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Lipids, apoptosis, and cross-presentation: links in the chain of host defense against *Mycobacterium tuberculosis*

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Abstract

Eicosanoids regulate whether human and murine macrophages infected with *Mycobacterium tuberculosis* die by apoptosis or necrosis. The death modality is important since apoptosis is associated with diminished pathogen viability and should be viewed as a form of innate immunity. Apoptotic vesicles derived from infected macrophages are also an important source of bacterial antigens that can be acquired by dendritic cells to prime antigen-specific T cells. This review integrates in vitro and in vivo data on how apoptosis of infected macrophages is linked to development of T cell immunity against *M. tuberculosis*.

Introduction.

Despite the worldwide application of BCG-vaccination and other interventions, *Mycobacterium tuberculosis* (Mtb) remains one of the most successful human pathogens. Eight to ten million new cases of active tuberculosis occur each year due in large part to the large reservoir of asymptomatic people chronically infected with Mtb. A vaccine that prevents pulmonary tuberculosis, the primary contagious form of the disease, could have a major impact on containment of the global epidemic. However, all vaccines that have been developed to date, lead to improved containment of the Mtb, but do not lead to sterilizing immunity. A better understanding of the strategies that Mtb uses to evade host immunity may lead to the development of a more effective vaccine. As an intracellular pathogen that resides primarily in the phagosomal compartment of infected macrophages, Mtb avoids detection by the humoral immune system. Furthermore, despite eliciting a T cell response, Mtb is able to evade many of the anti-bacterial mechanisms mediated by cellular immunity. One idea that several vaccine strategies are trying to exploit is inducing a stronger Mtb-specific CD8+ T cell response. CD8+ T cells play an important immunological role in defending the host against virulent Mtb infection (reviewed in [1]). In support of these objectives, a detailed understanding of how CD8+ T cells are activated during Mtb infection is required. Here we will consider different ideas about how Mtb-specific CD8+ T cells are primed following infection.

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Priming of CD8+ T cells.

CD8+ T cells recognize short peptide antigens that are presented by the class I MHC antigen presentation pathway [2]. The class I MHC pathway, which is expressed by nearly every cell type, samples the cytosolic environment (reviewed in [3]). Consequently, CD8+ T cells detect cells with a perturbation of their intracellular environment. As such, it is no surprise that CD8+ T cells play a crucial role in immunity to many different intracellular pathogens. For example, viral proteins synthesized in the cytosol of infected cells are sampled and presented by the class I MHC pathway. Peptides in the cytosol of the cell are transported into endoplasmic reticulum (ER) through the action of the transporter associated with antigen processing (TAP) and tapasin. Once in the ER, peptides are trimmed and form a trimeric complex with the MHC I heavy chain and $\beta 2$ microglobulin. This peptide-loaded class I MHC complex is transported to the cell surface. Antigen-specific CD8+ T cells are able to identify infected cells by recognizing viral peptide epitopes bound to class I MHC. However, recognition of virally infected cells by CD8+ T cells only leads to CD8+ T cell activation if they have been **previously** primed. T cell priming refers to the initial activation of naïve T cells (see glossary), which occurs when a T cell recognizes its cognate antigen presented by dendritic cells (DC), an event that usually occurs in the lymph node (LN).

The plasticity of macrophages and DC, their overlapping features and functions, and inability to clearly distinguish between the two cell types, presents problems in studying these two cell types [4-7], both of which are important for immunity to tuberculosis. DC are generally acknowledged to be crucial for the priming of T cells because, in contrast to parenchymal cells, they efficiently pick up antigen in the periphery – at sites of infection or inflammation – and traffic to the LN, where T cell priming occurs. Despite the semantics difficulties, for the discussion that follows we refer to DC as CD11c+ cells that traffic to LN and prime naïve T cells, although we acknowledge this function may not be limited to DC.

In the draining LN, thousands of naïve T cells transiently interact with the DC. If a naïve T cell's TCR recognizes a peptide antigen presented by the DC, a high affinity and longer lasting interaction occurs [8, 9]. During such a cognate interaction, the accessory and costimulatory molecules expressed by the DC bind their counter-receptors on the T cell forming an immune synapse. The end result of this interaction is activation of the naïve T cell. An important consequence of priming is that the activation threshold of the T cell is lowered so it is more easily activated during its next encounter with antigen. Once primed, activated CD8+ T cells proliferate, exit the LN and home to sites of inflammation.

