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Commentary

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New TB vaccines: is there a requirement for CD8+ T cells?

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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 (TB) (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 disseminated *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* (Δ*secA2*) 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 Δ*secA2* mutant causes increased caspase expression and macrophage apoptosis compared with WT *M. tuberculosis*. When extracellular SodA expression was restored in the Δ*secA2* 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+

Nonstandard abbreviations used: BCG, bacille Calmette-Guérin; Δ*secA2*, *secA2* gene–deleted *Mycobacterium tuberculosis*; SodA, superoxide dismutase A; TB, tuberculosis.

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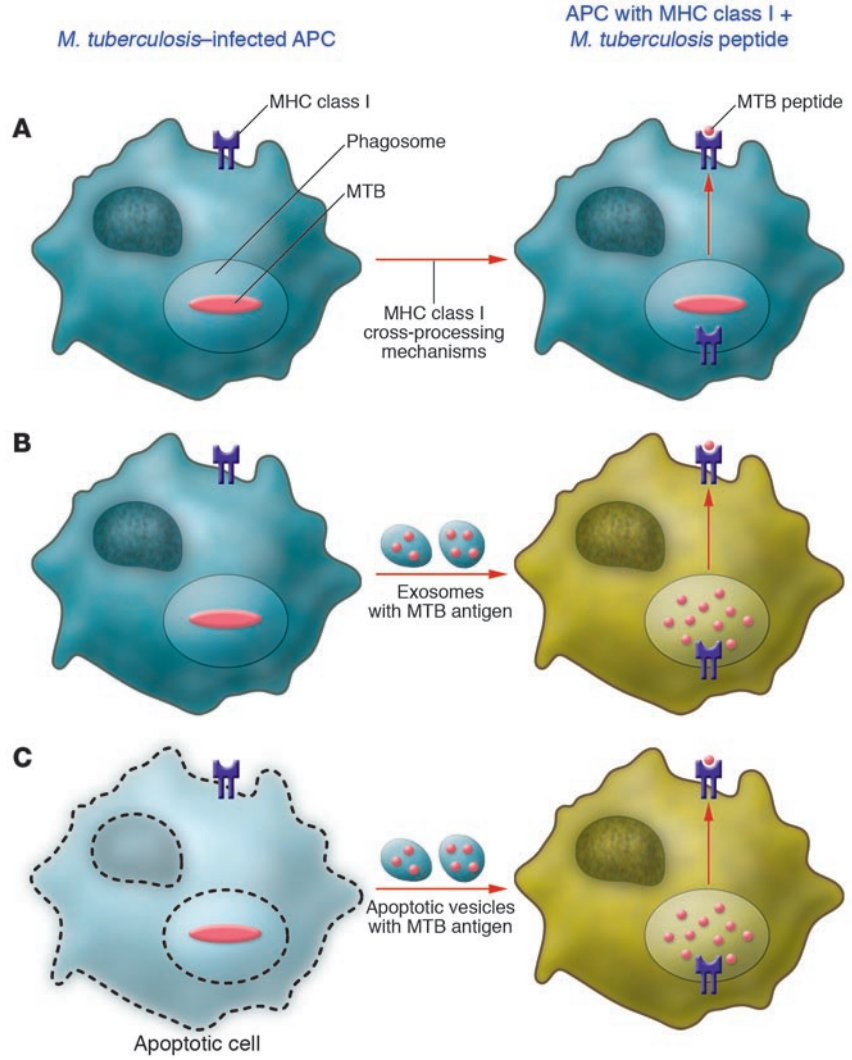

Figure 1

M. tuberculosis antigen entry into MHC class I cross-processing pathways for $CD8^+$ T cell activation. (**A**) Direct MHC class I cross-processing of *M. tuberculosis* antigens in an infected APC. (**B**) Cross-processing by a bystander APC of *M. tuberculosis* antigen–containing exosomes extruded by infected cells. (**C**) Cross-processing by a bystander APC of *M. tuberculosis* antigen–containing apoptotic vesicles. MTB, *M. tuberculosis*.

