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CD8 T cells and *Mycobacterium tuberculosis* infection

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Abstract

Tuberculosis is primarily a respiratory disease that is caused by *Mycobacterium tuberculosis*. *M. tuberculosis* can persist and replicate in macrophages in vivo, usually in organized cellular structures called granulomas. There is substantial evidence for the importance of CD4 T cells in control of tuberculosis, but the evidence for a requirement for CD8 T cells in this infection has not been proven in humans. However, animal model data support a non-redundant role for CD8 T cells in control of *M. tuberculosis* infection, and in humans, infection with this pathogen leads to generation of specific CD8 T cell responses. These responses include classical (MHC Class I restricted) and non-classical CD8 T cells. Here, we discuss the potential roles of CD8 T cells in defense against tuberculosis, and our current understanding of the wide range of CD8 T cell types seen in *M. tuberculosis* infection.

Mycobacterium tuberculosis

Tuberculosis remains a major cause of morbidity and mortality worldwide, and is responsible for 9 million new cases and 1.5 million deaths each year(1). The causative agent, *Mycobacterium tuberculosis*, is an acid-fast bacillus with a complex cell wall containing long-chain fatty acids (mycolic acids) as well as other lipid molecules. The waxy cell wall contributes to its ability to persist inside of host cells.

M. tuberculosis is primarily transmitted via aerosolized droplets and inhaled into the lungs where it is able to establish infection. Epidemiologic evidence suggests that only 30% of people exposed to *M. tuberculosis* result in established infections in humans, so in many instances the innate responses must be sufficient to kill the few bacilli that make their way to the respiratory tract. Established infection is measured in humans by development of T cell reactivity against a relatively crude mixture of mycobacterial antigens (tuberculin or purified protein derivative, PPD) using a tuberculin skin test (delayed hypersensitivity reaction), or by interferon gamma (IFN-g) release assays (ELISA or ELISPOT) for T cells that react against *M. tuberculosis*-specific antigens. Infection with *M. tuberculosis* can lead to active tuberculosis, defined as having symptoms consistent with disease (persistent cough, weight

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loss), radiographic evidence of lesions in lungs, and culture of *M. tuberculosis* from sputum or other anatomic sites. Active tuberculosis occurs in 5–10% of infected persons. The majority of humans infected with *M. tuberculosis* control but do not eliminate the infection, have no clinical signs of disease and are not contagious. This clinically silent infection is termed “latent tuberculosis infection” (LTBI). It is estimated that one-third of the world’s population has LTBI. Reactivation occurs in ~10% of latently infected humans, sometimes decades after the initial infection, and presents with the same symptoms as active tuberculosis. Thus, the more than 2 billion people with LTBI serve as an enormous reservoir of potential disease and transmission.

The immune response plays a major role in controlling initial infection (i.e. preventing development of active tuberculosis) and preventing reactivation of LTBI. Upon entering the airways, *M. tuberculosis* is engulfed by alveolar macrophages and begins to replicate. The organism can then enter the lung parenchyma, infecting other macrophages and dendritic cells. This leads to the production of inflammatory cytokines and chemokines, which results in recruitment of additional immune cells to the site, including monocytes which differentiate into macrophages, and neutrophils. Dendritic cells in the airways and parenchyma phagocytose *M. tuberculosis* bacilli and migrate to lung draining lymph nodes, which also become infected. In the lymph nodes, a T cell response (both CD4 and CD8) is generated. The T cells migrate back to the site of infection in the lungs and participate in granuloma formation.