DC cross-presentation of intracellular cytosolic antigens.

How DC acquire intracellular cytosolic antigens is a particularly important question. For pathogens that directly infect DC, the cytosolic production of pathogen-encoded proteins is sufficient. However, a problem arises for pathogens that don't infect DC. For example, certain viruses are **tropic** for certain cell types, such as epithelial cell, and do not infect DC. If only professional APC such as DC can prime T cells, how is the antigen transferred from an infected epithelial cell to a DC? Cellular mechanisms exist that facilitate the transfer of antigen from infected cells to DC. Uninfected DC can acquire viral antigens when lysis of infected cells releases viral proteins that can be taken up via endocytosis [10]. Alternately, DC can engulf infected cells that are dying. However, uptake of soluble antigen or dying cells results in the transfer of cytosolic antigen into the exogenous antigen-presenting pathway of APC. A second step is required for the transfer of antigen from the endocytic compartment into the cytosolic class I MHC antigen presentation pathway. This later step is referred to cross-presentation [11, 12]. Cross-presentation of antigen resulting in the activation, or priming, of naïve T cells is called cross-priming (see glossary) [13-16].

One way that DC can acquire intracellular viral or bacterial antigens is through the uptake of apoptotic vesicles derived from infected macrophages [17]. Albert et al showed that apoptosis but not necrosis of influenza-infected monocytes conferred upon uninfected DC the ability to prime human CTL in vitro [17]. Similarly, Yrlid et al found that virulent *Salmonella*, an intracellular bacterial pathogen, induces apoptosis of infected macrophages in a manner that is dependent upon its type II protein secretion system [9, 18]. DC are able to cross-present the bacterial antigens contained in the apoptotic macrophages via the class I MHC pathway. While neither of these studies show that these events occur in vivo, they provide important evidence that microbial antigens contained in apoptotic infected cells can be cross-presented by DC and may be important for priming of CD8+ T cell responses.

Priming of Mtb-specific CD8+ T cells.

Significant progress has been made in elucidating the events leading to priming of Mtb-specific CD8+ T cells. As Mtb is an intracellular pathogen that survives primarily in macrophage phagosomes, one envisions two possible mechanisms leading to the presentation of mycobacterial antigens to naïve CD8+ T cells (Figure 1). In the first scenario, infected DC in the lung traffic to the LN where they directly prime CD8+ T cells. The second possibility is that uninfected DC acquire mycobacterial antigens from dying cells and then migrate to the LN where they cross-present the antigens to CD8+ T cells. In both cases, the mycobacterial antigens still need to be transferred from the phagosomal compartment into the cytosolic compartment, which is sampled by the class I MHC pathway.

Direct infection of DC by Mtb.

Macrophages can present viral antigens to T cells [19], indicating that they are competent at cross-presentation. However, whether they are competent to cross-prime T cells, a function that is considered specific to DC, is unknown. Early data suggested that mycobacterial antigens from infected human macrophages were not transferred to uninfected DC, raising the possibility that priming of naïve T cells requires Mtb infected DC [20]. Furthermore, Mtb efficiently infects murine and human DC in vitro [21-23]. When DC infected with BCG in vitro are transferred into mice T cell priming occurs in vivo, suggesting that infected DC can directly prime T cells [21, 24]. How antigen is transferred from the phagosomal compartment to the class I MHC pathway in Mtb infected DC is unknown; however, two mechanisms have recently been described. First, is the evidence that Mtb translocates from the phagosome to the cytosol in both human macrophages and DC [25]. In this case, the class I MHC pathway could directly sample bacterial products. An argument against this process as the primary means of class I MHC presentation is the late kinetics of translocation (days) compared to presentation of antigens (hours) [26, 27]. In addition, translocation is dependent on the bacterial ESX-1 type VII secretion system; however, priming of Mtb-specific CD8+ T cells can occur independently of ESX-1 [28]. Secondly, increasing evidence from several labs indicates that ESX-1 can mediate membrane damage. It is possible, that ESX-1 or related proteins, damages the phagosomal membrane allowing bacterial proteins to leak into the cytosol of the infected macrophage [29]. Both of these mechanisms would be consistent with the experimental finding that proteins secreted by Mtb are over-represented as antigens recognized by CD8+ T cells [1].

