

Development of a potent invigorator of immune responses endowed with both preventive and therapeutic properties

Gursaran P Talwar¹
Jagdish C Gupta¹
Abu S Mustafa²
Hemanta K Kar³
Kiran Katoch⁴
Shreemanta K Parida⁵
Prabhakara P Reddi⁶
Niyaz Ahmed⁷
Vikram Saini⁸
Somesh Gupta⁹

¹Talwar Research Foundation, New Delhi, India; ²Department of Microbiology, Kuwait University, Kuwait; ³Department of Dermatology, Paras Hospital, Gurgaon; ⁴National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra, India; ⁵German Centre of Infection, Justus Liebig University, Giessen, Germany; ⁶Department of Comparative Biosciences, University of Illinois Urbana Champaign, IL, USA; ⁷Department of Biotechnology and Bioinformatics, University of Hyderabad, Hyderabad, India; ⁸Department of Microbiology, University of Alabama, Birmingham, AL, USA; ⁹Department of Dermatology and Venereology, All India Institute of Medical Sciences, New Delhi, India

Abstract: This article reviews briefly the making of an immunoprophylactic-cum-immunotherapeutic vaccine against leprosy. The vaccine is based on cultivable, heat-killed atypical mycobacteria, whose gene sequence is now known. It has been named *Mycobacterium indicus pranii*. It has received the approval of the Drug Controller General of India and the US Food and Drug Administration. Besides leprosy, *M. indicus pranii* has found utility in the treatment of category II (“difficult to treat”) tuberculosis. It also heals ugly anogenital warts. It has preventive and therapeutic action against SP2/O myelomas. It is proving to be a potent adjuvant for enhancing antibody titers of a recombinant vaccine against human chorionic gonadotropin, with the potential of preventing pregnancy without derangement of ovulation and menstrual regularity in sexually active women.

Keywords: leprosy, tuberculosis, anogenital warts, myeloma, adjuvant

Introduction

The immune system has the task of defending the body from infections caused by a variety of microorganisms foreign to the body. Vaccines were and are made to boost the immune response against a given bacteria, virus, or harmful toxins generated by microorganisms. While the body has an armamentarium in terms of cells and molecules endowed with the property of responding and reacting with various “invaders”, the process to build up adequate immunity takes time. Vaccination with a killed or attenuated microbe performs this function and builds up adequate immunity against an eventual infection. Interestingly, some vaccines also have therapeutic properties.

This communication describes briefly the making of an immunoprophylactic-cum-immunotherapeutic vaccine initially developed against leprosy. The vaccine has received the approval of the Drugs Controller General of India (DCGI) and also of the US Food and Drug Administration (FDA). It is at present the only vaccine of its type in the world. It has been taken up by the industry and is available to the public. Besides leprosy, it has found therapeutic utility in the treatment of category II (“difficult to treat”) tuberculosis. Astonishingly, it cures ugly anogenital warts, presumably by potentiating both cellular and humoral immune responses. Its efficacy in prevention and treatment of SP2/O myeloma in mice has also been reported. It is employed as an adjuvant in a potential birth control vaccine against human chorionic gonadotropin (hCG) for preventing pregnancy.

Leprosy

Leprosy is caused by *Mycobacterium leprae*, a mycobacteria isolated by Armauer Hansen in Norway in 1873. He was unable to grow it in any of the many media that he

Correspondence: Gursaran P Talwar
Talwar Research Foundation,
E-8 Neb Valley, Neb Sarai, New Delhi
110068, India
Tel +91 11 6502 2405
Email gptalwar@gmail.com

tried. His thesis is one of the shortest on record. The majority (~99%) of humans can resist infection of *M. leprae* successfully, and do not become leprosy patients. The few who become victim to the disease manifest a spectrum ranging from a single lesion with few if any bacteria, classified as tuberculoid (TT) to multibacillary lepromatous leprosy (LL) with multiple lesions loaded with *M. leprae*. This spectrum is a reflection of the existence of complete or variable degrees of immune response against *M. leprae* in patients manifesting the different forms of leprosy. Our first task was to learn of what goes wrong or is deficient in humans who become victims of the disease. After learning this, the next task obviously was to see whether anything can be done to prevent humans contracting the disease.

Nature of immune deficit in leprosy

Those who contract leprosy have T cells that are unable to react against some key antigen(s) of *M. leprae*. Their immune system is otherwise fairly normal, and they respond normally to cholera or typhoid vaccines.¹ T lymphocytes generate the signal for macrophages to prevent the proliferation of phagocytosed *M. leprae*. Table 1 gives data clearly supporting the role of T cells in this process. In this experiment, monocyte-derived macrophages from either TT leprosy patients or those suffering from the LL form of the disease were infected with *M. leprae* derived from patients. Radioactive thymidine (³H-thymidine) was used as a precursor in the medium. It was incorporated into DNA by *M. leprae* engulfed in macrophages derived from either LL or 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 property.²

Can anything be done to restore this deficiency?

This was the basic requirement of an eventual vaccine against leprosy. Vaccines are usually made using the killed

or attenuated forms of infecting microorganisms. Such a homologous approach was illogical for leprosy, as the basic defect in LL patients is their inability to respond to key antigen(s) of *M. leprae*. Therefore, a heterologous approach was adopted.