Granulomas are the pathologic hallmark of tuberculosis. These are complex organized spherical structures consisting of macrophages, lymphocytes, and neutrophils (Figure 1.) Often, the center of the granuloma is necrotic, termed caseous necrosis. This structure is the result of the host’s attempt to contain and limit the infection. In fact, studies in non-human primates indicate that some granulomas are capable of sterilizing the infection, while others in the same host are not. The success of the granuloma in killing the bacilli is likely a major factor in outcome of infection. Poor killing of the bacilli appears to lead to dissemination and formation of additional granulomas, or development of more complex pathologies such as pulmonary consolidations, tuberculosis pneumonia, and cavities. *M. tuberculosis* has devised mechanisms for survival within the granuloma, and this structure can serve as a niche for persistent infection. Thus, immune responses at the site of infection (granulomas) are extremely important for control of *M. tuberculosis* infection. However, in humans, it is nearly impossible to assess immune responses in granulomas. Instead, T cell responses in humans are primarily studied in blood, since this is the sample most commonly obtained from patients. Our data from macaques suggests that peripheral (blood) responses are a poor indicator of T cell responses in granulomas (2).

Although *M. tuberculosis* is considered to be an intracellular pathogen, it can also survive and replicate extracellularly pathogen in vivo and in vitro. The primary host cell is the macrophage, where *M. tuberculosis* can block phagolysosome fusion and replicate within the phagosome while other reports suggest that the bacillus can also enter the cytoplasm of host cells (reviewed by (3)). It has also been reported that *M. tuberculosis* can enter and live within epithelial cells, although the evidence for this in vivo is sparse(4). Within the granuloma, the bacillus can be found in primarily macrophages and in the caseous necrotic

region (5). Although the necrotic region is quite low in oxygen, *M. tuberculosis* can survive there extracellularly, although replication is likely limited. To be effective, T cells must be activated by interaction with infected macrophages and release cytokines to activate the antimicrobial capacity of macrophages, or kill the cell via cytotoxic mechanisms. The structure of the granuloma may limit the interaction of T cells with infected macrophages, since the lymphocyte-rich region of the granuloma is usually in the outer cuff, while the infected macrophages are primarily in the inner regions of the granuloma (Figure 1). Our data from macaques indicate that only a small fraction of the T cells in granulomas can produce cytokines upon re-stimulation ex vivo, suggesting that either cytokine production is inhibited in the granuloma, or that many of the T cells in the granuloma are not specific for mycobacterial antigens (2).

T cell responses to *M. tuberculosis*

T cells play a critical part in the host immune response against *M. tuberculosis* infection. Humans deficient in CD4 T cells, particularly those that are HIV+, have dramatically increased susceptibility to both primary and reactivation tuberculosis. Mice and macaques depleted of CD4 T cells, or macaques that are SIV-infected, also show increased susceptibility to active or reactivation tuberculosis(6–11). While CD8 T cells were initially thought be less important than CD4 T cells in the immune response to *M. tuberculosis*, it has now been recognized that they play a critical but complex role. As intracellular organisms, *M. tuberculosis* antigens can be processed either by cytosolic, transporter associated with antigen processing (TAP)-dependent mechanisms, vacuolar or phagosomal compartments (that includes cross-priming) (reviewed in (12)). Like CD4 T cells, CD8 T cells are able to produce IL2, IFN-γ, and TNF, cytokines that are known to have critical functions during *M. tuberculosis* infection. Importantly, CD8 T cells have cytolytic functions to kill *M. tuberculosis*-infected cells via granule-mediated function (via perforin, granzymes, and granulysin) or Fas-Fas ligand interaction to induce apoptosis. In humans, CD8 T cell can produce granulysin, which can kill *M. tuberculosis* directly (13).

CD8 T cells are able to recognize *M. tuberculosis* specific antigens (as peptides) presented by classical and non-classical MHC molecules. Classically restricted CD8 T cells have been identified that recognize antigens presented by antigen presenting cells in the context of classical MHC Ia (HLA-A, -B, -C) molecules. Non-classically restricted CD8 T cells include those CD8 T cells that are capable of recognizing Mg antigen in the context of HLA-E molecules (non-MHC 1a), glycolipids associated with group 1 CD1 molecules and MHC I-related molecules (MR1) such as mucosal associated invariant T cells (MAIT). Finally, γδ T cells represent a separate population of CD8 (and CD4) T cells that have both innate and adaptive functions in response to *M. tuberculosis* infection. CD8 T cells have been shown to play direct functions in response to *M. tuberculosis* infection but they also play important roles in orchestrating many different functions in the overall host immune response (e.g., interaction to provide optimal CD4 T cell function). The roles of both conventional and unconventional CD8 T cells in the host immune response to *M. tuberculosis* infection will be reviewed here, using human and animal model data.

