

Development of an extra-ordinary Vaccine with Multiple Applications

G.P. Talwar, Jagdish C. Gupta

Talwar Research Foundation, New Delhi, India

Corresponding Author: Prof. G.P. Talwar, Talwar Research Foundation, E-8, Neb Valley, New Delhi-110068, India

Although, the causativemicro-organism of leprosy (*M.leprae*) was discovered by Armauer Hansen nearly two centuries backin Norway, the load of leprosy is high in India and in other economically developing countries. We developed a therapeutic vaccine against leprosy, based on a non-pathogenic cultivable mycobacteria coded as Mw (1), which was employed as adjunct to standard multi-drug regime to treat multi-bacillary leprosy patients. Its inclusion for therapy *expedited* the time for recovery of the patients and what is remarkable is that the blemishes usually associated with leprosy, which drugs are unable to remove despite their ability to kill *M. leprae*, *disappear* in patients receiving the vaccinein addition to the standard drugs. Fig. 1 illustrates the recovery experienced by patients receiving the Mw vaccine.

Another highly useful impact of the vaccine is the upgrading of their immunity to resist *M.leprae* infection on re-exposure to the pathogen. Patients developing multibacillary BL, LL type of leprosy are negative to Delayed Hypersensitivity skin response to Lepromin(a homogenate of *M. leprae*), and they remain lepromin negative even when they are rendered bacterial negative on prolonged treatment with drugs. On the other hand, the inclusion of M.w. (now MIP) vaccine in the treatment renders these patients lepromin positive. Fig. 2 reflects the upgrading of the immunological deficit of these patients to this pathogen.

1. RENAMING OF MW AS MIP AFTER GENOME SEQUENCING

Genome of Mycobacterium w (*M.w.*) has been sequenced and being hitherto an unlisted mycobacteria in International Depository, it has been deposited there and has been named as *Mycobacterium indicuspranii* (2,3). Pran is my family name and nii is the National Institute of Immunology, New Delhi, of which I was the Founder Director, and from where field trials on MIP as adjunct to MDT were conducted.

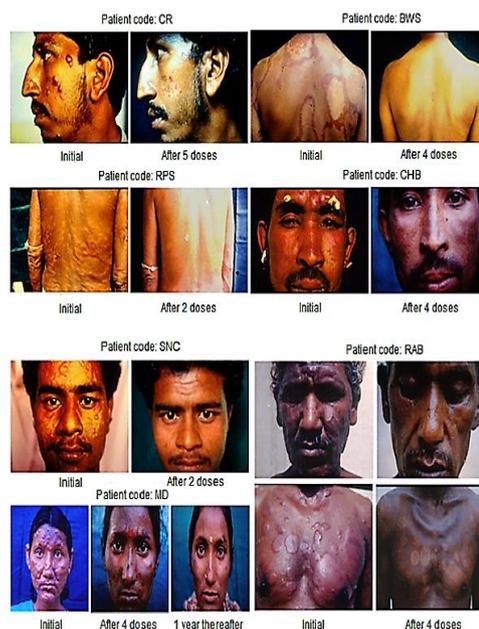


Fig 1: Some representative cases of LL/BL multibacillary patients treated with MDT plus Mw.

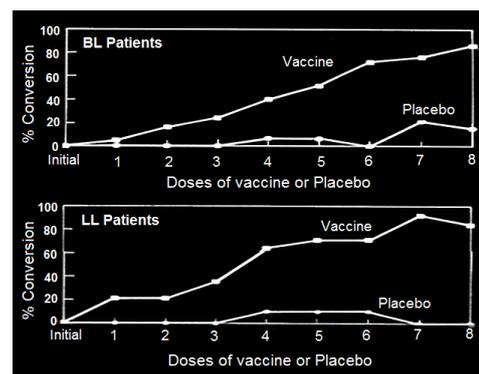


Fig 2: Conversion of lepromin status of BL/LL patients treated with MDT ± Mw

2. TREATMENT OF TUBERCULOSIS

Mycobacterium indicusspranii (MIP) shares also antigens with *M. tuberculosis* in addition to *M. leprae*. A trial was carried out employing MIP along with standard drugs for treatment of “Difficult to treat” Category II tuberculosis patients. Table 1 summarizes the observations. What was further noteworthy was

that these patients showed low relapse rate in comparison to those treated with drugs only (Fig. 3).

Table 1: 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

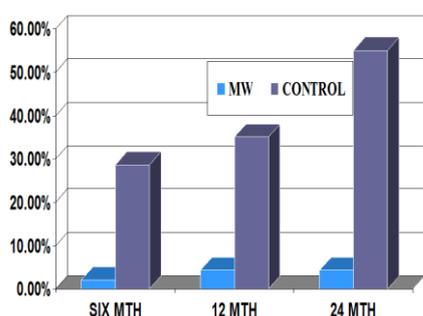


Fig 3: Relapse rate of Category II tuberculosis patients after treatment with MDT alone or MDT+ Mw (MIP).

3. ANO-GENITAL WARTS AND LESIONS ELSEWHERE ON THE BODY

Prof. Somesh Gupta at the All India Institute of Medical Sciences New Delhi employed MIP as intra-lesion injection (4,5). Figures 4, 5 and 6 show that these warts were astonishingly all cleared.



Fig 4: Effect of MIP on ugly anogenital warts. (a) A patient with giant condylomata. (b) The lesions completely subsided with intralesional immunotherapy with MIP.



Fig 5: Action of MIP on ugly ano-genital warts (A) Before treatment (B) After treatment with MIP.



