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Revisiting the Current Assays associated to Host Immune Responses against Tuberculosis Infection

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Mini Review

As an ancient microbe, Mycobacterium tuberculosis (MTB) is an extremely successful pathogen. MTB causes more deaths worldwide now than at any previous time in history as the World Health Organization (WHO) estimates that approximately one-third of the world's population (roughly 2 billion total) is infected with MTB [1]. MTB is a major health threat, causing 9 million new infections and between 2 and 3 million deaths annually. Future prospects look bleak due to the increasing impact of HIV and drug resistance MTB strains (MDR) on the TB epidemic [1].

The clinical course of TB provides clues as to the mechanisms that underlie MTB's success as a pathogen. First, MTB establishes infection with a small inoculum, suggesting that it inhibits innate immune responses. Second, it often persists throughout the life of the host, suggesting evasion of adaptive immunity. Third, the transmission of MTB from one host to another typically depends upon the formation of lung cavities in which aerosols are generated by coughing or sneezing. The mechanisms of lung cavitation are complex but include bystander damage of healthy tissue by the host cellular immune response. It is generally thought that granuloma formation is a means by which the host controls certain pathogens, most notably mycobacteria and fungal species [2]. Granulomas form in animal models of TB and in human infection the scarred result of the granulomatous response to initial infection is sometimes observed as a calcified lesion abutting the pleural in a lower lobe of the lung, the "Ghon lesion" [3]. Although the granuloma limits the extent of early infection, it is ultimately cell-mediated immunity involving T-cells that control MTB replication.

Protective host immune responses involve T-cells and macrophages (Mø), whereas antibodies are unimportant by comparison [4]. MHC class I pathways and $CD8^+$ T-cells appear to be important in the host containment of virulent MTB [5-7]. Previous studies demonstrated that $CD8^+$ T-cell lines were capable of inhibiting MTB growth in vitro [8]. $CD8^+$ T-cells function as cytotoxic effector cells and it has been suggested that these cells play the greatest role in lysing infected cells in lesion that still contain few bacteria and therefore sterilize the granuloma [9,10]. It has been shown that in mice, moderate levels of protection can be obtained upon transferring $CD8^+$ T-cells even at late stages of infection [9,11]. In contrast, in most cases, the protection afforded by the $CD4^+$ T-cells is much greater than that seen by $CD8^+$ T-cells [12]. $CD4^+$ T-cells, mediated by MHC class II pathways, secrete IFN-γ and activate Mø to produce reactive oxygen species (ROS) and nitric intermediates (RNI), thereby enhancing their microbicidal activity. In mice, depletion of $CD4^+$ T-cells prior to infection leads to increased bacterial burden and shortened survival [12-15]. These results are also shown in knockout models as both CD4–/– and MHC-II–/– mice are extremely susceptible to MTB infection [16]. One of the difficulties in discerning the nature of the T-cell responses that correlate with protection has been that the rodent models used to study TB do not naturally "contain" infection in a manner analogous to how humans "contain" infection. Clinical observations and studies in humans provide insight into the components of true "containment."

The development of safe and effective vaccines for both drug-sensitive MTB and MDR remains a high priority for the scientific community. For decades, a number of factors have been considered responsible for the variable efficacy of *M. bovis* BCG vaccine, the only vaccine still available against TB. These factors are related to the strain, the dose, and protocol for administer the vaccine [17]. Ideally, a vaccine to prevent TB would confer lifelong protection. Despite significant efforts over the course of several decades, several important issues related to the immune responses or epitopes recognized by MTB remain to be addressed. An NIH-sponsored workshop of leading scientists and experts held in June 2007 reviewed data on T-cell and B-cell epitopes derived from MTB, and identified several important knowledge gaps:

- Epitopes have been described for only a small fraction of the MTB genome
- more systematic data is necessary, addressing the recognition of different antigens and epitopes in different stages of MTB infection and disease, and in different ethnicities
- definition of epitopes recognized in small animals and non-human primates would also be beneficial to further evaluations of new vaccine candidates and vaccination strategies.

$CD4^+$ T-cell populations appear to be very important as HIV-infected persons with latent TB infection are at high risk of progressing to active TB, and frequently present with extrapulmonary or disseminated infection [18,19]. Furthermore, treatment of HIV-TB co-infected persons with antiretroviral therapy often causes increased clinical and radiographic evidence of TB in the lungs as the $CD4^+$ count recovers – the so-called "paradoxical reaction", or "immune-reconstitution inflammatory syndrome-IRIS" [18,20]. In summary, both HIV and TB are life-threatening pathogens in their own right, but their synergic effects on the immune system during co-infection markedly enhance their effect on the host [20-22].

In terms of assays to detect cellular immune responses in the context of TB, there are few that are clinically available. One of the main issues that exist is what specific antigens from MTB are eliciting immune responses. The tuberculin skin test (TST) does not measure antigen-specific $CD4^+$ or $CD8^+$ T-cells and often gives false results [23]. The IFN-gamma release assays-IGRA, such as Quantiferon-Gold (QFT) and T-SPOT.TB, however, are more appropriate in detecting

TB-specific responses [24]. The three antigens composing the QFT test, ESAT-6, CFP-10, and TB7.7, are represented as peptides and detected using whole blood [25]. Nevertheless, there are now more specific assays to detect immune responses after infection with MTB.

In all of these assays, a clear understanding of which antigens from MTB elicit immune responses is direly needed. Recent studies have focused on the identification of new antigens [26-31]. These studies have varied from in silico approaches, [31] to gene expression approaches [29] as well as MHC-specific methods [26,27,30]. The identification of TB-specific antigens that elicit immune responses provides the framework for new assays to assess cellular immunogenicity.

New assays to detect $CD4^+$ and $CD8^+$ T-cell responses after MTB infection includes cell specific detection systems, such as flow cytometry and enzyme based assays (such as ELISPOT) [24,32-37]. These assays will quantitatively and qualitatively measure specific $CD4^+$ and/or $CD8^+$ T-cell responses that are specific for MTB. However, albeit TB is the leading cause of death in AIDS patients worldwide, very little is known about early TB infection or MTB/HIV co-infection in infants. Thus, clinically relevant newborn animal model to study TB infection is urgently needed. An aerosol model in neonatal nonhuman primates which mimics clinical and bacteriological characteristics of MTB infection, with the potential to allow the establishment of a TB co-infection model of pediatric AIDS, as seen in human newborns/infants has been established [38]. There, post infection specific cell-mediated immune responses and lesions were detected suggestive of the classic Ghon focus in human children, as it represents the first example of early MTB infection of newborn macaques. Using ELISPOT assays, the IL-12 production correlated with early MTB infection lesions seen by routine thoracic radiographs. That study indicates a unique opportunity to further characterize immunopathogenesis and establish a MTB/SIV co-infection model for pediatric AIDS.

In sum, MTB is an extremely well adapted pathogen which has co-existed with the human host for millennia, and it has learned how to modulate potentially protective host immune responses to insure its own survival. Although there is still no correlate for protection in TB, there might be a harmonization of laboratory assays which ideally can be used to evaluate the immunogenicity, safety and parameters, such as vaccine "take" of a TB vaccine candidate. Better understanding of the human immune response to MTB infection and disease, plus the recent progress in immunology, microbiology, and molecular genetics, will provide fundamental shifts that promise to have extraordinary impact on the approaches to TB control. Ultimately, it is hoped that progress in the field of vaccinology will lead to a more rational approach towards the improvement of the BCG vaccine.

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