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REVIEW ARTICLE

The Role of Adjunctive *Mycobacterium w* Immunotherapy for Tuberculosis

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Mycobacterium w (*Mw*) is a potent immunomodulator based on saprophytic cultivable autoclaved atypical *Mycobacterium* (*M.*), and shares T cell and B cell antigenic determinants with *M. tuberculosis* and *M. leprae*. *Mw* enhances T-helper 1 response, resulting in the release of type-1 cytokines, predominantly interferon-γ, and thereby propagates cell-mediated immune responses. In experimental models, *Mw* has shown a protective effect against tuberculosis in mice and guinea pigs challenged with live *M. tuberculosis* H_{37}Rv. Clinical trials have shown significant clinical benefits of *Mw* in leprosy and tuberculosis. Used as an adjuvant to multidrug therapy in multibacillary leprosy, *Mw* resulted in expedited and distinct clinical improvement, a decline in the bacterial index, and enhanced the immunologic recovery of patients. In tuberculosis, *Mw* has resulted in higher curative rates and a significant reduction in the time to sputum conversion in patients with a high bacterial load. In addition, *Mw* has shown potential for tuberculin conversion and increased $CD4^+$ cell count in human immunodeficiency virus (HIV)-infected people. With the increasing incidence of multidrug resistant tuberculosis and the high burden of HIV infection, there is a need to evaluate the role of *Mw* as an adjunctive treatment in tuberculosis, and to examine its immunomodulatory effects in large, well-designed, randomized, controlled clinical trials. An effective immunotherapeutic vaccine, particularly for HIV-positive patients, would greatly affect the profile of tuberculosis at the population level.

1. Introduction

Tuberculosis (TB) is a major infectious disease worldwide, with a high rate of morbidity and mortality.[1] In 2005, an estimated 8.8 million new cases of TB occurred. This led to 1.7 million deaths, of which 195,000 were associated with human immunodeficiency virus (HIV) infection.[2] Of the new cases, 7.4 million occurred in Asia and sub-Saharan Africa.[2] The incidence of TB is steadily increasing. This has been attributed to its coexistence with HIV infection, and the emergence of multidrug resistant as well as the recently reported extreme drug resistant strains of *Mycobacterium (M.) tuberculosis*.[3] A high pill burden, as well as long treatment duration, have negative impacts on treatment compliance. These factors culminate in the high rate of treatment discontinuation leading to high relapse rates, making global TB elimination programs problematic. Therefore, there is an urgent need to develop new treatment regimens that may have shorter treatment durations and are effective at achieving rapid, sustainable bacterial clearance in tissues.[3] This would increase the curative rates, reduce morbidity and mortality, and control the spread of *M. tuberculosis* in the population.

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Based on these factors, potential new immunoadjuvants to complement current chemotherapies need to be considered because they would help enhance the mycobactericidal immune responses of the host. *Mycobacterium w* (*Mw*) immunotherapy is a potent immunomodulator based on saprophytic cultivable autoclaved *Mw*, which shares T cell and B cell antigenic determinants with *M. tuberculosis* and *M. Leprae*, and confers protection against TB in mice and guinea pigs challenged with live *M. tuberculosis* $H_{37}Rv$.[4]

Mw immunotherapy is derived from a new fast-growing, nonpathogenic atypical mycobacterium species that has metabolic and growth properties resembling those belonging to Runyon's group IV class of mycobacteria.[5] However, *Mw* is not identical to the strains currently listed in this group. The *Mw* species has been identified through polymerase chain reaction DNA sequence determination as a unique species distinct from other known mycobacterium species,[6] including: *M. avium*, *M. intracellulare*, *M. scrofulaceum*, *M. kansasii*, *M. gastri*, *M. gordonae*, *M. shimoidei*, *M. malmoense*, *M. haemophilum*, *M. terrae*, *M. nonchromogenicum*, *M. triviale*, *M. marinum*, *M. flavescens*, *M. simian*, *M. szulgai*, *M. xenopi*, *M. asciaticum*, *M. aurum*, *M. smegmatis*, *M. vaccae*, *M. fortuitum* subspecies *Fortuitum*, *M. fortuitum* subspecies *Peregrinum*, *M. chelonae* subspecies *Chelonae*, *M. chelonae* subspecies *Abscessus*, *M. genavense*, *M. tuberculosis* and $H_{37}R_v$ *M. paratuberculosis*.