Search for an atypical *Mycobacterium* sharing antigens with *M. leprae*

Whereas *M. leprae* is noncultivable, a candidate for a vaccine against leprosy has to be cultivable in one medium or another to enable its production on a large scale for public use. We collected 16 cultivable, atypical mycobacteria from various sources, some already named and classified, others lying in various atypical collections. Each of them was coded and investigated for its ability to cause blast transformation of T cells from not only TT but also LL patients.³ Their ability to generate cytokines influencing macrophage function was investigated.⁴ The ensemble of these investigations led to the shortening of the list of 16 to 5 mycobacteria. These were *M. vaccae*, *M. phlei*, *M. goodnae*, ICRC bacillus, and *Mycobacterium w* (*Mw*).

To confirm their closeness to *M. leprae*, lepromin-like preparations were made of these five atypical mycobacteria and evaluated alongside lepromin prepared from *M. leprae* in TT and LL patients to evoke *M. leprae* lepromin-like responses in leprosy patients. An atypical mycobacteria (*Mw*) appeared to possess these molecular traits.⁵

Could *Mw* serve as a vaccine? Can it convert lepromin-negative leprosy patients to lepromin-positive status? This investigation was carried by Chaudhary et al at the School of Tropical Medicine, Kolkata. Twenty of 32 leprosy patients who were consistently lepromin negative were converted from lepromin-negative to lepromin-positive status after a single intradermal injection of autoclaved *Mw*.⁶ This was confirmed by Kar et al⁷ in lepromin-negative family members and contacts of leprosy patients in Delhi. A total of 67 of 68 (98.5%) converted

Table 1 Mycobacterial multiplication in cultivated macrophages derived from peripheral blood monocytes of leprosy patients

Patient	Clinical status	CPM ³ H-thymidine incorporated per 5×10 ⁵ phagocytic cells	
		Macrophages + lymphocytes + <i>Mycobacterium leprae</i>	Macrophages + <i>Mycobacterium leprae</i>
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

Note: Reproduced from Talwar GP, Krishnan AD, Jha P, Mehra V. Intracellular growth of an obligatory parasite *Mycobacterium leprae*: host bacterial interactions. *Biochimie*. 1974;56:231–237.²

Abbreviations: CPM, counts per minute; LL, lepromatous leprosy; TT, tuberculoid leprosy.

to lepromin-positive status after two immunizations with *Mw*. These observations pointed out the potential of *Mw* to serve as an immunoprophylactic vaccine to protect susceptible family members from contracting leprosy on continuous exposure to *M. leprae* from a multibacillary leprosy patient.

Therapeutic action of *Mw*

Zaheer et al administered autoclaved *Mw* as adjunct to standard multidrug therapy (MDT) to treat multibacillary leprosy patients. A parallel group received a tenth of the dose of tetanus toxoid as adjunct. It was observed that the inclusion of *Mw* with the drugs expedited bacterial clearance and shortened the period of complete recovery.⁸ Figure 1 illustrates the

remarkable property of *Mw* in not only expediting recovery but also clearing granulomas in patients.⁹

Efficacy in slow responders

Fourteen patients who were not responding adequately to standard treatment with MDT were referred to our treatment center. They were randomly distributed into two groups: one group continued to receive MDT, and the second group was also given MDT with *Mw* vaccine as an adjunct. Figure 2 shows the dynamics of bacterial index values in two of these patients. Soon after immunization with *Mw*, the bacterial index started declining noticeably, followed by full recovery of the patients.¹⁰ This was not the case in the group receiving drugs alone, in whom the decline continued to be extremely slow (Figure 3).



Figure 1 Representative cases of LL/BL multibacillary patients treated with MDT plus *Mycobacterium w* (*M. indicus pranii*).

Note: Reproduced from Talwar GP. Leprosy is in principle eradicable: a possible approach. *Curr Sci*. 2014;106:1344–1345.⁹

Abbreviations: MDT, multidrug therapy; LL, lepromatous leprosy; BL, borderline leprosy.

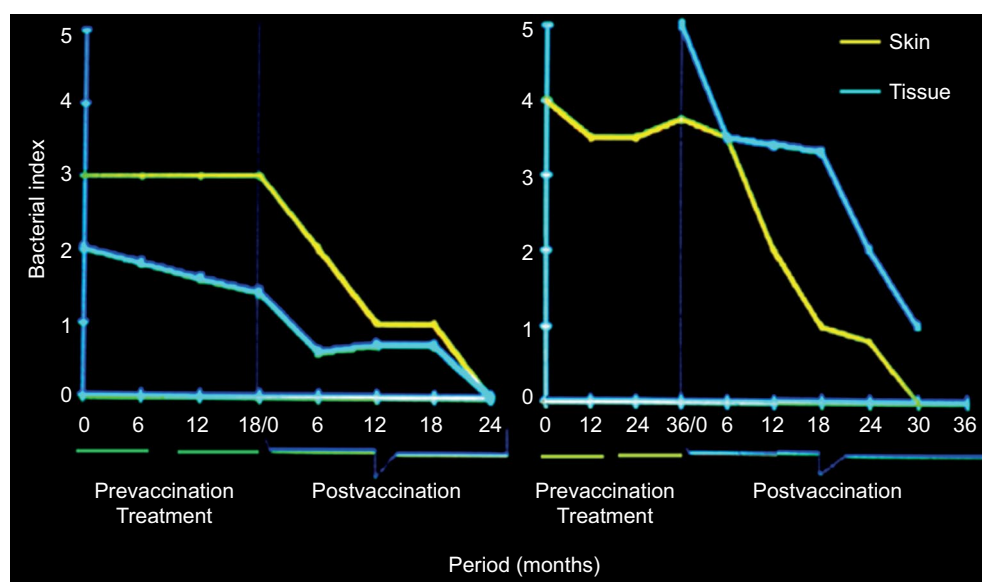


Figure 2 Synergistic effect of *Mycobacterium w* vaccine in slow responders to drugs in two patients.