Fig 6: Cure by MIP of warts on feet. (A) Before treatment and (B) After 5 months of treatment with MIP.

4. MYELOMAS

Prof. Dipankar Nandi at the Indian Institute of Sciences, Bangalore, employed MIP for prevention of SP2/0 myelomas in mice (6). Fig.7 summarizes their observations.

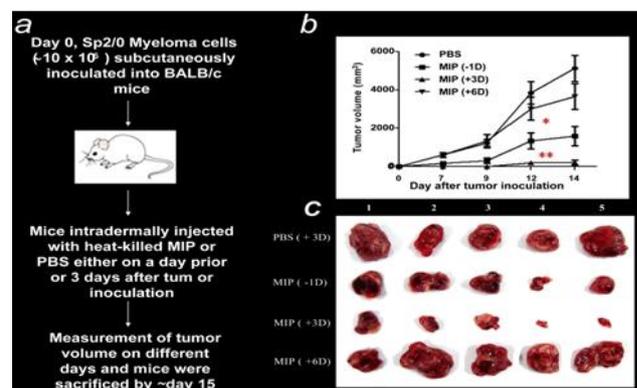


Fig.7. MIP treatment suppresses tumor growth and induces a Th1 cytokine response. (a) General outline of the *in vivo* experimental protocol. (b) Comparison of the anti-tumor effects of MIP administered at different time points. Cohorts of ten mice were inoculated s.c. with $\sim 10^7$ Sp2/0 cells. Mice were injected i.d. with a single dose of MIP ($\sim 5 \times 10^8$) 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 \pm SD mm³) at indicated days post implantation. (c) Representative photographs of solid tumors from different treatment groups dissected on day 14 [from (6)]

5. MIP, A POTENT INVIGORATOR OF IMMUNE RESPONSE

We are in process of reviving a vaccine inducing anti-hCG antibodies for preventing unwanted pregnancy. Antibody titres against hCG by employing a genetically engineered vaccine along with MIP as adjuvant, induced notably higher antibody titres in mice as shown in Fig. 8 (7).

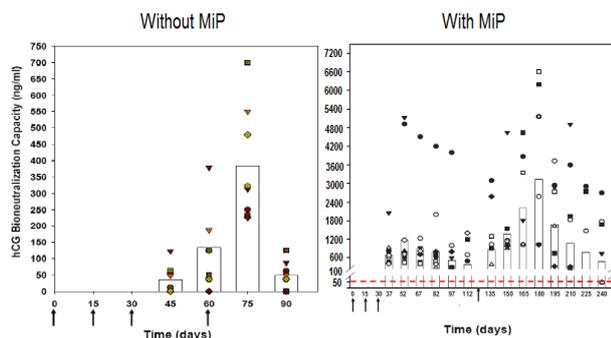


Fig. 8: Enhancement of antibody response to hCG β -LTB vaccine in Balb/c mice by MIP. Mice were immunized intra-muscularly with 2 μ g of the vaccine adsorbed on alum with or without MIP. Primary immunization consisted of 3 injections given at fortnightly intervals followed by a booster on day 60 or 120. The symbols represent the titres in a given mouse. Bars give the geometrical Means (From 7).

6. SUMMARY

A vaccine based on a non-pathogenic cultivable mycobacteria was developed against leprosy. As adjunct to the standard drugs, it expedited bacterial clearance, and shortened recovery period. A remarkable benefit was the disappearance of granulomas and ugly blemishes in the patients. The genome sequence of this mycobacteria has been determined, and it has been named as *Mycobacterium indicuspranii* (MIP).

Besides leprosy, MIP has therapeutic effect in Category II, 'Difficult to treat' tuberculosis patients. MIP cures dramatically ano-genital warts. MIP has also preventive and therapeutic action on myelomas. It is a potent invigorator of immune response and is being used as adjuvant in a vaccine directed against hCG.

REFERENCES

- [1] Talwar GP. An immunotherapeutic vaccine for multibacillary leprosy. *Int Rev Immunol.* 1999; 18(3): 229-249.
- [2] 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.
- [3] Saini V, Raghuvanshi S, Talwar GP, Ahmed N, Khurana JP, Hasnain SE, TyagiAkhilesh K, Tyagi Anil K. Polyphasic taxonomic analysis establishes *Mycobacterium indicuspranii* as a distinct species. *PLoS One.* 2009; 4: e6263.
- [4] Gupta S, Malhotra AK, Verma KK, Sharma VK. Intralesional immunotherapy with killed *Mycobacterium w* vaccine for the treatment of ano-genital warts: an open label pilot study. *J EurAcadDermatolVenereol.* 2008; 22(9):1089-93.
- [5] Singh S, Chouhan K, Gupta S. Intralesional immunotherapy with killed *Mycobacterium indicuspranii* vaccine for the treatment of extensive cutaneous warts. *Indian J DermatolVenereolLeprol.* 2014; 80:509-514.
- [6] Rakshit S, Ponnusamy M, Papanna S, Saha B, Ahmed A, Nandi D. Immunotherapeutic efficacy of *Mycobacterium indicuspranii* in eliciting anti-tumor T cell responses: critical roles of IFN γ . *Int J Cancer.* 2012; 130: 865-875.
- [7] Purswani S, Talwar GP, Vohra R, Pal R, Panda AK, Lohiya NK, Gupta JC. *Mycobacterium indicuspranii* is a potent immunomodulator for a recombinant vaccine against human chorionic gonadotropin. *J ReprodImmunol.* 2011; 91: 24-30.