Mw immunotherapy is thought to enhance T-helper 1 (Th-1) response resulting in the release of type-1 cytokines [interleukin (IL)-2, IL-12, IL-15 and interferon (IFN)-γ] and putatively augments cell-mediated immunity, which may impact on disease progression.[4,7] Since it enhances T cell activity, *Mw* has been used as an immunoadjuvant to chemotherapy for sputum-positive pulmonary TB patients in clinical trials and has resulted in more rapid sputum conversion.[8,9] *Mw* has also been tested as an adjuvant therapy for the treatment of lung cancer and showed a significant effect on tumor regression.[10] *Mw* has been used in other trials as an adjuvant to multidrug therapy in multibacillary leprosy, showing expedited and distinct clinical improvements, a significant decline in the bacterial index and enhanced immunologic recovery.[3,11,12] *Mw* was reported to be safe and well tolerated in these patients.

This systematic review summarizes the evidence from trials done to date that assessed the effect of adjunctive *Mw* immunotherapy on multidrug resistance, morbidity, mortality and improved survival of patients with TB.

2. Search Strategy

We carried out a comprehensive search to identify all relevant studies in English, regardless of publication status (published, unpublished, in press and in progress). Using the search terms tuberculosis AND ("mycobacterium w" OR immunotherapy OR immunoadjuvant OR vaccine), we searched the following databases:

- Cochrane Library (latest issue);
- MEDLINE 1966 to October 2007;
- EMBASE 1980 to October 2007;
- LILACS 1982 to October 2007 (La Literatura Latinoamericana y del Caribe de Information en Ciencias de la Salud) (www.bireme.br).

We also manually reviewed the reference lists of the identified articles and relevant review articles. In addition, we manually searched the abstracts and proceedings of the following conferences: The International Union Against Tuberculosis and Lung Disease World Congress, The American Thoracic Society International Congress and The European Respiratory Society World Congress. We also approached individuals and organizations within the field of TB immunotherapy for information regarding unpublished data and work in progress.

3. Phase 1 Studies: Immunogenicity, Safety, Adverse Reactions and Summary of Data in Leprosy

The *Mw* vaccine is manufactured under the trade name Immuvac by Cadila Pharmaceuticals, Ahmadabad, India. *Mw* has been assessed extensively as an adjunct to standard (multidrug) chemotherapy in leprosy patients in phase II and phase III studies.[7] The reports have shown significant clinical responses and faster bacteriological clearance of *M. leprae* bacilli and a shorter duration of treatment in patients who received *Mw* vaccine compared with patients who received placebo. The vaccine was generally well tolerated, showing only mild adverse reactions such as ulcerations at injection site, scarring, and transient fever.

In chronic infections such as TB, the cellular immune response of the host plays a crucial role in protective immunity. *Mw* induces T cells to produce high levels of IL-2 and IFN-γ, which activates cytolytic effector T cells and other macrophage functions.[13] This indicates that Th-1 cells are essential in cellular immunity against many intracellular pathogens.[4,13,14]

4. Putative Mechanisms of Action

The precise mechanisms of action of *Mw* are not fully understood. It has been demonstrated from several studies that *Mw* shares B cell and T cell antigenic determinants with *M. tuberculosis* and *M. Leprae*.[15,16] Some studies have reported a 28–31-kDa immunodominant antigen in *Mw* carrying B and T cell determinants.[17] In a study done on leprosy, Zaheer et al demonstrated a significant shift in the cell-mediated immune response that was reflected by a progressive lepromin conversion from

negativity to positivity in vaccinated patients (approximately 70% in borderline lepromatous/lepromatous, 100% in borderline-borderline type).[7] In murine models, *in vitro* characterization of the cellular immune response induced *in vivo* by *Mw* vaccination resulted in preferential activation of T cells, which produce high levels of IL-2 and IFN-γ but not IL-4 and IL-5.[7,18] *In vitro* restimulation of T cells could be done by *Mw* as well as *M tuberculosis* $H_{37}Ra$ or $H_{37}Rv$, indicating that these mycobacteria likely share major T cell antigens.[4] Based on these findings, several assumptions have emerged that have attempted to explain the mechanisms of action of *Mw*. *Mw* is thought to elicit a Th-1 response, resulting in the secretion of cytokines that play a crucial role in resistance against TB. IFN-γ is a predominant cytokine secreted by the antigen stimulated by lymphocytes that activates macrophages.[18] This is associated with enhanced secretion of reactive oxygen intermediates (O_2^-, H_2O_2) in the macrophages, which leads to rapid death and clearance of *M. leprae* bacilli.[19] A similar mechanism of action may be applicable to TB.