Note: In both, the bacterial index started declining on immunization with vaccine. Reproduced from Zaheer SA, Mukherjee A, Ramesh V, et al. Immunotherapy benefits multibacillary patients with persistently high bacteriological index despite long term multidrug therapy. *Immunol Infect Dis.* 1995;5:115–122.¹⁰

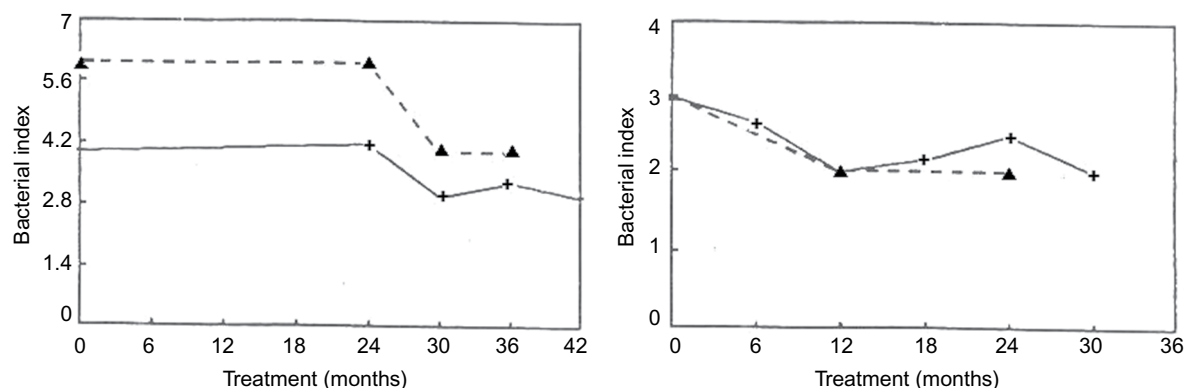


Figure 3 Bacterial index in two patients who continued to receive drugs with no vaccine given.

Note: Reproduced from Zaheer SA, Mukherjee A, Ramesh V, et al. Immunotherapy benefits multibacillary patients with persistently high bacteriological index despite long term multidrug therapy. *Immunol Infect Dis.* 1995;5:115–122.¹⁰

Conversion of borderline leprosy/LL patients to lepromin-positive status by *Mw*

LL patients are all lepromin negative, one of the criteria employed for classifying the patient in this category, and they continue to be lepromin negative even after bacterial clearance is achieved with drugs. Drugs kill the bacteria but do not improve the immune response. It was pertinent to examine whether the inclusion of *Mw* for treatment had any effect on the delayed skin-hypersensitivity response to *M. leprae*, manifested as a positive response to lepromin. Figure 4 shows the dynamics of the conversion of patients from lepromin-negative to lepromin-positive status.

Clearance of bacilli from peripheral nerves

M. leprae infects the peripheral nerve cells, causing loss of sensitivity. Zaheer et al¹⁰ had the good idea to investigate

whether inclusive treatment with *Mw* in patients eliminated fully or partially the bacilli infecting the nerve cells. Table 2 gives the observations. In contrast to drugs alone, where bacilli persisted in peripheral nerves of some patients, in the group receiving *Mw*, bacilli were eliminated in the peripheral nerves of all patients investigated.

Approval by drug regulatory authorities

Phase-wise clinical trials were conducted with due approval of ethics committees and drug regulatory authorities in recognized centers, followed by field trials in Kanpur Dehat in 272 villages inhabited by 420,823 people. Kanpur Dehat used to be one of the endemic districts for leprosy. Patients suffering from leprosy were treated with drugs and the *Mw* vaccine. Their household contacts who had been exposed received the vaccine based on *Mw* in the hope that they would

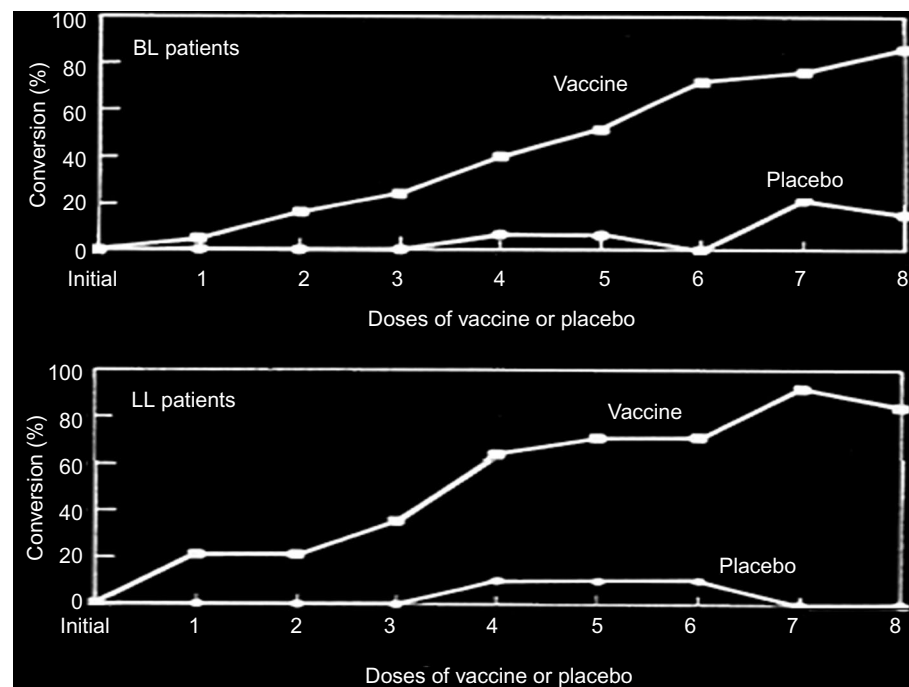


Figure 4 Conversion of lepromin status of BL/LL patients treated with MDT \pm *Mycobacterium w* (*M. indicus pranii*).