Whether Mtb infects DC in vivo is less clear. High dose BCG administered intravenously leads to the early infection of splenic DC (defined by their CD11c expression), although they represent only a small proportion of the total cells infected [30]. However, in contrast to macrophages, Mtb-infected DC express costimulatory molecules, a sign of activation and an indication that they are competent to prime T cells. Furthermore, using the CD11c/DTR tg

mouse model, DC have been shown to be required for priming of Mtb specific T cells [31]. In this model, the expression of the simian diphtheria toxin receptor is under the control of the CD11c promoter [32]. Administration of diphtheria toxin leads to the transient deletion of DC – transient because the progenitors are unaffected. DT-induced DC deletion, even though transient, significantly delayed initiation of the ESAT6-specific CD4+ T cell response and impaired control of Mtb infection in the lung [31]. This study only addressed priming of CD4+ T cells; the role of DC in the priming of CD8+ T cells may differ. More importantly, while these data indicate that DC are required for T cell priming during Mtb infection, they do not discriminate between a requirement for directly infected DC vs. acquisition of mycobacterial antigen by uninfected DC.

Role of DC in vivo.

Priming of the T cell response in vivo can be tracked by measuring the initiation of T cell immunity to Mtb. Following aerosol infection of C57BL/6 mice with virulent Mtb, T cell immunity was first initiated in the draining LN [33]. Curiously, bacterial dissemination to the draining LN was always detected prior (day 8-9 post-infection) to detection of T cell priming in the LN (day 11-12 post-infection). The correlation between dissemination of viable bacteria to the LN and initiation of T cell immunity suggests that infected cells act as a Trojan horse. As the infected cells traffic from the lung via the lymphatics to the draining LN, they inadvertently lead to the dissemination of the bacteria. This data also suggest that DC, the major cell type capable of trafficking from the lung to the LN, is directly infected and is both aiding the spread of infection and priming T cells.

The kinetics of dissemination and initiation of the immune response has subsequently been confirmed by other labs using more sensitive and sophisticated approaches [34-36]. A major advance in understanding dissemination of Mtb from the lung came from the lab of Dr. Joel Ernst. Using GFP-labeled bacteria, Wolf et al showed that by two weeks after infection, the infected cells in the lung represented diverse cell types including neutrophils, macrophages, and DC. However, the major cell type infected with Mtb were CD11c^{high}CD11b^{high} cells consistent with myeloid DCs. Infected DC of a similar phenotype were detected in the LN, suggesting these cells trafficked from the lung. Unfortunately, the low frequency of infected cells prior to day 14 makes it difficult to determine the identity of the infected cells that traffic to the lung and primes the T cell response between days 8-12. However, it is likely to be a DC.

Mtb, as a successful pathogen, has developed many ways to evade, or inhibit, immunity. Given the important role of DC in T cell priming and transporting Mtb from the lung to the LN, it is not surprising that Mtb interrupts this process by impairing activation of DC [37] and their subsequent trafficking to the LN. Myeloid DC obtained from the lung or draining LN of infected mice were inefficient at stimulating T cells and it appears that infection with virulent Mtb inhibits antigen presentation independently of any effect on MHC II levels [38]. This raises the possibility that even if infected myeloid DC transport bacteria to the LN, they may not be competent to prime T cells. An alternate possibility is that antigen is transferred from infected DC trafficking to the LN, to other uninfected DC that can efficiently prime naïve T cells.

Cross-presentation of mycobacterial antigens.

As discussed above, an alternate possibility is that phagocytosis of dying infected macrophages leads to acquisition of bacterial antigens by DC, as has been shown for influenza and Listeria [17, 18]. The relevance of these processes to mycobacterial antigen presentation was first investigated by Schaible et al [39]. Extracellular vesicles derived from infected DC and macrophages were identified that were free of viable bacteria but contained

mycobacterial lipids and proteins. The origin of these vesicles was not entirely clear, but they appear to be apoptotic blebs or possibly exosomes. While infected macrophages were able to directly stimulate CD8⁺ T cells, their coculture with uninfected DC led to the transfer of mycobacterial antigens to DC, which became competent to cross-present the antigens to CD8⁺ and CD1b-restricted T cells. Presentation was TAP-1-dependent and required an intact class I MHC pathway. Thus, the antigenic cargo contained in these vesicles could be cross-presented by DC to CD8⁺ T cells. As these studies were done with previously activated T cells, the observed T cell activation was not true cross-priming but would be more accurately categorized as cross-presentation. Nevertheless, the uptake of antigen-containing vesicles by DC provides a mechanism by which uninfected DC can acquire Mtb antigens and prime naïve T cells.