The importance of CD8 T cells in control of M. tuberculosis infection

There are several lines of evidence that point to the importance of CD8 T cells in control of initial or long term *M. tuberculosis* infection. Initial studies using adoptive transfer of T cells to mice suggested a role for these cells in protection against *M. tuberculosis*(8). As knockout mice became available, a series of studies in B2microglobulin (β2M), Class I heavy chain (KbDb), TAP, and CD1d deficient mice confirmed that classically restricted CD8 T cells were necessary to control the infection (8, 14–16). Results from knockout mice can be difficult to interpret, as gene deletion can result in unexpected additional phenotypes. For example, β2M−/− mice have higher iron levels due as β2M regulates iron uptake, which could result in increased bacterial growth (17). In addition, β2M is necessary for loading the alternative MHC molecule, CD1d. Thus, the use of several mouse strains deficient in CD8 T cells due to different gene deletions was necessary to demonstrate the importance of this T cells subset in murine tuberculosis. Additional work in MHC Class II deficient mice (that lack CD4 T cells) indicated an important role for CD4 T cells in inducing an appropriate CD8 T cell response in *M. tuberculosis* infected mice (9). Finally, depletion of CD8 T cells in the chronic stage of infection in mice resulted in increased bacterial burden, suggesting these cells are necessary for long-term control of infection (18).

Although the mouse data support the importance of CD8 T cells in the immune response against *M. tuberculosis*, it is much more difficult to determine the contribution of CD8 T cells to protection against tuberculosis in humans. The loss of CD4 T cells in HIV+ subjects correlates with increased susceptibility to tuberculosis, but similar human associations of susceptibility to tuberculosis related to CD8 T cells do not exist, simply because there are few recognized situations where humans are specifically deficient in CD8 T cells and in contact with *M. tuberculosis*. It is clear that *M. tuberculosis* infection in humans induces CD8 T cells that recognize a wide range of *M. tuberculosis* antigens, and that these CD8 T cells can be cytokine producing and cytotoxic. Macaques develop disease that recapitulates nearly all aspects of tuberculosis in humans. Rhesus macaques depleted of CD8 T cells and challenged with high dose *M. tuberculosis* are no longer partially protected by BCG vaccination (19). Our data support that CD8 T cells are essential in acute and chronic low dose infection in cynomolgus macaques (Lin and Flynn, unpublished), and CD8 T cells producing cytokines are present in granulomas ((2, 7, 20, 21) and unpublished). Thus, there is a body of evidence building in small and large animals, and circumstantial evidence in humans, that CD8 T cells have the potential to play a major role in protection against tuberculosis.

CD8 T cell function

CD8 T cells are sometimes called cytotoxic T lymphocytes (CTL), due to their potential to kill target cells. The killing mechanism is generally dependent on production of perforin, which acts as a pore for delivery of other proteins into the target cell. Perforin is expressed by a number of cells in granulomas, including CD8 T cells ((10) and unpublished). MHC Class I restricted CD8 T cells from the lungs of *M. tuberculosis*-infected mice express perforin and lyse infected macrophages(10). This lysis was dependent on perforin, since strontium abrogated the ability of CD8 T cells to lyse infected macrophages. CD8 T cells

from human blood also lysed *M. tuberculosis* infected macrophages and killed the intracellular bacilli in an antigen specific fashion (22). However, assessing the importance of perforin in CD8 T cell function in vivo has been challenging, since the absence of perforin in mice results in higher production of cytokines, including IFN-γ, by T cells, both in chronic viral and (23) in *M. tuberculosis* infection. Thus, perforin knockout mice were not more susceptible to *M. tuberculosis* infection(24), but had higher overall IFN-γ production(9), which could compensate for the lack of CD8 T cell cytotoxicity.