Abbreviations: BL, borderline leprosy; LL, lepromatous leprosy; MDT, multidrug therapy.

Table 2 Clearance of *Mycobacterium leprae* from peripheral nerves by *Mw*

Group	Type	Initial BI	Final BI	Initial H/P	Final H/P	Nerve BI ^a
Vaccine	LL	4	0	LL	NSI	0
	BL	3	0	BL	NSI	0
	LL	5	0	LL	BL	0
	BL	2.33	0	BL	NSI	0
Control	LL	4.33	0	LL	LL	2
	BL	3.16	0	BL	BL	1
	LL	2.16	0	LL	LL	0

Note: ^aAll nerve biopsies done on sural nerve.

Abbreviations: *Mw*, *Mycobacterium w*; BI, bacterial index; H/P, histopathology; LL, lepromatous leprosy; BL, borderline leprosy; NSI, nonspecific infiltration.

be protected from becoming patients. Observations made during these trials are reported elsewhere.^{11,12} Vaccination with *Mw* shortened treatment time of patients. In addition, no side effects of any significance were seen by vaccination of healthy contacts with *Mw*. Our vaccine has received the approval of the DCGI and was licensed to a company to enable its availability to the public. In due course, it also received the approval of the FDA.

Move for eradication of leprosy

Eradication of small pox was achieved by planned use of a vaccine against that infectious disease. An Indian government committee led by Soumya Swaminathan, Director-general of the Indian Council of Medical Research, has decided to evaluate the use of *M. indicus pranii* (MIP) to eliminate if not eradicate leprosy in India. To begin with, a field project will

be launched in five districts of high endemism in the country. The index patient will receive the vaccine in addition to MDT. Their family members and contacts will be immunized with the MIP vaccine twice at 6-month intervals with the hope that they will not become patients.

Tuberculosis

The company licensed to manufacture *Mw* vaccine asked from us a biological test to confirm that they were growing the right bacteria. As *Mw* shares antigens with *M. tuberculosis* besides *M. leprae*, we thought of testing whether *Mw* could prevent tuberculosis in guinea pigs following infection with the pathogenic strain *M. tuberculosis* H₃₇R_v. This was indeed the case. As shown in Figure 5, after 3 weeks the lungs had nodules full of *M. tuberculosis* and the spleen was enlarged. Immunization with *Mw* prevented these from happening.

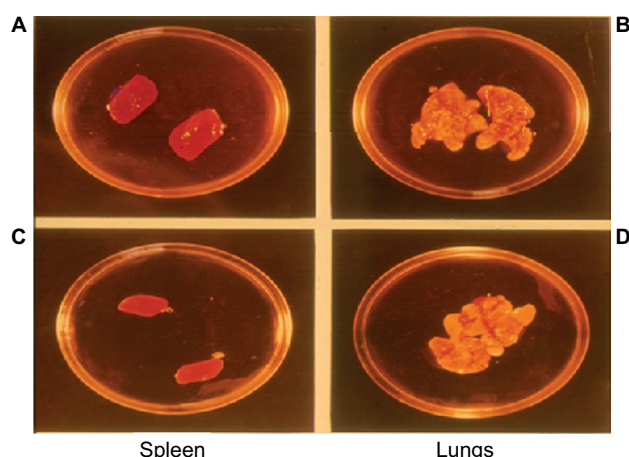


Figure 5 Protection test of *Mycobacterium w* (Mw) against tuberculosis in guinea pigs. **Notes:** (A, B) Guinea pigs challenged with *M. tuberculosis* H₃₇Rv. (C, D) Guinea pigs immunized with Mw before challenge with *M. tuberculosis* H₃₇Rv.

Similarities and differential properties of Mw with respect to BCG

Bacillus Calmette–Guérin (BCG) is effective only in live form and loses immunizing capability in a dead state, whereas Mw is effective in both live and in autoclaved form. Furthermore, in contrast to BCG, Mw seems to have no genetic restrictions. As shown in Figure 6, Mw prevented the growth of pulmonary lesions in all four genetic strains (C3H, CBA, BALB/c, C57BL) of mice investigated, whereas BCG was effective in BALB/c and C57BL but not in CBA or C3H mice.¹³

Treatment of category II tuberculosis patients

Mw was employed as adjunct for treatment of category II (difficult to treat) tuberculosis patients in an exploratory trial conducted in Ahmedabad. Table 3 gives the results. Inclusion of Mw with drugs improved recovery considerably. What is further significant is that patients receiving Mw with drugs had much lower relapse rates (Figure 7).