5. Treatment Outcomes in TB Patients Treated With *Mw*

5.1. Sputum conversion

In a controlled pilot trial using intradermal *Mw* immunotherapy given every 15 days as adjunct to standard anti-TB chemotherapy in sputum smear-positive TB patients, Patel et al showed that *Mw* immunotherapy reduced the duration to sputum conversion.[8] Sputum conversion was achieved within 30 days after starting treatment in 69 of 134 (51.5%) patients given *Mw* immunotherapy, rather than the 60 days which is common in patients given anti-TB chemotherapy alone. Hence, the duration of sputum conversion was reduced almost by 50%. There was also an accelerated sputum conversion rate in patients who received *Mw* immunotherapy irrespective of bacterial load (1+minimum, 3+maximum), stage of treatment, initial or retreatment category. The rapid sputum conversion was associated with improved quality of life, and reduced treatment duration and nosocomial spread of mycobacterium. *Mw* immunotherapy was well tolerated by patients in this study.[8]

In the same trial, Patel and Trapathi also found that pulmonary TB patients on a retreatment regimen (category II) who received adjunctive *Mw* immunotherapy showed improved cure rates.[9] All patients recruited in this trial received anti-TB chemotherapy according to World Health Organization guidelines. A laboratory technician blinded to the treatment allocation conducted the sputum examination and assessed sputum positivity. The sputum test results were graded 3+, 2+ or 1+ by the same technician. Sputum testing was done at baseline and was repeated every 15 days for up to 3 months, and repeat checks were done at 3, 5 and 8 months if the sputum test was negative at 3 months, or at 4, 6 and 9 months if the sputum test was positive at 3 months.[9]

Table 1 summarizes the sputum results, showing significant differences in the proportions of bacterial load in the two treatment arms ($\chi^2 = 15.9$, $p \leq 0.01$).

At the end of the treatment phase for category II patients, the sputum conversion was 75.5% in the *Mw* immunotherapy group versus 51.8% in the control group ($\chi^2 = 4.4$, $p \leq 0.05$) (Table 2).

Table 3 shows the sputum conversion rates at 4 months (extended intensive phase) after starting treatment. The curative rate was 97.9% with *Mw* immunotherapy versus 77.7% in the control group, while treatment failure was 2.0% and 22.2%, respectively ($\chi^2 = 8.6$, $p \leq 0.01$). The sputum conversion rate and progressive cure rate, as described above, were significantly

Table 1 Progressive improvement in sputum conversion 2 months after completion of the intensive treatment phase

Bacterial load	*Mw* vaccine + TB treatment, *n* (%)	TB treatment alone, *n* (%)	Total, *n*
1+	12 (24.5)	11 (40.7)	23
2+	17 (34.7)	2 (7.4)	19
3+	20 (40.8)	14 (51.9)	34
Total	49 (100)	27 (100)	76

Mw = *Mycobacterium w*; TB = tuberculosis.

Table 2 Sputum conversion after 4 months of treatment

Sputum results	*Mw* vaccine + TB treatment, *n* (%)	TB treatment alone, *n* (%)	Total, *n*
Sputum negative	37 (75.5)	14 (51.8)	51
Sputum positive	12 (24.4)	13 (48.1)	25
Total	49 (100)	27 (100)	76

Mw = *Mycobacterium w*; TB = tuberculosis.

Table 3 Sputum conversion at 2 months after completion of the intensive treatment phase

Sputum results	*Mw* vaccine + TB treatment, *n* (%)	TB treatment alone, *n* (%)	Total, *n*
Sputum negative	48 (97.9)	21 (77.7)	69
Sputum positive	1 (2.0)	6 (22.2)	7
Total	49 (100)	27 (100)	76

Mw = *Mycobacterium w*; TB = tuberculosis.

higher in those who received *Mw* immunotherapy compared with the control group while the treatment failure rate was significantly lower in the *Mw* immunotherapy group compared with the control group. This observed difference appears to be statistically significant. However, the authors of the original papers did not use statistical tests to examine these differences (the χ^2 values above were calculated by me).

Another methodological flaw noted with these studies is the lack of clarity on whether categories I and II were defined *a priori*, or in *post hoc* analysis. The numbers of patients were unbalanced between categories I and II, suggesting that randomization was not done with stratification for this factor, or that the analysis was done *post hoc*.