CD8 T cells express proteins, primarily the serine proteases granzymes, that are stored in granules and delivered across the immunologic synapse via perforin when the CD8 T cell engages a target cell by TCR-MHC Class I recognition. There are five granzymes in humans, each with specific sets of substrates. Mice have a larger set of granzymes. These proteins traditionally were described as inducing pro-apoptotic pathways and inducing cell death in target cells. However, more recently, granzymes have been implicated in degradation of extracellular matrix and in regulation of pro-inflammatory cytokines (25). Granzymes are expressed in cytotoxic T cells as well as NK and NKT cells. Granyzme B is expressed in peripheral blood CD8 T cells from *M. tuberculosis* infected humans, with higher expression seen in those with latent infection compared to active TUBERCULOSIS (26). Granzyme A expressing T cells were observed in human granulomas (27). However, mice lacking granzyme B did not appear more susceptible to *M. tuberculosis* infection (24). Recently, granzyme B expression by neutrophils (which do not express perforin) was described to correlate with bacterial load in individual granulomas from cynomolgus macaques, suggesting that there is still much to be learned about granzymes and tuberculosis (28).

A major difference in the functional potential of CD8 T cells between mice and humans is that human CD8 T cells express another important cytolytic protein found in granules, granulysin (29, 30). The absence of granulysin in murine CD8 T cells makes it difficult to assess the full potential of this T cell subset in protection against tuberculosis in mice. Granulysin is synthesized as a 15kDa molecule and is cleaved to produce a 9 kDa form that is within granules in both CD8 T cells and NK cells. This protein can use perforin to enter the target cell, but it was recently shown that granulysin can also form a pore and deliver granzymes to target cells (31). Granulysin was shown to be responsible for killing *M. tuberculosis* within macrophages (13). This is a direct mechanism as purified granulysin can kill cell free *M. tuberculosis* in vitro. Although the mechanism by which *M. tuberculosis* is killed by granulysin is not completely understood, this protein damages the cell wall and disrupts lipid metabolism in the bacterium. It is present in the granulomas of humans (27) and macaques (unpublished), as well as in leprosy granulomas (32). Thus, granulysin is a potent anti-microbial protein of CD8 T cells that is likely to be important in vivo.

CD8 T cells can also make a variety of cytokines, including IFN-γ, IL-17, TNF, IL-10, IL-2 and TGF-β, and these have been reported in human studies with TB patients(26). The production of IFN-γ by CD8 T cells is thought to be an important mediator of protection, however little work has been done to demonstrate the importance of the other cytokines produced by CD8 T cells in protection against *M. tuberculosis*. Often, CD8 T cell activity is measured by ELISPOT for IFN-γ, but intracellular cytokine staining and flow cytometry

studies have demonstrated that these cells can produce other pro-inflammatory cytokines, such as TNF, IL-2, and even IL-17, often in similar frequencies to CD4 T cells (reviewed in (2, 33, 34)). The production of pro-inflammatory cytokines is assumed to be important for activation of cells that harbor *M. tuberculosis*, particularly macrophages. Although the cytokine cocktail necessary for activation of human macrophages to kill *M. tuberculosis* is controversial, most believe that IFN-γ and TNF are important for this function. IFN-γ can also induce production of chemokines (such as CXCL9, 10, 11), which may induce migration of cells to the granuloma. IL-2 production by CD8 T cells is likely to be important for T cell proliferation, although no studies on IL-2 production by CD8 T cells in tuberculosis specifically have been performed. Our data from murine models supports that in the absence of IFN-γ production from CD4 T cells (using adoptive transfer of CD4 T cells from mice deficient in IFN-g production), IFN-g production from CD8 T cells increases but is not sufficient to protect the mice from exacerbated tuberculosis(35). There has been substantial discussion in the literature about the importance of “multi-functional” T cells, i.e. those that express multiple instead of single cytokines, for protection against infections. However, in tuberculosis, the protective value of multifunctional T cells is quite controversial, with studies suggesting that multi-functional T cells are important for protection, and other studies that support that the presence of multi-functional T cells is more closely linked to the presence of active tuberculosis. In our mouse tuberculosis studies, we found that, surprisingly, CD8 T cells from the lungs expressed either IFN-γ or CD107 (as a marker of cytotoxic cells) but generally not both (36). This is different from viral infections where cytotoxicity and IFN-γ production are generally produced in the same CD8 T cell. Our unpublished data from macaque granulomas supports that most CD8 T cells did not co-express IFN-γ and CD107. Many studies in the literature use IFN-γ as a readout of CD8 T cell function, but if the cytotoxic and cytokine producing functions are truly present in different cells, this may be an inadequate measure of true CD8 T cell potential. However, it remains to be seen if this is true in both blood and granulomas, and in humans, and whether the expression of CD107 actually correlates to in vivo cytotoxic potential in *M. tuberculosis* infection. Co-expression of IFN-γ with granulysin, perforin or granzymes has been demonstrated in the blood *of M. tuberculosis* infected humans, with increased expression of granulysin and perforin following ex vivo stimulation (37), but multifunctional phenotypes in human granulomas have not been reported. In our macaque studies, the vast majority of T cells in granulomas were single cytokine producers, rather than multifunctional. However, in each granuloma the collection of single cytokine producers could lead to a “multifunctional” granuloma, although the complete cytokine phenotype of each granuloma varied substantially within and across macaques (2). Thus, CD8 T cells have the potential to be a very potent subset of T cells in preventing or even potentially exacerbating *M. tuberculosis* infection and disease.