Gene sequencing of Mw

Reddi et al carried out a molecular analysis of Mw, which indicated it as a unique species.¹⁴ Three recognized centers were given a grant by the Department of Biotechnology, Government of India to determine the genome sequence and carry out molecular definition of Mw, an atypical cultivable mycobacterium with remarkable therapeutic traits of relieving leprosy and tuberculosis. Their findings are reported

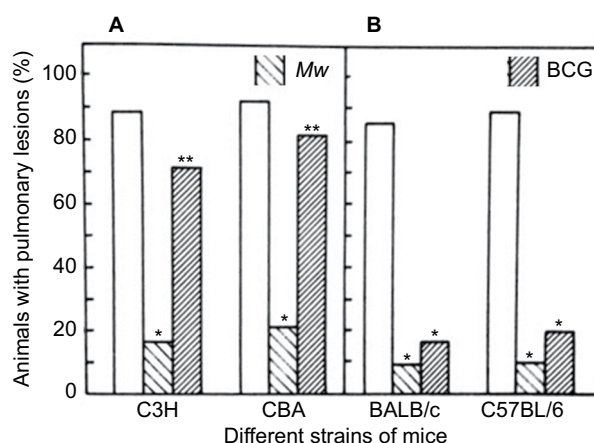


Figure 6 Effect of immunization with killed Mw or live BCG.

Notes: Effects on development of pulmonary lesions in C3H and CBA (A) and in BALB/c and C57BL/6 (B) strains of mice. Animals were immunized with either 10⁷ heat-killed Mw subcutaneously or 10⁴ live BCG intravenously. Four weeks later, the animals were challenged with 10⁷ *Mycobacterium tuberculosis* H₃₇Rv intravenously. Four weeks postchallenge, the animals were killed and visible lesions in the lungs recorded. Data plotted are the means of results obtained from three sets of experiments with Mw and two sets of experiments with BCG, with n=7–10 animals per group. Data for the nonimmunized group of animals are also presented (blank bars). Reprinted from Vaccine, 9, Singh IG, Mukherjee R, Talwar GP, Resistance to intravenous inoculation of *Mycobacterium tuberculosis* H₃₇Rv in mice of different inbred strains following immunization with a leprosy vaccine based on *Mycobacterium w*, 10–14, Copyright 1991, with permission from Elsevier.¹³

Abbreviations: Mw, *Mycobacterium w*; BCG, bacillus Calmette–Guérin.

Table 3 Outcome of the additive effect of MIP (Mw) in comparison to MDT alone for therapy of category II tuberculosis patients

Treatment description	Cured	Cured (%)
MIP + MDT (n=49)	48/49 ^a	97.96
MDT alone (n=27)	21/27 ^b	77.77

Notes: ^aOne patient defaulter for six doses, sputum negative after intensive phase; ^bsix patients, no effect of therapy.

Abbreviations: MIP, *Mycobacterium indicus pranii*; Mw, *Mycobacterium w*; MDT, multidrug therapy.

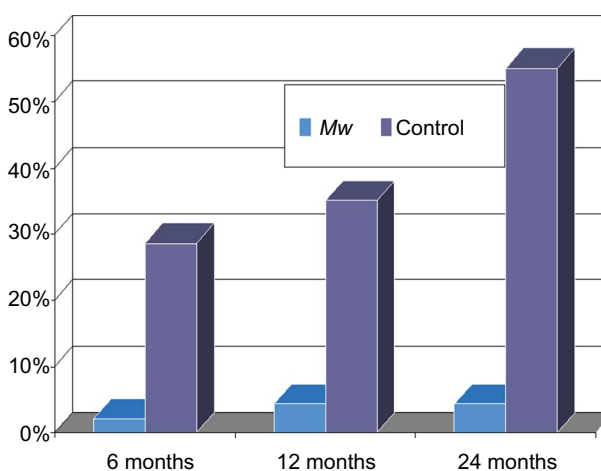


Figure 7 Relapse rate of category II tuberculosis patients after treatment with MDT alone (control) or MDT + *Mycobacterium w* (*M. indicus pranii*) (Mw).

Abbreviation: MDT, multidrug therapy.

elsewhere.¹⁵ *Mw* was considered an ancestor of both *M. leprae* and *M. tuberculosis*. No bacteria of this definition had heretofore existed in the International Depository. It was named *Mycobacterium indicus pranii* (MIP).¹⁶

Unexpected astonishing properties of MIP

Every now and then, SG at the All India Institute of Medical Sciences receives patients harboring ugly anogenital warts. He found that administration of MIP caused remarkable recovery of these warts. Figure 8 shows the clearance of the warts. Figure 9 shows an ugly lesion on the anus. Among the first nine patients suffering from such anogenital warts, three were positive for HIV. In spite of that, they benefited from this treatment. These observations have been published elsewhere.¹⁷ Similar warts occurring elsewhere in the body were also healed (Figure 10).¹⁸

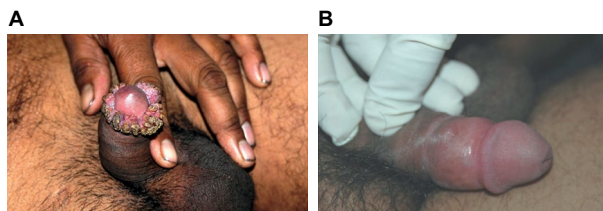


Figure 8 Effect of MIP on ugly anogenital warts.

Notes: (A) A patient with giant condylomata. (B) The lesions completely subsided with intralesional immunotherapy with MIP. Reproduced from Gupta S, Malhotra AK, Verma KK, Sharma VK. Intralesional immunotherapy with killed *Mycobacterium w* vaccine for the treatment of anogenital warts: an open-label pilot study. *J Eur Acad Dermatol Venereol*. 2008;22:1089–1093 with permission from John Wiley and Sons.¹⁷

Abbreviation: MIP, *Mycobacterium indicus pranii*.