Overall, the treatment of category II pulmonary TB is associated with a suboptimal curative rate worldwide.[20,21] This confirms the need to investigate new treatment strategies that would improve the curative rates in category II pulmonary TB. Therefore, more definitive clinical studies are needed to investigate the efficacy of *Mw* immunotherapy as an adjunctive treatment in category I and category II treatment regimens.

5.2. Treatment outcomes in cancer

Chaudhuri and Mukhopadhyay reported positive findings for *Mw* immunotherapy as an adjunct to radiation therapy in the management of invasive bladder cancer.[22] At the end of the 24-month follow-up period, all five patients under observation were disease-free with no evidence of a residual tumor mass.[22] Similar findings were reported by Sur and Dastidar for non-small cell lung cancer.[10] The use of *Mw* immunotherapy as an adjuvant to chemotherapy plus radiotherapy in non-small cell lung cancer improved the quality of life with a significantly better Karnofsky performance status and a significant regression of tumor size in the intervention group.[10]

5.3. Treatment outcomes in HIV infection

Mw immunotherapy has been shown to enhance immune recovery in HIV infection. Kharkar compared three treatments for HIV infected patients who had comparable mean $CD4^+$ T cell counts at baseline.[23] Group A received *Mw* immunotherapy alone, group B received *Mw* plus two antiretroviral drugs, and group C received *Mw* immunotherapy plus highly active antiretroviral therapy. The mean baseline $CD4^+$ cell counts were 204.7 cells/μL in group A, 200.9 cells/μL in group B and 213.2 cells/μL in group C. After treatment, the mean baseline $CD4^+$ cell count increased by 108.9% (to 445.5 cells/μL) in group C, by 80.2% (to 368.9 cells/μL) in group A, and by 68% (to 338.3 cells/μL) in group B. In 15 of the 17 patients in group C, the mean $CD4^+$ cell count increased by 88.4% compared with 41.2% in Group A in patients who received immunomodulator alone.[23] These findings demonstrate that *Mw* immunotherapy is a potent immunostimulator; however, this study was small, non-blinded, and did not include a control group. The treatment allocation sequence was not described, suggesting this was a nonrandomized study. The observed differences between groups were not subject to rigorous statistical testing, increasing the suspicion that these differences might have occurred by chance. However, the consistent increase in mean $CD4^+$ cell count in all three groups has generated a hypothesis that needs to be tested in a larger, well-designed trial with an appropriate control group.

The tuberculin response, a delayed hypersensitivity reaction, is a strong indicator of the cellular immunity status against TB. It should be noted that individuals with severe immunosuppression, such as HIV-positive patients with $CD4^+ < 200$ cells/μL, have a negligible ability to react to tuberculin protein.[24] In brief, they are anergic to the tuberculin response. Laxman demonstrated that *Mw* immunotherapy for HIV-positive patients enhanced tuberculin conversion.[24] Fifty HIV-positive individuals, who were initially tuberculin-negative, were enrolled into the study and given a single intradermal dose of 0.1 mL *Mw*. All patients underwent a Mantoux test to determine delayed hypersensitivity reactions before receiving the vaccine. The diameter of induration was measured to determine the tuberculin response and this measurement was repeated 90 days later. Forty-eight of 50 people had tuberculin conversion at 90 days, showing a high rate of positive tuberculin response. These findings are important, particularly in areas where HIV seroprevalence is high, because HIV-positive individuals are at an increased risk of developing TB.[25–27] Therefore, there is a greater need to identify appropriate prophylactic agents to minimize the incidence of TB among HIV-positive individuals. Previous trials evaluating *M. vaccae* as an agent that could be used to induce the immune response and convert tuberculin-anergic individuals to tuberculin-positive proved unsuccessful. In such trials, *M. vaccae*, even following three intradermal injections, did not yield a positive response in terms of tuberculin conversion.[28–30] Based on these findings, *Mw* immunotherapy offers the potential to enhance immune reactivity in HIV-positive anergic individuals, making it a potential candidate immunoprophylactic vaccine against TB in HIV-positive individuals. Therefore, larger randomized controlled trials are urgently required to test the efficacy of such novel vaccines in areas with high HIV seroprevalence.

