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Review

Role of CD8$^+$ T lymphocytes in control of *Mycobacterium tuberculosis* infection

Jeff E. Grotzke [a], David M. Lewinsohn [a,b,*]

[a] *Department of Molecular Microbiology and Immunology, Oregon Health and Science University 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA*
[b] *Division of Pulmonary and Critical Care Medicine, Oregon Health and Science University, R&D 11, Portland VA Medical Center, 3710 SW US Veterans Hospital Road, Portland, OR 97239, USA*

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Abstract

Tuberculosis remains a global health concern. Control of infection is dependent on cell-mediated immune responses, with CD4$^+$ T lymphocytes playing a central role. In this article, data supporting the importance of CD8$^+$ T lymphocytes is reviewed, with an emphasis on the unique functional roles that this lymphocyte subset may play.

Keywords: Tuberculosis; CD8-positive T-lymphocytes; Antigen presentation; Immunologic surveillance

1. Introduction

Despite widespread use of the bacillus Calmette-Guerin (BCG) vaccine and increased availability of effective drug therapy, *Mycobacterium tuberculosis* (Mtb) remains one of the most common causes of infectious disease morbidity worldwide. In 2002, the World Health Organization (WHO) estimated 8.8 million new cases, with an estimated two million tuberculosis (TB)-related deaths. At present, the WHO estimates that one third of the world's population is infected with Mtb [1].

Through the WHO-sponsored DOTS (directly observed therapy, short-course) program, effective TB therapy is now available to 69% of the world's population. However, the likelihood of DOTS therapy resulting in the eradication of TB is limited by the large reservoir of latently infected individuals as well as delays in diagnosis. Indeed, it has been estimated that very high case detection rates would be required to significantly decrease the global burden of TB. However, a vaccine of even modest efficacy when combined with existing DOTS programs offers the potential to dramatically reduce the burden of TB [2].

Control of infection with Mtb relies heavily on the cellular immune system, that is, the interaction of lymphocytes and Mtb-infected macrophages and dendritic cells (DC). As a result, new vaccines for TB will need to elicit and maintain these critical effector mechanisms. Potential Mtb vaccines can be broadly grouped into those that are prophylactic and those that are therapeutic. Prophylactic vaccines are those that could confer either sterilizing immunity or could significantly decrease the likelihood of disease following exposure to Mtb. Therapeutic vaccines are those that minimize the risk of reactivation in those already infected.

Among lymphocyte subsets, CD4$^+$ T cells play a central role in the control of Mtb growth. Additionally, a growing body of evidence derived from both human and non-human models has suggested that CD8$^+$ T lymphocytes play an essential and unique role. In this article, the literature supporting a role for CD8$^+$ T cells in response to Mtb infection will be reviewed. Additionally, the mechanisms by which CD8$^+$ T

Abbreviations: BCG, *Bacillus Calmette-Guerin*; β2 m, beta(2)-microglobulin; DC, dendritic cell; ER, endoplasmic reticulum; HLA, human leukocyte antigen; IFNγ, interferon-gamma; LTBI, latent TB infection; MHC, major histocompatibilty complex; Mtb, *Mycobacterium tuberculosis*; NOS, nitric oxide synthase; RNI, reactive nitrogen intermediates; TAP, transporter associated with antigen processing; TB, tuberculosis; TNF-α, tumor necrosis factor-alpha; TST, tuberculin skin test; WHO, World Health Organization.

* Corresponding author. Tel.: +1 503 721 1020; fax: +1 503 402 2816.
E-mail address: lewinsod@OHSU.edu (D.M. Lewinsohn).

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cells may contribute uniquely to the containment of Mtb infection in vivo will be discussed.

2. Cellular immunity to *M. tuberculosis* infection

Following exposure to Mtb, it has been estimated that 90% of those who develop evidence of a cellular immune response (+TST) remain healthy throughout their lifetime. This persistent infection has been termed latent TB infection (LTBI) and reflects successful immune-mediated containment of Mtb. As a result, a comprehensive understanding of relevant host defense mechanisms will facilitate rational vaccine design.

A vigorous Th1 response, while effective in containing the growth of Mtb, cannot eradicate the bacteria. While little is known about the bacterial burden in humans latently infected with Mtb, it is clear that the organism can persist for many years [3,4]. Furthermore, those with LTBI are characterized by the presence of high frequency Mtb-specific T cells, consistent with the hypothesis that persistent antigen is driving these responses. Hence, LTBI might be better termed persistent TB infection. In vivo, the development of a specialized immune structure, the granuloma, plays a central role in the containment of Mtb. Granulomas consist of macrophages, macrophage-derived giant cells, $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, neutrophils, and B lymphocytes in a tightly structured and hence histologically recognizable pattern. The integrated and localized response conferred by the granuloma may be central to immune containment. For example, dysregulated granuloma formation has been associated with impaired immunity to Mtb [5–7].