Winau et al used similar vesicles purified from BCG infected murine macrophages to immunize mice [40]. Again, the purified apoptotic bodies contained bacterial antigens but no bacteria. CD8⁺ T cell priming was observed and required an intact class I MHC pathway. Successful T cell priming was associated with DC homing to the tissue sites where the purified vesicles were injected. Interestingly, initiation of the endosomal processing pathway abrogated CD8⁺ T cell priming— a feature that may be unique to cross-presentation of class I MHC-restricted peptides. The generation of CD8⁺ T cell responses in naïve mice indicates that true cross-priming occurred. Remarkably, not only did a CD8⁺ T cell response develop, but also vaccination with the vesicles generated immunity that protected mice against challenge with virulent Mtb.

The studies by Schaible and Winau provide the foundation for the “Detour Model” [41]. They convincingly show that the mycobacterial antigens contained in purified vesicles are taken up by both human and murine DC and enter the class I MHC pathway. However, these studies stop short of ascertaining whether apoptosis of infected macrophage is required for the transfer of antigens to DC, whether this process occurs *in vivo* and if so, whether its occurrence is of physiological significance. The apoptotic vesicles used in the studies by Winau et al and Schaible et al were derived from BCG infected macrophages [39, 41] and it isn't clear whether infection of macrophage with wild-type virulent Mtb would lead to apoptosis and enhanced T cell immunity, or whether virulent Mtb can inhibit antigen presentation or delay initiation of adaptive immunity. Finally, while immunization with purified vesicles cross-primed antigen-specific T cells, it is uncertain whether the generation of vesicles from infected macrophages is required for CD8⁺ T cell priming *in vivo*. As discussed above, the finding that Mtb infected DC traffic from the lung to the regional LN with kinetics mirroring T cell priming could be consistent with Mtb infected DC directly priming Mtb-specific T cells and could indicate the existence of a priming pathway independent of the “Detour Pathway” [41]. Thus, the role of apoptosis and cross-priming in the generation of adaptive immunity during virulent Mtb infection remained an important unanswered question. To confirm the existence of these pathways and to begin to elucidate their relevance, a better understanding of the host factors regulating cell death during Mtb infection was required.

The role of apoptosis in cross-priming.

Eicosanoids have been identified as important host lipid mediators that regulate inflammation and susceptibility following mycobacterial infection in fish, rodents, and man [42-45]. One effect of eicosanoids is the regulation of cell death in both human and murine macrophages infected with Mtb [45, 46]. Prostanoids such as the host lipid mediator PGE₂ induce plasma membrane repair and prevent mitochondrial damage; together these events protect infected macrophages against necrosis and instead promote apoptosis. Importantly, products of the 5-lipoxygenase (5-LO) enzyme including lipoxin A4 (LXA4) are produced

by macrophages after infection with virulent Mtb. LXA4 inhibits cyclo-oxygenase-2 production, which shuts down the synthesis of prostaglandins. Mouse mutants lacking these various enzymes exist. As predicted, the macrophages from mice that lack 5-LO, produce prostaglandins even after infection with virulent Mtb, and undergo more apoptosis than necrosis. Interestingly, 5-LO^{-/-} mice are more resistant to Mtb. Studies from Bafica et al found that a more pronounced Th1 cytokine response is detected in the lungs of infected 5-LO^{-/-} mice compared to WT **control** mice [43].

In order to determine whether apoptotic macrophages contribute to adaptive immunity, Divangahi et al established a novel adoptive transfer model in which macrophages from wild-type or knockout mice were infected *in vitro* with Mtb and then transferred by intra-tracheal instillation into normal recipient mice (Figure 2). This strategy was used to determine whether the macrophage genotype influences the T cell response and control of infection [44]. By using knockout macrophages that are prone to either apoptosis (e.g., 5-LO^{-/-}) or necrosis (e.g., mPGES^{-/-}) following infection, Divangahi et al determined how these two different cellular fates alter the course of infection *in vivo*. One advantage of this adoptive transfer infection model is that the development of tuberculosis occurs in a developmentally normal host with an intact immune system, which avoids the pitfalls of studying Mtb infection in knockout mice in which the genetic lesion affects multiple cell types and physiological processes.

