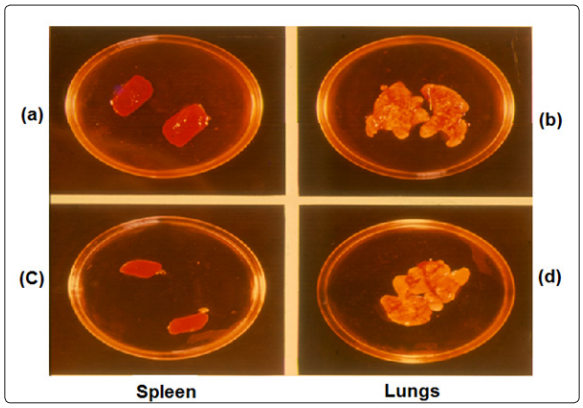

Figure 4: Protection test of Mw against tuberculosis in Guinea pigs. (a) And (b) Guinea pigs challenged with M. tuberculosis H37Rv. (c) and (d) Guinea pigs immunized with Mw before challenge

MIP has been found to be highly efficacious in treatment of Category II "Difficult to treat" tuberculosis patients (Table 2).

Table 2: Outcome of the additive effect of MIP in comparison to MDT alone for therapy of Cat II Tuberculosis patients

Treatment Description	Cured	Cured (%)
MIP + MDT (n =49)	48/49*	97.96
MDT alone (n=27)	21/27**	77.77

*One patient defaulter for 6 doses, sputum negative after intensive phase.
** Six patients- No effect of therapy

Figure 5 shows that the relapse rate of patients treated with MIP is much lower than those receiving drugs alone [13].

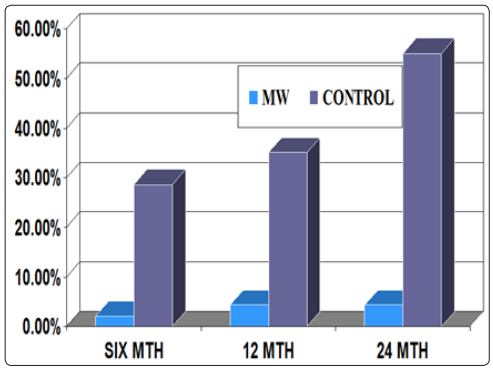

Figure 5: Relapse rate of Category II tuberculosis patients after treatment with MDT alone or MDT+ Mw (MIP)

Clearance of ugly Ano- genital warts

Prof. Somesh Gupta at the All India Institute of Medical Sciences, New Delhi employs MIP for treatment of warts on ano-genital parts of the body or on feet (Figs 6-8) [17,18].

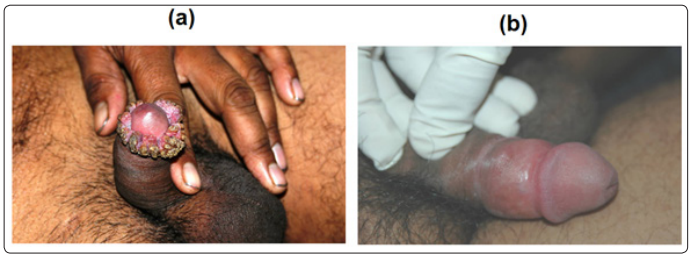

Figure 6: Effect of MIP on ugly anogenital warts. (a) A patient with giant condylomata. (b) The lesions completely subsided with intraregional immunotherapy with MIP.

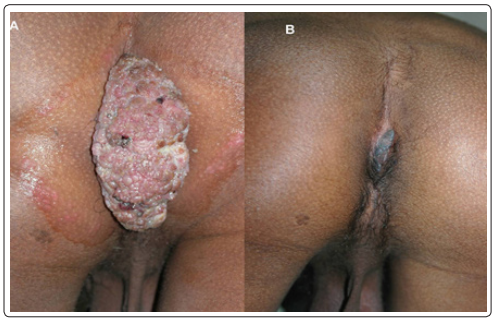

Figure 7: Action of MIP on ugly ano-genital warts (A) Before treatment (B) After treatment with MIP

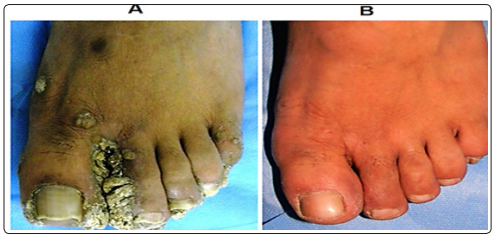

Figure 8: Cure by MIP of warts on feet. (A) Before treatment and (B) After 5 months of treatment with MIP

Cancers

MIP prevents and cures SP2/o Myelomas in mice (Figure 9) [19]. The Company that manufactures and sells MIP makes more money for its sale for treatment of a variety of cancers than for the leprosy.

