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
Shreemanka K Parida⁵
Prabakara 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, India; 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
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. 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 ($^3$H-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 $^3$H-thymidine by *M. leprae* was restricted, whereas T lymphocytes derived from LL patients lacked this property.

**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. Their ability to generate cytokines influencing macrophage function was investigated. The ensemble of these investigations led to the shortening of the list of 16 to 5 mycobacteria. These were *M. vaccae*, *M. phlei*, *M. gordonae*, 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.

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. This was confirmed by Kar et al in lepromin-negative family members and contacts of leprosy patients in Delhi. A total of 67 of 68 (98.5%) converted

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical status</th>
<th>CPM $^3$H-thymidine incorporated per $5 \times 10^6$ phagocytic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Macrophages + lymphocytes + <em>Mycobacterium leprae</em></td>
</tr>
<tr>
<td>1 LL</td>
<td>36,458</td>
<td>45,628</td>
</tr>
<tr>
<td>2 LL</td>
<td>53,929</td>
<td>59,596</td>
</tr>
<tr>
<td>3 LL</td>
<td>52,354</td>
<td>83,476</td>
</tr>
<tr>
<td>4 TT</td>
<td>6,332</td>
<td>54,969</td>
</tr>
<tr>
<td>5 TT</td>
<td>32</td>
<td>78,447</td>
</tr>
<tr>
<td>6 TT</td>
<td>381</td>
<td>26,260</td>
</tr>
</tbody>
</table>


**Abbreviations:** CPM, counts per minute; LL, lepromatous leprosy; TT, tuberculoid leprosy.
to lepromin-positive status after two immunizations with \textit{Mw}. These observations pointed out the potential of \textit{Mw} to serve as an immunoprophylactic vaccine to protect susceptible family members from contracting leprosy on continuous exposure to \textit{M. leprae} from a multibacillary leprosy patient.

**Therapeutic action of \textit{Mw}**

Zaheer et al administered autoclaved \textit{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 \textit{Mw} with the drugs expedited bacterial clearance and shortened the period of complete recovery.\textsuperscript{8} Figure 1 illustrates the remarkable property of \textit{Mw} in not only expediting recovery but also clearing granulomas in patients.\textsuperscript{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 \textit{Mw} vaccine as an adjunct. Figure 2 shows the dynamics of bacterial index values in two of these patients. Soon after immunization with \textit{Mw}, the bacterial index started declining noticeably, followed by full recovery of the patients.\textsuperscript{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* (\textit{M. indicus pranii}).


*Abbreviations*: MDT, multidrug therapy; LL, lepromatous leprosy; BL, borderline leprosy.
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...
be protected from becoming patients. Observations made during these trials are reported elsewhere.\textsuperscript{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 endemicism 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.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Type</th>
<th>Initial BI</th>
<th>Final BI</th>
<th>Initial H/P</th>
<th>Final H/P</th>
<th>Nerve BI$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine</td>
<td>LL</td>
<td>4</td>
<td>0</td>
<td>LL</td>
<td>NSI</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>BL</td>
<td>3</td>
<td>0</td>
<td>BL</td>
<td>NSI</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>5</td>
<td>0</td>
<td>LL</td>
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</tr>
<tr>
<td></td>
<td>BL</td>
<td>2.33</td>
<td>0</td>
<td>BL</td>
<td>NSI</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>LL</td>
<td>4.33</td>
<td>0</td>
<td>LL</td>
<td>LL</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>BL</td>
<td>3.16</td>
<td>0</td>
<td>BL</td>
<td>BL</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>2.16</td>
<td>0</td>
<td>LL</td>
<td>LL</td>
<td>0</td>
</tr>
</tbody>
</table>

*Note:* All 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.

#### 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 $M. tuberculosis$ $H_{37}R_{v}$. 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.
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.
elsewhere.\textsuperscript{15} Mw was considered an ancestor of both \textit{M. leprae} and \textit{M. tuberculosis}. No bacteria of this definition had heretofore existed in the International Depository. It was named \textit{Mycobacterium indicus pranii} (MIP).\textsuperscript{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.\textsuperscript{17} Similar warts occurring elsewhere in the body were also healed (Figure 10).\textsuperscript{18}

![Figure 8](image1.png) **Figure 8** Effect of MIP on ugly anogenital warts. **Notes:** (A) A patient with giant condylomata. (B) The lesions completely subsided with intrallesional immunotherapy with MIP. Reproduced from Gupta S, Malhotra AK, Verma KK, Sharma VK. Intrallesional immunotherapy with killed \textit{Mycobacterium} w vaccine for the treatment of anogenital warts: an open-label pilot study. \textit{J Eur Acad Dermatol Venereol.} 2008;22:1089–1093 with permission from John Wiley and Sons.\textsuperscript{17} **Abbreviation:** MIP, \textit{Mycobacterium indicus pranii}.

![Figure 9](image2.png) **Figure 9** Action of MIP on ugly anogenital warts (A) before treatment and (B) after treatment with MIP. **Abbreviation:** MIP, \textit{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.\textsuperscript{19} Figure 11 gives a summary of their findings. They have also reported the generation by MIP of cytokines IL-2 and IFN\textgreek{y}, which may be involved in this action.

**Potent adjuvant properties of \textit{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 \textit{M. leprae} and \textit{M. tuberculosis}. No bacteria of this definition had heretofore existed in the International Depository. It has been named \textit{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 \textit{M. leprae}, which is not achieved...
MIP treatment suppressed tumor growth and induced a Th1 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 ~10^7 SP2/0 cells. Mice were injected intradermally with a single dose of MIP (~5 x 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 ± 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, Papanna S, Saha B, Ahmed A, Nandi D. Immunotherapeutic efficacy of Mycobacterium indicus pranii in eliciting anti-tumor T cell responses: critical roles of IFNγ. Int J Cancer. 2012;130:865–875, with permission from John Wiley and Sons.19

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

Figure 12 Enhancement of antibody response to hCGβ-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 hCGβ-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: hCGβ, 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 invocator of both humoral and cellular immune responses. Used as an adjuvant, it enhances considerably the antibody response to the hCGβ-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 I cytokines.

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**Disclosure**

The authors report no conflicts of interest in this work.

**References**