Tregs, and others (9). $CD4^+$ and $CD8^+$ T cells are essential for protective immunity to *M. tuberculosis* and thus a major focus for vaccine development (10). It is speculated, but not proven, that an inability to adequately prime $CD8^+$ T cells is responsible for the failure of *M. bovis* BCG to adequately protect against TB and that optimal activation of both $CD8^+$ and $CD4^+$ T cells is necessary for developing an improved TB vaccine.

The antigen repertoire for $CD8^+$ T cells and the processing mechanisms of *M. tuberculosis* antigens for MHC class I presentation by dendritic cells and macrophages remain poorly defined. Conventional MHC class I antigen processing requires de novo synthesized antigens (e.g., viral proteins) in the cytosol for proteolysis by proteasomes and transport of peptides into the ER by a transporter associated with antigen processing (TAP) molecule for loading onto MHC class I. An alternative mechanism allows processing of exogenous (i.e., those taken up by phagocytosis) or vacuolar antigens (i.e., those from *M. tuberculosis* bacilli in phagosomes) for presentation by MHC class I molecules to $CD8^+$ T cells (11, 12). This alternative form of MHC class I antigen processing is called cross-processing and is responsible for in vivo cross-priming. Cross-processing of *M. tuberculosis* antigens can occur through a number of distinct mechanisms: Antigens 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-$2K^b$ 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 Δ*secA2*-OVA–infected mice compared with WT *M. tuberculosis*–infected mice. These $CD8^+$ T cells proliferated and were cytotoxic in vivo. Subcutaneous immunization with Δ*secA2*-OVA increased the number of SIINFEKL-specific $CD8^+$ memory T cells as measured by H-$2K^b$ 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 Δ*secA2*-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 Δ*secA*-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 an aerosol challenge. For animal studies, vaccination with *M. bovis* BCG remains the gold standard against which all other vaccines need to be compared. Establishing the superiority of a new TB vaccine over *M. bovis* BCG in these animal models is difficult, as demonstrated by the study by Hinchey et al. (7). Modest differences in mycobacterial CFU in the lungs after 1 month between Δ*secA*- and *M. bovis* BCG–vaccinated mice translated into significant differences in survival. In guinea pigs, vaccination with *M. bovis* BCG or Δ*secA* yielded similar levels of protection (decrease in CFU) in lungs and spleen for the two vaccines. Vaccination with Δ*secA* reduced CFU and pathology in mediastinal lymph nodes, suggesting that Δ*secA* might be better at limiting pulmonary pathology and bacterial dissemination.

New TB vaccines

Progress in standardizing animal models of *M. tuberculosis* infection has allowed ready comparison of genetically manipulated mycobacteria and new TB vaccines across studies and research centers around the world. This has resulted in rapid development of a new generation of TB vaccines using four general approaches (reviewed in ref. 18): (a) developing subunit vaccines of fused *M. tuberculosis* proteins (72F and ESAT6-85B, both fusions of 2 proteins) with novel adjuvants (19, 20); (b) developing heterologous vectors such as modified vaccinia Ankara (MVA) or adenovirus expressing *M. tuberculosis* proteins (21); (c) improving the efficacy of *M. bovis* BCG by overexpressing *M. tuberculosis* proteins or heterologous proteins such as listeriolysin (22, 23); and (d) attenuating *M. tuberculosis* by removing virulence genes such as *secA* (18). A number of these new TB vaccines are beyond preclinical testing and in phase I and II clinical trials in uninfected and latently *M. tuberculosis*–infected tuberculin skin test–positive (TST^+) healthy volunteers. The challenge will be to select vaccine(s) for phase III trials that will require 10,000 or more participants in TB-endemic settings. There are no surrogate markers for protection against progression of *M. tuberculosis* infection and development of TB to use in phase II studies to triage vaccine candidates. Whether enhanced cross-priming of MHC class I–restricted $CD8^+$ T cells is a requirement for new 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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