Exogenous Re-infection by Multiple Exposures to *Mycobacterium tuberculosis* Contributes to Subsequent Development of Active Tuberculosis

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Abstract: The majority of tuberculosis (TB) exists in the world’s poorest countries, where costly biosafety level three facilities for containment of infectious TB patients and diagnostic facilities are not affordable. Health care workers (HCWs), in countries with high burdens of tuberculosis (TB) are at risk of nosocomially acquired TB, as there are increased numbers of cases of TB on open hospital wards and minimal or absent TB infection control. This setting provides a means to study development of immune profiles associated with human exposure to *Mycobacterium tuberculosis* (*Mtb*). Individuals with multiple exposures to *Mtb* develop a Th1 response, involving IFN-γ. However early expression of a Th2 response, consisting of IL-4, was found to be associated with development of active TB disease. A Th2 response was confined to T cells of the CD8 and γδ T cell phenotype which can result in reduced bactericidal function of mycobacterial infected cells. The facets of the immune response which are responsible for failure of elimination of intracellular *Mtb* leading to active disease are poorly understood.

Key words: *M. tuberculosis*, T cells, health care workers, review

INTRODUCTION

At present time about 2 million people in the world die of TB every year; 98% of the mortality cases occur in developing countries[1, 2]. Most individuals infected with *Mtb* do not develop TB, however the pathogenic success of *Mtb* can be attributed to its ability to persistence over long periods of time without causing overt disease. However, for about 10 percent of these infections, the disease can progress to active status, a condition where bacterial replication and dissemination occurs and may now be found in the secretions that emanate from the pulmonary tree. During this stage, the infected patient can release mycobacterium-containing microdroplets and individuals who are in close proximity to this active TB patient, can become infected.

The vast majority of global TB infections occur in economically disadvantaged countries that cannot support the construction and maintenance of adequate health care facilities to protect patients and their healthcare workers (HCW) from acquiring TB. HCWs in such facilities are exposed repeatedly for prolonged periods to aerosolized *Mtb* from patients who are being interviewed, or evaluated for active TB, or are occupying a bed during the weeks and possibly months that they are receiving treatment. Obligatory preventive therapy for TB infection many times is not implemented for HCWs in these hospital facilities. The question of whether such repeated exposure to aerosolized *Mtb* contributes to the progression to active disease and or virulence of the infection among HCWs is of primary importance if control of TB is to be achieved[3, 4]. Although it is now known that during a working period of 5-10 years a considerable number of HCWs of countries whose healthcare facilities facilitate exposure to TB develop active TB[5, 6] and that repeated exposure is the cause for this rapid development to active TB, the role of immunity during this period of repeated exposure is yet to be defined. Nevertheless, we
have found in a small study that 6 out 10 HCWs who progressed to active TB had a consistent Th2 type of response that was not present among those 4 HCW who worked at similar locations under similar conditions and whom remained healthy\cite{3,4}.

Portugal has the highest incidence of pulmonary tuberculosis in Western Europe with 47 new cases of active TB per hundred thousand inhabitants\cite{5-7}. HCWs in Portugal are repeatedly exposed to aerosolized Mtb from TB patients due to the lack of infection control practices\cite{8-12}. Because TB potentially can readily result from the inhalation of as few as 10 bacilli\cite{13} these HCWs are not only at proven risk\cite{14} but are themselves sources for new infections in the community, particularly, within the hospital environment itself. Given the high probability that repeated exposure of the Portuguese HCW to expelled microdroplets containing \textit{Mtb} will result in a large number of TB infections and given the probability that some of these infections will progress to active TB disease, this population of HCW provides an opportunity to study the progression of TB and its relationship to a variety of markers of immunity that are known to be involved in general infectious diseases processes. In this review, the hypothesis that multiple TB exposure can lead to induction of Th2 responses contributing to active disease will be examined.

**The immune response against \textit{Mycobacterium tuberculosis} infection:** Breathing in microdroplets containing \textit{Mtb} may result in the organism eventually reaching the sites of the alveolae where specialized alveolar cells phagocytose the organism. Some of the organisms in passing through the bronchial tree will be phagocytosed by aveolar macrophages that have the capacity to kill the organism\cite{15}. The products of the phagocytosed bacteria have antigens that are presented by macrophages as antigen bound to class-I MHC molecules to CD8+, as class-II MHC molecules to CD4+ and non-MHC class to γδ+ T cells\cite{15-19}. The presentation of these molecules to T cells causes the secretion of interferon gamma (IFN-γ), IL-12 and the subsequent signalling from TNF-α results in the activation of other macrophages to phagocytose and kill the intracellular organism\cite{20}. Dendritic cells participate in the generation of acquired immunity after carriage of \textit{Mtb} antigens to draining lymph nodes where recognition of specific antigens by naïve T cells results in T cell activation and subsequent effectors functions in the lung\cite{20,21}. At this time generation of acquired immunity in part involves specialised dendritic cells which tend to coordinate the processes that result in the recognition and subsequent killing of the organism after re-exposure to the organism\cite{22,23}. Failure of one or more of these effectors functions, particularly IFN-γ production, results in bacterial survival (latent disease) and possible later development of active TB disease.

