MHC class I–restricted CD8+ T cells are necessary to mount an immune response against Mycobacterium tuberculosis. M. tuberculosis antigens can enter MHC class I cross-processing pathways through a number of different mechanisms, including via the uptake of antigen-containing apoptotic vesicles released by infected cells. A study in this issue of the JCI by Hinchey and colleagues shows that M. tuberculosis inhibits host cell apoptosis and thus may interfere with optimal cross-priming and action of CD8+ T cells (see the related article beginning on page 2279). M. tuberculosis genetically modified to induce apoptosis is shown to be more effective in priming CD8+ T cells in vivo and therefore may be a more effective vaccine against tuberculosis than the currently utilized M. bovis BCG vaccine.

Mycobacterium tuberculosis continues to cause widespread morbidity and mortality in children and adults worldwide, despite the availability of relatively simple diagnostic tools, inexpensive and effective drugs, and public health infrastructures in most countries for control and treatment of tuberculosis (1). In adolescents and adults, TB is primarily caused by reactivation of latent/persistent M. tuberculosis bacilli and progression to active pulmonary disease. M. bovis bacille Calmette-Guérin (BCG), widely used as TB vaccine for newborns and effective in preventing dissemination of M. tuberculosis disease in young children, is unable to prevent pulmonary (reactivation) TB in adolescents and adults (2, 3). The latter finding was reconfirmed in a recent study of BCG revaccination of more than 15,000 7- to 14-year-old school children in Brazil (4). Thus, an effective vaccine for the prevention of pulmonary TB in adolescents and adults, many of whom are latently infected with M. tuberculosis in countries in which TB is endemic, is urgently needed to control the TB pandemic.

**Macrophage apoptosis and M. tuberculosis**

During the last 20 years, great progress has been made in areas essential for new TB vaccine development, including mycobacterial genetics, TB immunology, and animal models of M. tuberculosis infection. Completion of the M. tuberculosis genome sequence combined with genetic tools to delete, add back, or complement mycobacterial genes allows one to determine the M. tuberculosis genes essential for survival in macrophages and animal models and those genes involved in resisting host immune responses (5, 6). M. tuberculosis readily infects macrophages, and macrophage apoptosis has developed as one host defense mechanism against infection. However, virulent M. tuberculosis has evolved to be capable of inhibiting macrophage apoptosis. The study by Hinchey et al. in this issue of the JCI (7) represents an elegant example of a combination of approaches from the 3 areas of research described above to determine the role of mycobacterial genes secA2 and sodA in resisting macrophage apoptosis and to determine whether enhanced apoptosis of secA2 gene–deleted M. tuberculosis (ΔsecA2) is associated with increased cross-presentation of antigens to CD8+ T cells and improved immunity against an aerosol challenge with M. tuberculosis in vivo (7). Earlier studies established that SecA2 was required for secretion of superoxide dismutase A (SodA) by M. tuberculosis and that knocking out secA resulted in a less virulent organism (8). Superoxide anions can kill mycobacteria directly and induce macrophage apoptosis. Apoptosis kills intracellular mycobacteria by a superoxide-independent mechanism. Hinchey et al. (7) now show that, in vitro, a ΔsecA2 mutant causes increased caspase expression and macrophage apoptosis compared with WT M. tuberculosis. When extracellular SodA expression was restored in the ΔsecA2 mutant by adding an N-terminal signal sequence to sodA, the level of macrophage apoptosis were reduced to that observed in response to WT M. tuberculosis. Thus a link between SecA2–dependent SodA secretion and inhibition of macrophage apoptosis was established.