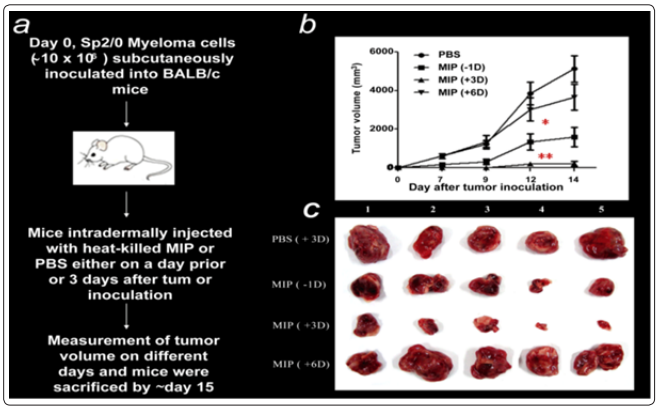

Figure 9: MIP treatment suppresses tumor growth and induces a Th1 cytokine response. (a) General outline of the in vivo experimental protocol. (b) Comparison of the anti-tumor effects

of MIP administered at different time points. Cohorts of ten mice were inoculated S.C. with ~10^7 Sp2/0 cells. Mice were injected i.e. with a single dose of MIP (~5×10^8) either one day (-1D) before or 3 (+3D) or 6 (+6D) days after tumor inoculation. Mice injected i.d. with PBS on day 3 were included as controls. The growth of tumors (mean ± SD mm3) at indicated days post implantation. (c) Representative photographs of solid tumors from different treatment groups dissected on day 14.

Potent Invigorator of Immune Response

MIP enhances substantially the antibody titres in mice by inclusion as adjuvant in the anti-hCG vaccine that we are developing for Birth Control (Figure 10) [20].

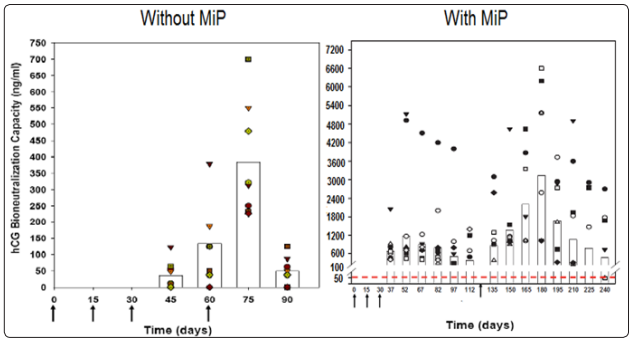

Figure 10: Enhancement of antibody response to hCGβ-LTB vaccine in Balb/c mice by MIP. Mice were immunized intra-muscularly with 2µg of the vaccine adsorbed on alum with or without MIP. Primary immunization consisted of 3 injections given at fortnightly intervals followed by a booster on day 60 or 120. The symbols represent the titres in a given mouse. Bars give the geometrical means

Conclusion

To sum up, we have developed a vaccine based on a cultivable non-pathogenic Mycobacteria, whose genome sequence is known which has been named as Mycobacterium indicus pranii (MIP). The vaccine has both immuno-therapeutic and immuno-prophylactic action against Leprosy. It shares antigens with M. tuberculosis and is highly effective in treatment of Category II Difficult to treat tuberculosis.

It is a potent invigorator of immune responses. It enhances significantly antibody titres on use as adjuvant to our anti-hCG vaccine. It also cures marvellously ugly ano-genital warts.