Divangahi et al used the CD8+ T cell response to TB10.4, a mycobacterial antigen that elicits an immunodominant response following low dose aerosol Mtb infection, to track the CD8+ T cell response following intra-tracheal transfer of Mtb-infected macrophages [44]. An earlier TB10.4-specific CD8+ T cell response was detected both in the draining pulmonary LN and in the lung following transfer of pro-apoptotic macrophages compared to wild-type macrophages. Importantly, the cellular fate of the infected macrophages was crucial; pre-treatment of the pro-apoptotic macrophages with inhibitors of caspase-8 and caspase-9, which prevented apoptosis of the infected macrophages, abrogated the enhancement of the CD8+ T cell response.

To determine how the Mtb infected macrophages enhanced the CD8+ T cell response, the infected macrophage adoptive transfer model was adapted for use with OT-I TCR-transgenic mice, so early events in T cell priming could be easily assessed. Similar to the intra-tracheal adoptive transfer of Mtb-infected macrophage, OT-I CD8+ T cell priming was detected earlier after the transfer of Mtb-infected OVA-pulsed 5-LO^{-/-} macrophages compared to wild-type macrophages. Importantly, the infected macrophage did not directly activate CD8+ T cells; instead, T cell priming required endogenous DC, since DC depletion abrogated OT-I CD8+ T cell expansion. Similar to the results of Winau et al [40], CD8+ T cell priming required TAP-1 and an intact class I MHC pathway. These experiments show that CD8+ T cell priming requires cross-presentation of antigen acquired by DC from apoptotic Mtb-infected macrophage via the detour pathway.

Harnessing the power of death.

The finding that apoptosis enhances T cells cross-priming naturally leads to the proposition of whether it is possible to enhance vaccine-induced immunity by using pro-apoptotic vaccines. Although it may be difficult (although not impossible) to enhance apoptosis by manipulating host pathways, considerable progress has been made in manipulating mycobacterial strains. For example, the *secA2* mutant of Mtb is unable to secrete certain bacterial proteins including SodA. Instead of inducing necrosis, this mutant primarily induces apoptosis of infected macrophage *in vitro* and *in vivo* [47]. *In vivo* infection of mice with this mutant induces a more robust CD8+ T cell response than the parental WT strain.

This was manifested as greater proliferation, greater CTL activity, and more IFN γ production. Importantly, after in vivo infection, an enhanced antigen-specific CD8 $^{+}$ T cell response is elicited, presumably a consequence of increased cross-priming. This indicates that there is a relationship between the induction of apoptosis and better induction of CD8 $^{+}$ T cell immunity and importantly, better protection. The identification of other mycobacterial genes that inhibit macrophage apoptosis or induce macrophage necrosis may also be amenable to deletion. Construction of such pro-apoptotic mutant could be constructed in Mtb or in BCG [47-50]. Another possibility is to add to the vaccine a pharmacological agent capable of inducing host cell apoptosis. Of course one of the critical issues is whether a pro-apoptotic vaccine will give rise to a quantitatively (e.g., increased frequency of memory T cells) or qualitatively (e.g., eliciting different T cell subsets or T cells with different functions) better response.

What is the nature of the vesicle derived from the infected macrophage that transfers bacterial antigens to DC?