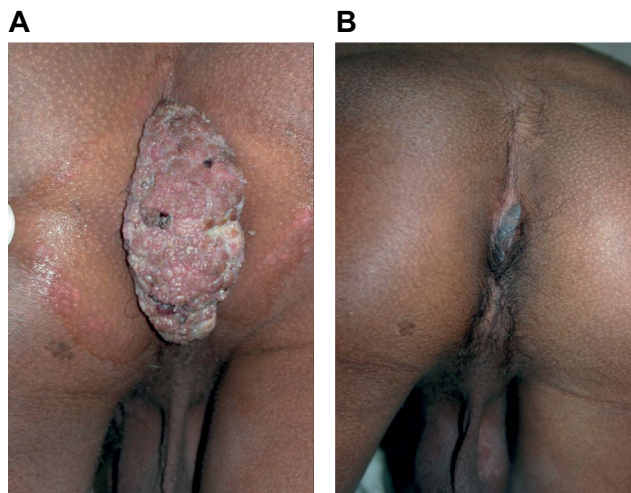


Figure 9 Action of MIP on ugly anogenital warts (A) before treatment and (B) after treatment with MIP.

Abbreviation: MIP, *Mycobacterium indicus pranii*.

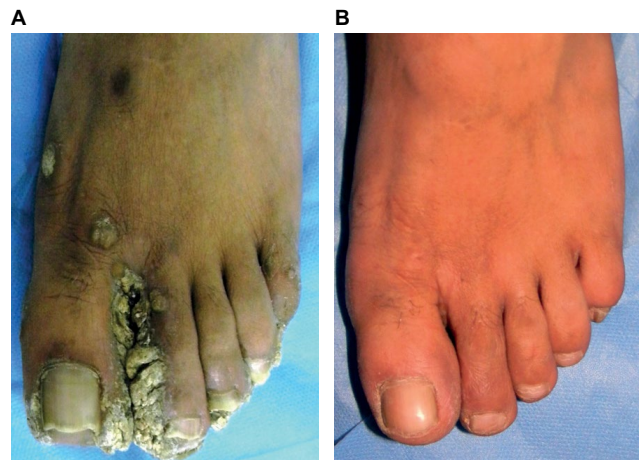


Figure 10 Cure of warts on feet: (A) before treatment and (B) after 5 months of treatment with MIP. Copyright ©2014. Reproduced from IJDLV. Singh S, Chouhan K, Gupta S. Intralesional immunotherapy with killed *Mycobacterium indicus pranii* vaccine for the treatment of extensive cutaneous warts. *Indian J Dermatol Venereol Leprol*. 2014;80:509–514.¹⁸

Abbreviation: MIP, *Mycobacterium indicus pranii*.

Prevention and therapy of SP2/O myelomas in mice

Rakshit et al showed the prevention and anticancerous action of MIP on development of SP2/O myelomas in BALB/c mice.¹⁹ Figure 11 gives a summary of their findings. They have also reported the generation by MIP of cytokines IL-2 and IFN γ , which may be involved in this action.

Potent adjuvant properties of *Mycobacterium indicus pranii*

Our laboratory is involved in making a recombinant vaccine against hCG, which has the potential of preventing pregnancy in sexually active women without derangement of ovulation and menstrual regularity. Inclusion of MIP as an adjuvant in the vaccine enhances antibody titers remarkably (Figure 12). MIP is a potent invigorator of immune response.

Summary

This review recapitulates briefly the making of an immunotherapeutic-cum-immunoprophylactic vaccine against leprosy. The vaccine is based on an atypical, cultivable mycobacteria, originally coded *Mw*. The gene sequence analysis of *Mw* and its molecular definition have been done. It is an ancestor of both *M. leprae* and *M. tuberculosis*. No bacteria of this definition had heretofore existed in the International Depository. It has been named *Mycobacterium indicus pranii* (MIP).

MIP given as an adjunct to standard MDT expedites bacterial clearance and shortens the period of complete recovery of leprosy patients. It also upgrades the immune response of patients to *M. leprae*, which is not achieved

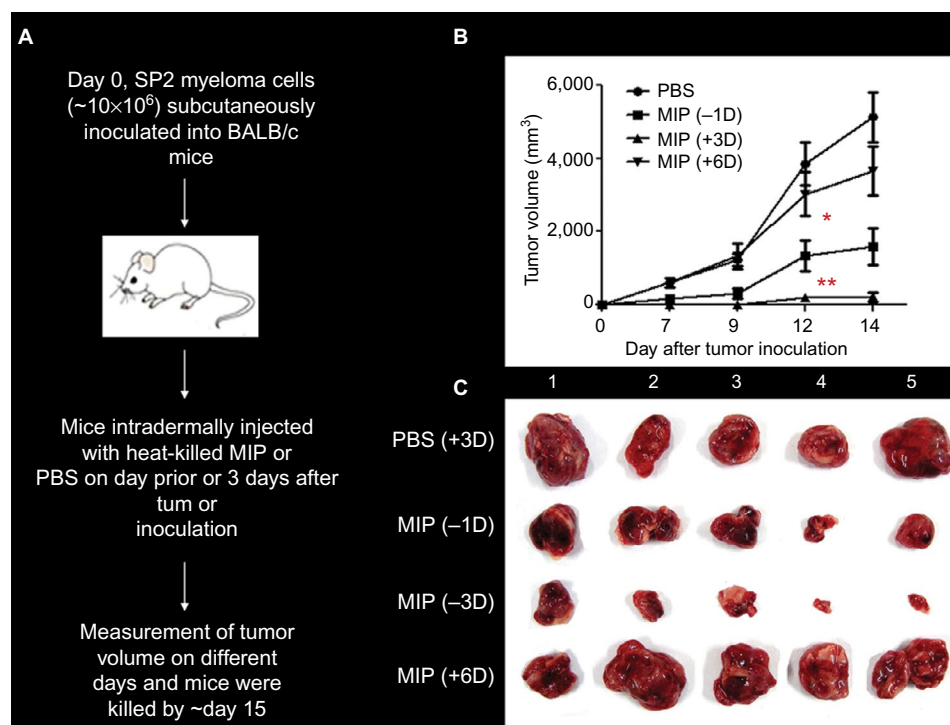


Figure 11 MIP treatment suppressed tumor growth and induced a T_H1 cytokine response.