Numerous studies have also found that patients with active TB have a Th2 response characterized by production of IL-4, IL-5 and IL-10. These studies suggest that a progressive decline in Th1 response along with the maintenance and dominance of the steady-state of a Th2 response is associated with the progression from latent infection to active TB disease. Therefore, it is the declining of a Th1 response and a concomitant presence of a Th2 response which may prevent the progression of latent to active TB status\cite{24,27}. It is the purpose of this review to provide support for the hypothesis that multiple TB exposure can lead to modification of Th1 responses and expression of Th2 responses and that these contribute to the development of active disease.

**Evidence of exogenous reinfection:** Exogenous reinfection has been viewed as a rare phenomenon due to the belief that primary \textit{Mtb} infection confers resistance sufficient for prevention of subsequent infection\cite{28}. Adult TB was therefore attributed to the reactivation and dissemination of tubercle bacilli that was latent for a period of time. Although active TB that takes place in the 5 to 10% of individuals who had been infected decades earlier can be identified as reactive cases of TB by X-ray, namely, by the position of the lesions\cite{28}, the cause for this latent appearance of disease-\textit{i.e.}, reduction of immunity vs repeated exposure to the mycobacterium, cannot be determined by clinical, radiological, or conventional laboratory means. Early studies by Lurie\cite{29} on spontaneous infection showed that vaccinated rabbits kept in contact with \textit{Mtb}-infected rabbits progressed to active disease very quickly as opposed to the infected, non-immunised group. Longitudinal studies on the incidence of TB in individuals exposed to a familial source of TB infection demonstrated that adults exposed to TB developed clinical pulmonary TB more frequently than those infected adults who were not exposed to TB\cite{30-32}. According to the American Thoracic Society, individuals in close contact with infectious TB patients have a 25-50% chance of being infected with \textit{Mtb}\cite{14}. Persistence and reinfection of \textit{Mtb} occurs despite an aggressive adaptive immune response resulting from primary infection, the importance of the latter has only been recently recognized\cite{3,4}.

More recent studies using molecular fingerprinting have shown that in countries with high burdens of TB, re-infection is responsible for a large number of active TB cases\cite{31,34}. TB has also been demonstrated to take place in patients who were successfully treated and cured of infection and this recurrence has been attributed to re-infection resulting from prolonged contact with other active TB patients\cite{33,34}. Furthermore, a high rate of \textit{Mtb} nosocomial transmission has also been demonstrated with brief contact with an infectious TB case\cite{35}. Hospitals where there is a very high rate of
TB are also sites where transmission of the infections takes place from patient to patient[36], from patient to HCW[34] as well as from and from HCW to HCW[34]. Studies using a mouse model that study the immune responses to post-primary TB support the hypothesis that re-infection contributes to active disease[37]. In addition, studies employing the zebrafish model for the tracking of a super-infection caused by \textit{M. marinum} show that upon secondary infection, macrophages containing intracellular mycobacteria that participate in the formation of a primary granuloma, the available immune responses fail to promote the killing of these newly deposited mycobacteria[38]. These studies also provide additional information on how a secondary infection may cause the disruption of the primary granuloma structure thereby affecting the metabolism of pre-existing quiescent bacilli. Therefore, if exogenous re-infection contributes to active disease, it is evident that secondary exposure induces immune responses which disrupt long term protective immunity.

Source of Th2 responses in countries endemic for TB: The majority of countries endemic for TB have obligatory neonatal \textit{Mycobacterium bovis Calmette-Guerin} (BCG) vaccination programs. Although more than 3 billion people have been vaccinated with BCG[39], protection seems to be limited against tubercular meningitis developing in infants [age lower than 24 months] and non-effective as a protective measure for the adult[40,41]. Endemic TB countries, such as, Portugal, obligatory Mantoux positivity (induration greater or equal than 5 mm) is required for school entry. Mantoux negative individuals can receive up to 4-5 BCG vaccinations in their lifetime even though multiple BCG vaccination has been associated with development of Th2 dominated responses[42-47]. In high TB burdened Malawi, environmental mycobacteria and subsequent BCG vaccination results in pro-inflammatory TNF-\(\alpha\) and IL-1\(\beta\) and anti-inflammatory IL-10 production[48]. Endemic TB countries have increased parasitic/helminth infections, may contribute to the induction of Th2 responses[49]. This is supported by studies that show that IL-4 responses are higher in TB patients of countries that are closest to the equator[40,50]. Figure 1 demonstrates the association of the Th2 responses, environmental mycobacterial load, frequency of parasitic infections and increased TB and frequency of new cases TB cases resulting from multiple exposure to \textit{Mtb}.