While the DC acquisition of antigen via phagocytosis of apoptotic cells or blebs is well described under a variety of conditions, it is not the only way that microbial antigens could be transferred between infected macrophages and DC. Necrotic cells can be immunostimulatory and DC uptake of necrotic debris can be cross-presented to T cells [51]. However, Divangahi observed that the intra-tracheal transfer of infected mPGES $^{-/-}$ macrophages, which are more prone to necrosis, elicited a T cell response that was similar in kinetics and magnitude to WT macrophages. A second possibility is that DC take up exosomes. Exosomes are derived from multivesicular bodies and are released by diverse cell types including macrophages. Exosomes isolated from the culture supernatant of infected macrophages have a diameter of 50-100 nm, express LAMP1, CD86 and MHC II, and contain bacterial products such as LAM and the 19 kD lipoprotein [52]. Interestingly, exosomes can stimulate a proinflammatory response both in vitro and in vivo. Furthermore, using purified exosomes, Giri et al has shown that exosomes can stimulate T cells, but that this requires APC. Thus, this is reminiscent of cross-presentation. Could the cross-presentation of mycobacterial antigens result from the uptake of exosomes in addition to, or instead of, apoptotic bodies? First, the vesicles isolated by Winau et al are similar in appearance and only slightly larger (200-500 nm) than exosomes (see Figure 1; [52]). Second, the purification scheme had some similarities (differential centrifugation with a final ultracentrifugation of 100,000 g). One of the distinguishing features of exosomes and apoptotic bodies is that exosome production is independent of caspase inhibition [52]. Treatment of the infected macrophages with a caspase inhibitor eliminated uptake of the vesicles by DC suggesting that the vesicles were indeed apoptotic bodies and not exosomes [39]. Finally, as discussed above, treatment of the infected macrophages with caspase-8 and -9 inhibitors abrogated the early CD8 $^{+}$ T cell response, indicating that cross-presentation required DC uptake of apoptotic macrophages [44].

Does cross-presentation enhance CD4 $^{+}$ T cell responses?

Mtb-specific CD8 $^{+}$ T cell responses are enhanced under conditions that favor or mimic apoptosis of Mtb infected macrophages. As discussed above, our understanding is that this results from cross-presentation of the antigenic cargo contained in the dying cells. This pathway is one way that “endosomal” bacterial antigens can be presented by the class I MHC pathway. Whether uptake of apoptotic bodies or cells by DC leads to any alterations in the CD4 $^{+}$ T cell response is less clear. The Mtb-specific CD4 $^{+}$ T cell response following infection with the pro-apoptotic secA2 mutant was unchanged compared to WT Mtb [47]. However, vaccination with vesicles from BCG-OVA infected macrophages generated both an OVA-specific CD8 $^{+}$ and CD4 $^{+}$ T cell response [40]. After the transfer of Mtb-infected

pro-apoptotic *Alox5*^{-/-} macrophages, not only was there an earlier and more robust Mtb-specific CD8+ T cell response, but the CD4+ T cell response to ESAT6 and Ag85B was also enhanced [44]. This may not be too surprising if DC phagocytosis of apoptotic vesicles transfers their cargo of Mtb antigens to the endocytic system, which intersects with the MHC II processing pathway. However, the mechanisms that govern this potential transfer have yet to be elucidated. Thus, while apoptosis has been directly linked to increased CD8+ T cell responses via cross-presentation, it also enhances class II MHC-restricted antigen presentation. This has important implications for the finding that vaccination with apoptosis-inducing bacterial vaccines or apoptotic vesicles induces protection against virulent Mtb: namely, the protective immunity elicited may be due to a combination of Mtb-specific CD4+ and CD8+ T cells.

Conclusion.

While many intracellular bacterial pathogens kill the infected cell, during the past decade it has become clear that the struggle between the host cell and the pathogen is not only about life and death, but if death occurs, how it is executed. Although there are no general rules, for the case of Mtb, it is now clear that apoptosis is an innate defense mechanism that the host employs to limit the infection; conversely, virulent Mtb subverts host pathways to inhibit apoptosis and instead induce necrosis, which allows the bacterium to evade host immunity and to infect other cells. The regulation of the death modality is complex and we are beginning to appreciate that in a wild type host infected with virulent Mtb, there is a mixture of apoptosis and necrosis, depending on the microenvironment of the infected cell.

In addition to being an innate host defense mechanism, apoptotic infected cells also provide a link to the initiation of acquired immunity. This can occur when DC take up apoptotic vesicles containing mycobacterial antigens. By providing professional APC with bacterial antigens in a concentrated form, the process of apoptosis can enhance priming of T cells. This is particularly true for CD8+ T cells. Cross-presentation provides a mechanism by which uninfected DC can prime CD8+ T cells. It is also clear that when DC acquire Mtb antigens from apoptotic vesicles, they not only are able to cross-prime CD8+ T cells, but they can also efficiently prime Mtb-specific CD4+ T cells. While it is possible that infected DC can also prime CD8+ T cells, more data suggests that Mtb infection may impair the ability of DC to mature, traffic, and present antigen, which would have the net effect of impairing the efficacy of T cell priming. Similarly, the inhibition of apoptosis and promotion of necrosis by Mtb impairs presentation of bacterial antigens acquired by DC via the “Detour Pathway,” and may represent an additional layer of immune evasion mediated by Mtb.