Conventional CD8 T cells, Unconventional CD8 T cells and Unique cell types

Conventional CD8 T cells

Conventional or “Classical” CD8 T cells recognize 9 amino acid peptide epitopes from antigens presented by MHC Class I. Although there are several mechanisms by which

peptides can be loaded into MHC Class I, the most common source of such peptides is the cytoplasm. *M. tuberculosis* is considered an intracellular pathogen but can have both intracellular and extracellular niches in vivo, at least in humans and non-human primates. In the macrophage, the bacillus is generally found in the phagosome. Although there are reports of the bacillus entering the cytoplasm, this remains controversial. Recent data support that even if the bacillus does not enter the cytoplasm, antigens from *M. tuberculosis* can be exported from the phagosome, possibly via the ESX secretion systems(3). The potential mechanisms by which MHC I molecules are loaded with *M. tuberculosis* peptides have been reviewed elsewhere (12, 38). In any event, it is clear the *M. tuberculosis* infection results in the induction of CD8 T cells that are MHC Class I restricted.

Unconventional CD8 T cells recognize *M. tuberculosis* specific antigens presented by a variety of molecules, including non-classical MHC Ib molecules (HLA-E, murine Qa1, murine H2M3, MR1) and CD1a-d. Using human derived CD8 T cell clones, it was shown that the majority of *M. tuberculosis*-specific CD8 T cells from infected subjects were classically restricted (via HLA-1A)(39). In contrast, *M. tuberculosis*-specific CD8 T cells from uninfected individuals are predominantly non-classically restricted CD8 T cells(40–42). Regardless of the way in which *M. tuberculosis* antigen is presented to the CD8 T cell, both classical and non-classical CD8 T cells can produce both cytokines or have cytotoxic functions discussed above.

Alternatively restricted CD8 T cells: MHC class Ib restriction

CD8 T cells from mice that lack MHC class Ia but have MHC class Ib molecules (H2M3 in the mouse) appear to be more susceptible to *M. tuberculosis* infection compared to normal mice, but were less susceptible compared to mice lacking MHC I completely (43). H2M3-restricted CD8 T cells from lungs were capable of producing IFN-γ but this was not fully protective. Because these cells are already activated in the naïve state, they are thought to bridge the innate and adaptive responses against *M. tuberculosis* infection. Taking advantage of the fact that H2M3 restricted CD8 T cells appear to recognize formylated M. tuberculosis peptides, vaccine strategies have been developed using formylated peptide of M. tuberculosis and have shown some protective effects in mice (44, 45). Although similar to human MHC Ib molecules, to date there is no known homologue for the H2M3 molecule in humans.