6. Dosing Frequency, Quality Control and Reproducibility

The immunotherapeutic vaccine is a suspension of killed *Mw* in physiologic saline at 10^{10} bacilli/mL.[7] It has been

used in doses of 1×10^9 autoclaved bacilli in 0.1 mL physiologic saline (0.85% NaCl) and continuation doses were given at half the number of bacilli of the first dose.[7] The vaccine was given as an intradermal injection in the deltoid region at intervals of 3 months for a total of eight doses in patients with multibacillary leprosy.[7] In TB, the *Mw* vaccine was given as an intradermal injection with a 0.2-mL loading dose given as 0.1 mL in both deltoid regions and then repeated with 0.1 mL intradermally every 2 weeks for four doses.[24] Laxman also gave the *Mw* vaccine as a single intradermal dose of 0.1 mL in a study assessing tuberculin conversion in HIV-seropositive individuals.[24]

"*Mw* was initially cultured in Lowenstein-Jensen medium taken from the master seed stock. The bacterial seed lots were then expanded in Middlebrook medium with bovine albumin, dextrose and casein enrichment, and harvest was done 8–9 days after being cultured. The pellet was centrifuged and washed three times with normal saline (0.085% NaCl). The bacilli suspended in saline were autoclaved for 15 minutes at 15 lb/inch pressure. To assess if the bacilli were completely killed, the autoclaved bacilli were cultured in Lowenstein-Jensen medium and assessed for growth of colonies after 2 weeks. Testing for sterility of the vaccine preparation thioglycolate and soya bean casein digest medium was used for this purpose. For preservative purposes, thiomersal was then added to a final concentration of 0.01%."[7] Placebo *Mw* was made by dissolving 1 g of micronized starch in 100 mL of distilled water, and autoclaved at 15 lb/inch pressure for 15 minutes and dispensed in sterile vials.[7] Other quality-control methods, such as cold chain, are not described in the paper.

7. Implications for Future Research and Recommendations

The incidence of TB is steadily increasing because of its close association with the HIV epidemic. It is worrying to note that multidrug resistant TB has spread globally and the newly reported extreme drug resistant TB is threatening current efforts to control TB. These factors are among many that contribute to the high mortality rate associated with TB. To date, there are no new interventions that could be used in many low-income countries. This supports the calls for new methods that could effectively arrest the spread of TB and improve outcomes in those who are infected. This review has provided a comprehensive summary of *Mw* immunotherapy and its potential role in TB. Based on the studies done to date, the data suggest that *Mw* immunotherapy is safe in humans and may induce anti-TB resistance. This is achieved through its ability to stimulate the production of IFN-γ by Th-1 cells. The cytokines form the basis for acquired resistance in TB.[4] Marthur, in his review, states that the current data highlight preliminary evidence suggesting that the use of *Mw* vaccine confers more rapid sputum conversion, irrespective of bacterial load, in patients on current short-course chemotherapy and retreatment regimens, reduces inflammation associated with extrapulmonary TB, and increases the $CD4^+$ cell count in HIV-infected people.[31] The vaccine has shown positive effects in psoriasis[32,33] and in different forms of cancer.[34–36] Other than in leprosy, the studies done in TB where *Mw* immunotherapy was used as an adjuvant to standard chemotherapy have been too small, often lacking a control group and proper randomization methods, to enable reliable conclusions to be drawn. However, the consistency of the findings from the studies reviewed here has generated a hypothesis for the role of *Mw* immunotherapy as an adjuvant treatment in TB, which must be rigorously tested in large, properly-designed, randomized, placebo-controlled trials. Such trials would strengthen the agenda of the Global Plan to Stop TB,[37] that is to develop new vaccines, drugs and diagnostic tests.

8. Conclusions

Mw immunotherapy has been widely used to control leprosy in India. The vaccine has reportedly shown significant beneficial effects in terms of bacterial clearance in multibacillary leprosy patients when used as an adjuvant to standard multidrug therapy. Preliminary evidence suggests that *Mw* immunotherapy has a similar effect in TB.[8,9,31] It is common knowledge that the rapid clearance of *M. tuberculosis* bacilli would reduce the duration of treatment and accelerate improvements in the quality of life. Treatments with shorter duration and more rapid bacterial elimination would have a huge impact on reducing the spread of TB in the population, which is the goal of the Global Plan to Stop TB agenda.[37] Immunotherapy based on *Mw* could be used as an immunoprophylaxis in HIV-positive individuals because they are at increased risk for TB. In HIV-infected individuals, *Mw* immunotherapy seems to increase the $CD4^+$ cell count. With many trials reporting findings for relatively small cohorts, short duration of follow-up and lacking appropriate control groups, the conclusions obtained from these studies are subject to many limitations. Nevertheless, these studies provide a good foundation for further prospective evaluation of this novel vaccine in well-powered trials with clinical outcomes, which the current literature does not provide for *Mw* immunotherapy.

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