Notes: (A) General outline of in vivo experimental protocol. (B) Comparison of antitumor effects of MIP administered at different time points. Cohorts of ten mice were inoculated subcutaneously with $\sim 10^7$ SP2/0 cells. Mice were injected intradermally with a single dose of MIP ($\sim 5 \times 10^8$) either 1 day before (-1 D) or 3 (+3 D) or 6 (+6 D) days after tumor inoculation. Mice injected intradermally with PBS on day 3 were included as controls. Growth of tumors (mean \pm SD [mm^3]) at indicated days postimplantation. (C) Representative photographs of solid tumors from different treatment groups dissected on day 14. Significance between MIP and PBS treated groups are indicated as follows: * $P < 0.05$; ** $P < 0.01$. Reproduced from Rakshit S, Ponnusamy M, Papanas S, Saha B, Ahmed A, Nandi D. Immunotherapeutic efficacy of *Mycobacterium indicus pranii* in eliciting anti-tumor T cell responses: critical roles of IFN γ . *Int J Cancer*. 2012;130:865–875, with permission from John Wiley and Sons.¹⁹

Abbreviations: MIP, *Mycobacterium indicus pranii*; T_H , T-helper; PBS, phosphate-buffered saline.

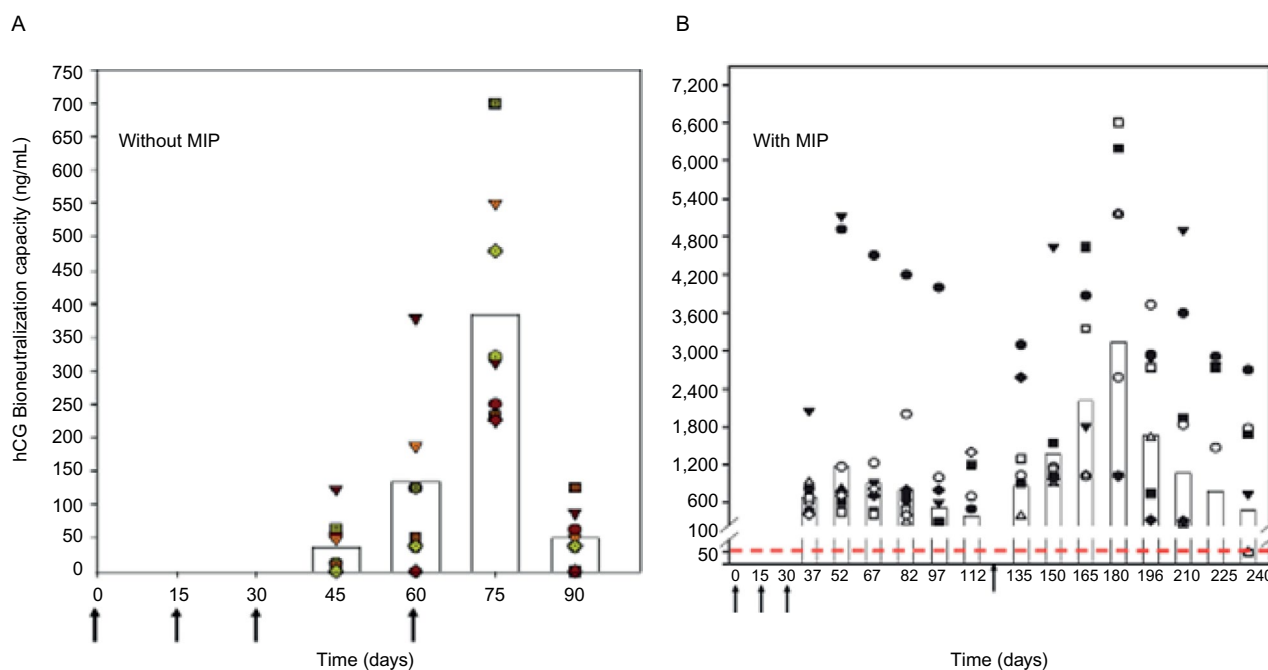


Figure 12 Enhancement of antibody response to hCG β -LTB vaccine in BALB/c mice by MIP.

Notes: Mice were immunized intramuscularly with 2 μg of the vaccine adsorbed on alum with or without MIP. Primary immunization consisted of three injections given at fortnightly intervals, followed by a booster on day 60 or 120. The symbols represent the titers in a given mouse. Bars give the geometrical means.¹⁹ (A) Observations in mice immunized with the hCG β -LTB vaccine adsorbed on alum; (B) titers of antibodies in mice immunized with the vaccine plus MIP as adjuvant. Reprinted from *Vaccine*, 29, Purswani S, Talwar GP, Development of a highly immunogenic recombinant candidate vaccine against human chorionic gonadotropin, 2341–2348, Copyright 2011, with permission from Elsevier.²⁰

Abbreviations: hCG β , human chorionic gonadotropin beta; MIP, *Mycobacterium indicus pranii*.

by drugs. MIP has received the approval of the DCGI and the FDA. Besides leprosy, it has been used successfully as an adjunct in the treatment of category II (difficult to treat) tuberculosis patients. MIP appears to be a potent invigorator of both humoral and cellular immune responses. Used as an adjuvant, it enhances considerably the antibody response to the hCG β -LTB vaccine, under development for control of fertility. Given intralesionally, it cures ugly anogenital warts. It suppresses growth of SP2/O myeloma tumors in BALB/c mice, presumably by inducing T-helper 1 cytokines.