References

1. Talwar GP (1999) An immunotherapeutic vaccine for multibacillary leprosy. Int Rev Immunol 18: 229-249.
2. Talwar GP, Krishnan AD, Gupta PD (1974) Quantitative evaluation of the progress of intracellular infection in vitro: DNA synthesis in Mycobacterium leprae in cultivated blood monocytes. Infect Immun 9: 187-191.
3. Talwar GP, Krishnan AD, Jha P, Mehra V (1974) Intracellular Growth of an obligatory parasite Mycobacterium Leprae. Host Bacterial Interactions. Biochimie 56: 231-237.
4. Talwar GP (1978) Entire Golden Jubilee Issue of Leprosy in India 50: 492-598.
5. Talwar GP, Krishnan AD, Mehra VL, Blum EA, Pearson JMH (1972) Evaluation of cell mediated immune responses in untreated cases of leprosy. Clin Exp Immunol 12: 195-203.
6. Chaudhuri S, Fotedar A, Talwar GP (1983) Lepromin conversion in repeatedly lepromin negative BL/LL patients after immunization with autoclaved Mycobacterium. Int J Lepr & Other Mycobact Dis 51: 159-168.
7. Kar HK, Sharma AK, Mishra RS, Mukherjee A, Mukherjee R, et al. (1992) Induction of lepromin positivity by a candidate anti-leprosy vaccine Mycobacterium w in lepromin negative healthy contacts of multibacillary leprosy patients. Indian J Lepr 64: 495-500.
8. Zaheer SA, Mukherjee R, Ramkumar B, Mishra RS, Sharma AK, et al. (1993) Combined multidrug and Mycobacterium w vaccine therapy in patients with multibacillary leprosy. J Infect Dis 167: 401-410.
9. Zaheer SA, Mukherjee A, Ramesh V (1995) Immunotherapy benefits multibacillary patients with persistently high bacteriological index despite long term multidrug therapy. Immunol Infect Dis 5: 115-122.
10. Walia R, Sarathchandra KG, Pandey RM, Parida SK, Zaheer SA, et al. (1993) Field trials on the use of Mycobacterium w vaccine in conjunction with multi-drug therapy in leprosy patients for immunotherapeutic and immune-prophylactic purposes. Lep Rev 64: 302-311.
11. Sharma P, Mukherjee R, Talwar GP, Sarathchandra KG, Walia R, et al. (2005) Immunoprophylactic effects of the anti-leprosy Mw vaccine in household contacts of leprosy patients: Clinical field trials with a follow up of 8-10 years. Lep Rev 76: 127-143.
12. Talwar GP (2014) Leprosy is in principle eradicable: a possible approach. Curr Sci 106: 1344-1345.
13. Talwar GP, Gupta JC, Mustafa AS, Kar HK, Katoch K, et al. (2017) Development of a potent invigorator of immune responses endowed with both preventive and therapeutic properties. Biologics: Target and Therapy 11: 1-9.
14. Reddi PP, Amin AG, Khandekar PS, Talwar GP (1994) Molecular definition of unique species status of Mycobacterium w, a candidate leprosy vaccine strain. Int J Lepr Other Mycobact Dis 62: 229-236.
15. Saini V, Raghuvanshi S, Talwar GP, Ahmed N, Khurana JP, et al. (2009) Polyphasic taxonomic analysis establishes Mycobacterium indicus pranii as a distinct species. PLoS One 4: e6263.
16. Talwar GP, Ahmed N, Saini V (2008) The use of the name Mycobacterium w for the leprosy immunotherapeutic bacillus creates confusion with M. tuberculosis-W (Beijing strain): a suggestion. Infect Genet Evol 8: 100-101.
17. Gupta S, Malhotra AK, Verma KK, Sharma VK (2008) Intraregional immunotherapy with killed Mycobacterium w vaccine for the treatment of ano-genital warts: an open label pilot study. J Eur Acad Dermatol Venereol 22: 1089-1093.
18. Singh S, Chouhan K, Gupta S (2014) Intralesional immunotherapy with killed Mycobacterium indicus pranii vaccine for the treatment of extensive cutaneous warts. Indian J Dermatol Venereol Leprol 80: 509-514.
19. Rakshit S, Ponnusamy M, Papanna S, Saha B, Ahmed A, et al. (2012) Immunotherapeutic efficacy of Mycobacterium indicus pranii in eliciting anti-tumor T cell responses: critical roles of IFNɣ. Int J Cancer 130: 865-875.
20. Purswani S, Talwar GP (2011) Development of a highly immunogenic recombinant candidate vaccine against human chorionic gonadotropin. Vaccine 29: 2341-2348.