Available data has demonstrated that growth inhibition of Mtb requires the adaptive acquisition of an Mtb-specific, Th1-type cellular immune response. Such a response reflects the coordinated interaction with macrophages and T cells. Abundant experimental evidence supports a central role for the interaction of Th1 $CD4^+$ lymphocytes and macrophages. In this model, Mtb-derived antigens are processed and presented via macrophage-associated major histocompatibilty complex (MHC)-II. Antigen recognition by $CD4^+$ T cells then leads to the release of pro-inflammatory cytokines, which in turn limit intracellular Mtb growth. For example, both human and mouse data support an essential role for $CD4^+$ T cells and MHC-II. In the mouse model, depletion of $CD4^+$ T cells prior to infection leads to increased bacterial burden and shortened survival [8–10]. These results are mirrored in knockout models as both $CD4^{-/-}$ and MHC-$II^{-/-}$ mice are extremely susceptible to Mtb infection [11]. Similarly in humans, patients with HIV-mediated $CD4^+$ depletion have a dramatically increased incidence of TB disease [1,12].

Antigen-specific activation of $CD4^+$ T cells leads to the release of the pro-inflammatory cytokines interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α). Mice with a targeted disruption of the IFN-γ gene are highly susceptible to Mtb infection, fail to produce nitric oxide synthase (NOS), and develop a disseminated form of disease characterized by irregular granulomas and large areas of tissue necrosis [13,14]. Humans with mutations in the IFN-γ receptor exhibit increased susceptibility to mycobacterial infections [15], while exogenously administered IFN-γ can improve outcome [16–18]. Furthermore, individuals with active TB have a relative deficiency of IFN-γ producing T cells [19,20]. TNF receptor knockout or mice depleted of TNF-α by monoclonal antibodies are extremely susceptible to Mtb, showing increased bacterial loads, dysregulated granuloma formation, and shortened survival [21]. Additionally, humans receiving anti-TNF-α therapy have increased vulnerability to mycobacterial infection [7].

One important mechanism by which both IFN-γ and TNF-α exert their protective role is through the activation of macrophages. Macrophage activation plays a crucial role in combating Mtb infection and leads to the upregulation of MHC-I and -II molecules as well as the production of reactive nitrogen and oxygen species. In the mouse model, reactive nitrogen intermediates (RNI) have been demonstrated to inhibit Mtb growth both in vivo and in vitro. This is most dramatically illustrated by the inducible nitric oxide synthase (iNOS) knockout mouse, which exhibits increased susceptibility to infection with Mtb [22,23]. In humans, a direct role for RNI has been more difficult to demonstrate, although iNOS has been demonstrated within the granuloma [24].

These and other data have unambiguously established a central role for the $CD4^+$ lymphocyte. However, the acquisition and maintenence of an effective adaptive $CD8^+$ T cell response is highly dependent on the presence of $CD4^+$ cells [25–33]. Hence, it is possible that the $CD4^+$ response is necessary but not sufficient.

3. Evidence for $CD8^+$ T lymphocyte involvement in Mtb containment

The anatomy of the adaptive immune response to infection with Mtb suggests that $CD8^+$ T cells are intimately involved in the host response. Following challenge with Mtb, antigen-specific IFN-γ producing $CD8^+$ T cells traffic to the lung as soon as 2 weeks post infection [34]. Also, $CD8^+$ T cells are present within the granuloma, where they have access to and are poised to prevent dissemination of infected cells [35]. Finally, Mtb-specific $CD8^+$ T cells specific for numerous antigens can be isolated at high frequencies from humans and mouse models, consistent with the hypothesis that $CD8^+$ T lymphocytes are constantly being stimulated with antigen.

Early mouse studies looking at the importance of T cell subsets in controlling Mtb infection gave conflicting results. Several studies showed little or no effect of depletion or adoptive transfer of Mtb-specific $CD8^+$ T lymphocytes [9,10,36]. However, studies using adoptive transfer of $CD8^+$ enriched cells from Mtb-infected mice or depletion of $CD8^+$ cells showed that this subset could mediate a modest decrease in bacterial load [8,37]. In most cases, the protection afforded by the $CD4^+$ subset was much greater than that seen by $CD8^+$