Acknowledgments

The work reviewed in this article received research grants from the Indian Council of Medical Research and the Department of Biotechnology, Government of India. The authors acknowledge SA Zaheer, S Choudhary, VM Katoch, Dipankar Nandi, Shilpi P Vyas, and Kripa N Nand for their valuable contributions to the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Talwar GP. An immunotherapeutic vaccine for multibacillary leprosy. *Int Rev Immunol*. 1999;18:229–249.
2. Talwar GP, Krishnan AD, Jha P, Mehra V. Intracellular growth of an obligatory parasite *Mycobacterium leprae*: host bacterial interactions. *Biochimie*. 1974;56:231–237.
3. Talwar GP, Krishnan AD, Mehra VL, Blum EA, Pearson JM. Evaluation of cell mediated immune responses in untreated cases of leprosy. *Clin Exp Immunol*. 1972;12:195–203.
4. Mustafa AS, Talwar GP. Five cultivable mycobacterial strains giving blast transformation and leukocyte migration inhibition of leukocytes analogous to *Mycobacterium leprae*. *Lepr India*. 1978;50:498–508.
5. Mustafa AS, Talwar GP. Early and late reactions in tuberculoid and lepromatous leprosy patients with lepromins from *Mycobacterium leprae* and five selected cultivable mycobacteria. *Lepr India*. 1978;50:566–571.
6. Chaudhuri S, Fotedar A, Talwar GP. Lepromin conversion in repeatedly lepromin negative BL/LL patients after immunization with autoclaved *Mycobacterium w*. *Int J Lepr Other Mycobact Dis*. 1983;51:159–68.
7. Kar HK, Sharma AK, Mishra RS, et al. Induction of lepromin positivity by a candidate anti-leprosy vaccine *Mycobacterium w* in lepromin negative healthy contacts of multibacillary leprosy patients. *Indian J Lepr*. 1992;64:495–500.
8. Zaheer SA, Mukherjee R, Ramkumar B, et al. Combined multidrug and *Mycobacterium w* vaccine therapy in patients with multibacillary leprosy. *J Infect Dis*. 1993;167:401–410.
9. Talwar GP. Leprosy is in principle eradicable: a possible approach. *Curr Sci*. 2014;106:1344–1345.
10. Zaheer SA, Mukherjee A, Ramesh V, et al. Immunotherapy benefits multibacillary patients with persistently high bacteriological index despite long term multidrug therapy. *Immunol Infect Dis*. 1995;5:115–122.
11. Walia R, Sarathchandra KG, Pandey RM, et al. Field trials on the use of *Mycobacterium w* vaccine in conjunction with multidrug therapy in leprosy patients for immunotherapeutic and immunoprophylactic purposes. *Lepr Rev*. 1993;64:302–311.
12. Sharma P, Mukherjee R, Talwar GP, et al. 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. *Lepr Rev*. 2005;76:127–143.
13. Singh IG, Mukherjee R, Talwar GP. Resistance to intravenous inoculation of *Mycobacterium tuberculosis* H₃₇R_v in mice of different inbred strains following immunization with a leprosy vaccine based on *Mycobacterium w*. *Vaccine*. 1991;9:10–14.
14. Reddi PP, Amin AG, Khandekar PS, Talwar GP. Molecular definition of unique species status of *Mycobacterium w*, a candidate leprosy vaccine strain. *Int J Lepr Other Mycobact Dis*. 1994;62:229–236.
15. Saini V, Raghuvanshi S, Talwar GP, et al. Polyphasic taxonomic analysis establishes *Mycobacterium indicus pranii* as a distinct species. *PLoS One*. 2009;4:e6263.
16. Talwar GP, Ahmed N, Saini V. 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*. 2008;8:100–101.
17. Gupta S, Malhotra AK, Verma KK, Sharma VK. Intralesional immunotherapy with killed *Mycobacterium w* vaccine for the treatment of anogenital warts: an open-label pilot study. *J Eur Acad Dermatol Venereol*. 2008;22:1089–1093.
18. Singh S, Chouhan K, Gupta S. Intralesional immunotherapy with killed *Mycobacterium indicus pranii* vaccine for the treatment of extensive cutaneous warts. *Indian J Dermatol Venereol Leprol*. 2014;80:509–514.
19. Rakshit S, Ponnusamy M, Papanna S, Saha B, Ahmed A, Nandi D. Immunotherapeutic efficacy of *Mycobacterium indicus pranii* in eliciting anti-tumor T cell responses: critical roles of IFN γ . *Int J Cancer*. 2012;130:865–875.
20. Purswani S, Talwar GP. Development of a highly immunogenic recombinant candidate vaccine against human chorionic gonadotropin. *Vaccine*. 2011;29:2341–2348.

Biologics: Targets and Therapy

Publish your work in this journal

Biologics: Targets and Therapy is an international, peer-reviewed journal focusing on the patho-physiological rationale for and clinical application of Biologic agents in the management of autoimmune diseases, cancers or other pathologies where a molecular target can be identified. This journal is indexed on PubMed Central, EMBase, and Scopus.

Submit your manuscript here: <https://www.dovepress.com/biologics-targets-and-therapy-journal>

Dovepress

The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.