**Cross-processing of M. tuberculosis for CD8+ T cells**

Adaptive immunity mediated by T cells and the cytokines they secrete is essential for controlling initial M. tuberculosis infection (usually in the lungs) and preventing reactivation of latent/persistent M. tuberculosis bacilli residing in granulomas. T cell failure induced by malnutrition, aging, HIV-1 infection, or immune-suppressive drugs allows latent infection to progress to active TB. Multiple T cell subsets are activated by M. tuberculosis antigens, including MHC class II–restricted CD4+ and MHC class I–restricted CD8+ T cells, as well as γδ TCR+ T cells, CD1–restricted T cells, CD25+CD4+
The antigen repertoire for CD8+ T cells is responsible for the long-term persistence of CD8+ T cells as measured by H-2Kb tetramer, CD44, and CD62 ligand staining during the first 1–2 months, with a suggestion of increased long-term persistence of CD8+ T cell responses in vivo. By adoptively transferring OT-I TCR-transgenic T cells, which recognize the SIINFEKL peptide of OVA presented by H-2Kb MHC class I molecules, into mice infected with mutant and WT M. tuberculosis expressing the SIINFEKL peptide (16), the authors performed a series of elegant in vivo experiments. After i.v. infection with these different M. tuberculosis strains, increased levels of SIINFEKL-specific CD8+ T cells were detected in spleens of ΔsecA2-OVA–infected mice compared with WT M. tuberculosis–infected mice. These CD8+ T cells proliferated and were cytotoxic in vivo. Subcutaneous immunization with ΔsecA2-OVA increased the number of SIINFEKL-specific CD8+ memory T cells as measured by H-2Kb tetramer, CD44, and CD62 ligand staining during the first 1–2 months, with a suggestion of increased long-term persistence of CD8+ T cell may translocate directly from phagosomes to the cytosol for processing or they may remain entirely within the vacuolar compartment. In a recently described pathway, the ER was shown to deliver protein translocation channels and peptide loading components to phagosomes. M. tuberculosis antigens then could transfer to the cytosol for proteasomal processing and peptides could be imported into phagosomes via TAP for binding to MHC class I (12).

These are at least three mechanisms through which M. tuberculosis antigens can enter these cellular cross-processing mechanisms. Via the first mechanism, antigens can directly be cross-processed by cells that have taken up M. tuberculosis bacilli (Figure 1A), as shown for human macrophages (13). Via the second mechanism, M. tuberculosis–infected cells can produce exosomes containing mycobacterial antigens, which can be taken up by bystander dendritic cells or macrophages for MHC class I cross-processing (Figure 1B) (14). Via the third mechanism, M. tuberculosis–infected cells can apoptose and release apoptotic vesicles with mycobacterial antigens for uptake by bystander APCs (Figure 1C) (15). Just which of these three mechanism(s) is operative or dominant during M. tuberculosis infection in vivo likely depends on the type and in vivo location of the APC. For vaccines, the adjuvant and/or vector used to deliver antigen will determine which mechanism will be used for CD8+ T cell priming.

In their current study, Hinchey et al. (7) sought to determine whether increased macrophage apoptosis in vitro translated into increased MHC class I–restricted CD8+ T cell responses in vivo. By adoptively transferring OT-I TCR-transgenic T cells, which recognize the SIINFEKL peptide of OVA presented by H-2Kb MHC class I molecules, into mice infected with mutant and WT M. tuberculosis–infected cells as measured by H-2Kb tetramer, CD44, and CD62 ligand staining during the first 1–2 months, with a suggestion of increased long-term persistence of CD8+ T cell...
memory in ΔsecA2-OVA– compared with M. tuberculosis H37Rv–OVA–immunized mice. Apoptosis is difficult to detect in vivo, and thus it isn’t clear whether apoptosis was responsible for the increased cross-priming of CD8+ T cells observed in vivo in ΔsecA-OVA–infected mice.

Animal models of M. tuberculosis infection

Mouse, guinea pig, and primates are the species most commonly used for experimental M. tuberculosis infection for pathogenesis and vaccine studies (17). These animals generally do not develop latent infection with reactivation TB as seen in humans, but they are useful as models of acute infection and for determining a vaccine’s immunogenicity and efficacy in reducing mycobacterial growth after vaccine’s immunogenicity and efficacy in CFU) in lungs and spleen for the two infection has allowed ready comparison of genetically manipulated mycobacteria and new TB vaccines across the world. This has resulted in rapid development of a new generation of TB vaccines or can be considered a surrogate for vaccine efficacy remains to be determined. The study by Hinchey et al. (7) increases our understanding of the role of MHC class I–restricted CD8+ T cells, the antigens they recognize, and their antigen-processing requirements in immunity against M. tuberculosis and indicates that activation of these cells is important and should be considered as new TB vaccines are designed and developed.

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Address correspondence to: W. Henry Boom, Tuberculosis Research Unit, Case Western Reserve University and University Hospitals’ Case Medical Center, 10890 Euclid Avenue, BRB 1031, Cleveland, Ohio 44106-4984, USA. Phone: (216) 368-4844; Fax: (216) 368-2034; E-mail: whb@case.edu.


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