**Contribution of Th2 responses to development of active TB disease:** Th2 cytokine IL-4 can be produced by T cells, natural killer cells (NK), mast cells, eosinophils and basophils. Antigen presenting cells (APC), such as the macrophage, can also produce IL-4 and IL-13[51]. This involves an alternative pathway which promotes a Th2 environment. The effects of IL-4 and IL-13 promote the clearance and presentation of the antigen by the macrophage which also participates in the clearing of cellular debris during allergy and parasite-induced granuloma formation[52]. In addition, the mannose receptors on dendritic cells recognize and bind polysaccharide antigens present on the surfaces of infectious agents[52]. This cross-linking activates an anti-inflammatory response that consists of increased levels of IL-10 and reduced IL-12 production resulting in a Th2 response[52]. T cells producing IL-4 act as antagonists to Th1 type responses, mediated by CD4+, CD8+ and \(\gamma\delta\) T cell production of IFN-\(\gamma\). The presence of a Th2 response in TB has been shown to reduce anti-mycobacterial responses such as macrophage activation[53], decrease toll-like receptor 2 (TLR2) signaling[53] and reduce nitric oxide synthase (iNOS)[54].

The majority of studies evaluating existing immune responses in TB disease are derived from clinically diagnosed TB patients (active TB). Therefore, immune responses associated with initial inhalation of tubercle bacilli, development and progression of disease have remained largely unknown. The peripheral blood monocyte derived macrophage (PBMC) of TB patients from endemic countries when stimulated with \textit{Mtb} causes CD4+[27], CD8+ and \(\gamma\delta\) T cell[3,26] to produce increased levels of IL-4 compared with that of healthy donors. In addition, TB patients with advanced disease have decreased percentages of the activation marker CD25 that is present on plasma membranes of CD4+ and CD8+ T cells[41]. Decreased activation of these cells is associated with an increase in IL-4 production by CD8+ and \(\gamma\delta\) TCR+ phenotypes compared with that from TB patients presenting minimal disease[41]. Increased production of IL-4 in both CD4+ and CD8+ T cells has also been associated with an increased degree of cavitary disease[55].

Household contacts (HHC) with TB patients in countries endemic for TB, such as, Mexico[56] and the Gambia[57], have increased levels of IL-4. Studies in Portugal demonstrated that 6 out of 10 HCWs that work with TB patients under conditions that afford little

![Fig. 1: Chronological progression of events which may influence of induction of a Th2 response, such as, exogenous reinfection associated with development of TB](image-url)
protection to repeated exposure to air-borne Mtb subsequently developed active TB[3,4]. The HCWs that developed active TB showed increased levels of IL-4+ within CD8+ and γδ+ T cells[3]. HCW who did not develop active TB had a higher percentage of CD8+ T cells expressing IFN-γ+, a higher percentage of γδ+ T cells expressing IFN-γ+ and low levels of IL-4 in CD8+ and γδ+ T cells[3]. These results suggest that IFN-γ producing T cells are not involved in either the prevention or progression to active TB disease. Rather, it is the early presence of IL-4 in T cells which leads to the progression of the infection to TB disease. The immune profile associated with the 6 HCWs who progressed to active TB status was detected years prior to the onset of active disease and therefore may serve as a predictor of progression to TB disease.

The interpretation of the presence of IL-4 in TB contacts or TB patients has become more difficult with the discovery of the spliced IL-4 variant, IL-4/482. Studies by Rook et al.[57,58] have demonstrated TB patients that expressed more of the spliced IL-4 variant (IL-4/482), presented with more extensive TB lung damage. In vitro studies[41] suggest that the spliced IL-4 variant IL-4/482, may be an antagonist to IL-4 induced cell proliferation and expression of CD23. Whereas in Portugal the immune profiles of HCW exposed to Mtb showed the presence of IL-4 prior to the progression to active TB status. Therefore, immune profiles of HCW exposed to Mtb showed the presence of IL-4 which could include or exclude IL-4/482. However, this particular phenotype was confined to HCWs who subsequently developed active TB. More studies are required to define the role of the IL-4 antagonist, spliced variant IL-4/482 which may have little to do with development of active disease itself but may have more to do with the acuteness and extension of the disease process itself.

**CONCLUSION**

The majority of these countries with high Mtb burdens are poorer countries in which health care facilities lack biosafety containment due to cost. Obligatory preventive therapy for TB infection many times is not implemented in these hospital facilities. HCWs, exposed to Mtb and who subsequently developed TB demonstrated early expression of a Th1 response with concomitant presence of a Th2 responses in CD8+ and γδ TCR+ cells. These results suggest that antigen persistence from repeated Mtb exposure leads to early expression of an immune profile that consists of the presence of a declining Th1 response with concomitant expression of a Th2 response. This presence of a Th2 response results in reduced DTH Mantoux induration and lack of an early clinical diagnosis. In this early phase, failing Th1 immunity, persistence of antigen and the early expression of Th2, IL-4 leads to eventual development of TB.

**ACKNOWLEDGMENTS**

This work was supported by a grant from the European Union (IC18*CT970236), Comissão Nacional de Luta contra a SIDA (001074) and Fundação Calouste Gulbenkian project “Pesquisa de novos marcadores de imunidade celular para a detecção precoce da infecção por M. tuberculosis”.

**REFERENCES**