This improved mechanistic understanding of how Mtb inhibits apoptosis to evade innate and adaptive immunity provides new strategies for optimizing vaccination. The idea of manipulating the host or bacterial pathways to create vaccines that induce more apoptosis, so called “pro-apoptotic” vaccines, may be a way to generate a more effective polyclonal immune response without having to resort to subunit or killed vaccines.

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Figure 1. Priming of naïve Mtb-specific T cells by DC.

Three models of how DC could acquire mycobacterial antigens during Mtb infection of the lung and prime naïve Mtb-specific T cells in the draining lymph nodes (LN) of the infected host. In the first model (A), infected macrophages in the lung undergoing apoptosis would generate apoptotic vesicles containing mycobacterial antigens. Pulmonary DC could take up these blebs and vesicles and migrate to the draining LN. Once there, the DC would be competent to prime naïve T cells. Antigen in the endocytic pathway could enter the class II MHC pathway leading to priming of CD4+ T cells. Cross-presentation of these antigens by the class I MHC pathway could prime CD8+ T cells. Alternately (B), infected DC could migrate from the lung to the draining LN, where the infected DC could directly prime CD4+ and CD8+ T cells. In the third model, Mtb infected DC may also undergo apoptosis in the lung. Analogous to (A), uninfected DC could engulf the apoptotic vesicles derived from infected DC, migrate to the LN and prime naïve T cells. Not shown here is another permutation in which infected DC or macrophages (B) could migrate to the draining LN, undergo apoptosis, and the mycobacterial antigen laden apoptotic vesicles taken up by LN resident DC. The antigens acquired in this manner by the LN-resident DC could then lead to the priming of naïve T cells.

Figure 2. Infected Macrophage Adoptive Transfer Model.

Macrophages are briefly infected *in vitro* with Mtb, washed and instilled into the lungs of recipient mice. While in the lung the transferred macrophage may undergo Mtb induced cell death. For example, macrophages lacking the enzyme 5-lipoxygenase (5-LO) are prone to die by apoptosis after infection with virulent Mtb. During apoptosis the plasma membrane remains intact as the nucleus fragments and cellular components along with bacterial debris and Mtb-containing phagosomes are contained in apoptotic blebs and vesicles. Apoptosis of infected macrophages is correlated with innate control of bacterial replication although the mechanism remains unclear. In addition, DC that engulf apoptotic vesicles that contain Mtb or Mtb products can cross-present the bacterial antigens to CD8⁺ T cells. In contrast, necrosis, which is characterized by the disintegration of the plasma membrane, swelling of the cell and organelle shrinkage and rupture, allows for the bacteria to escape into the extracellular space and infect surrounding macrophages. Mtb-infected macrophages that lack the enzyme prostaglandin E synthase (PGES) are prone to necrosis.

Table 1
Definitions

Priming	T cell priming refers to the initial activation of naïve T cells, which occurs when a T cell recognizes its cognate antigen presented by dendritic cells (DC). This event usually occurs in the lymph node (LN) and requires costimulatory and cytokine signals.
Presentation	Antigen presentation refers to the expression of antigen bound to an antigen-presenting molecule (for example peptides bound to class I or class II MHC molecules) on the cell surface. A T cell that produces a TCR specific for the antigen in the context of the antigen-presenting molecule can recognize the cell. Presentation does not presume whether the T cell has been previously activated (primed) or not.
Cross-priming	Cross-presentation of antigen to naïve T cells resulting in their activation, or priming, is called cross-priming
Cross-presentation	Class I MHC-restricted presentation of exogenous antigens that enter the endocytic compartment (e.g., by phagocytosis) to CD8+ T cells, which ordinarily recognize antigens in the cytosolic compartment.
Detour model	Process by which intracellular bacterial antigens contained in apoptotic blebs and vesicles are taken up by DC and cross-presented to CD8+ T cells.
Exosomes	Small microvesicles (50-100 nm in diameter) that are released from late endosomal compartments of a variety of cell types including macrophages. Exosomes isolated from the culture supernatant of infected macrophages express LAMP1, CD86 and MHC II, and contain bacterial products such as LAM and the 19 kD lipoprotein [52].