In humans, HLA-E molecules represent a unique class of non-classical HLA Class Ib molecules with limited genetic polymorphism. In mice, Qa-1 represents the murine homologue to HLA-E but no *M. tuberculosis* specific CD8 T cells restricted by Qa-1 have been identified (12). HLA-E molecules bind to nonamer peptides from both self and pathogen-derived antigens (including *M. tuberculosis*) that trigger CD8 effector function in a TCR dependent manner (46, 47). HLA-E dependent CD8 T cells have been identified in *M. tuberculosis* infected humans (33, 41, 47), with a variety of different functions that include proliferation and IFN-γ production. Caccamo et al demonstrated HLA-E restricted CD8 T cells were involved in Type 2 cytokine production (e.g., IL-4) and provided B cell help for antibody production but poor cytotoxic activity. These HLA-E restricted CD8 T cells decreased during effective chemotherapy against tuberculosis (33). Joosten et al

showed that HLA-E peptide associated CD8 T clones derived from *M. tuberculosis*-infected humans could inhibit T cell proliferation in a regulatory manner, while other clones had cytotoxic properties (47). The role these differing HLA-E restricted CD8 T cells play in the host immune response to *M. tuberculosis* infection has not been fully elucidated, but they may play a potentially important role in *M. tuberculosis*-HIV co-infected individuals. HIV is known to down-regulate expression of classical HLA molecules such as HLA-A and B to reduce antigen presentation (48). However, HLA-E is more resistant to downregulation by HIV and in some cases it may be up-regulated. Thus HLA-E restricted T cells may play a more critical role in *M. tuberculosis* control during co-infection (49, 50).

CD1 restricted CD8 T cells

CD1 molecules are another family of antigen presenting molecules with limited polymorphisms distinctly separate from the MHC genes. These molecules have the unique ability to present *M. tuberculosis*-derived lipid antigens to CD1-restricted CD8 T cells (but also αβ T cells [CD4−/CD8−], γδ T cells, CD4 and NK cells)(51). As mentioned above, the *M. tuberculosis* cell wall contains numerous lipids. CD1 molecules that engage TCRs can be classified as group 1 CD1 proteins that include CD1a-c and group 2 that includes CD1d. Expression varies based on antigen presenting cell type, as group 1 and 2 proteins are expressed on dendritic cells but only group 1 proteins are expressed on macrophages (reviewed in (52)). Differing components of mycobacterial lipids are presented by varying groups of CD1 molecules for processing. For example, polyketide lipids are presented by CD1c molecules whereas mannose-capped lipoarabinomannan (ManLAM) was identified to activate CD1b-retricted T cells (53).

Mice lack a complete set of CD1 encoding genes (they do not have Group 1 CD1 molecules) so most studies have been performed using human cells derived from blood in in vitro and ex-vivo studies. In humans, the relative frequency of these CD1 restricted T cells varies in the blood of *M. tuberculosis* infected individuals and can have naïve and memory phenotypes (54). Group 1 CD1 T cell responses correlate with mycobacterial load such that patients with active tuberculosis had higher frequencies of mycolic acid specific T cells (producing IL-2 and/or IFN-γ) than those subjects who had been treated for tuberculosis, although with substantial despite variability among patients (54). Although they have limited genetic variation, polymorphisms in CD1a are associated with susceptibility to *M. tuberculosis* infection (55). Nonetheless, the conserved conformation of the CD1 proteins makes CD1-restricted T cells an attractive target for vaccines and lipid based vaccination trials have shown partial protection in a highly susceptible animal model (56). Thus, it is likely that CD1 restricted responses have the potential to be protective during *M. tuberculosis* infection although the mechanisms and evidence for this are yet to be fully understood.

γδT cells

In humans, the Vγ2/Vδ2 T cells (or Vγ9/Vδ2 T cells) are the most common type of γδ T cells in the blood that contribute to both innate and adaptive responses to infection. While these cells are often double negative for both CD4 and CD8 markers, a substantial proportion of them express the CD8 marker. These cells only exist in primates and can be

induced to expand after stimulation with phosphoantigens (hydroxyl-3-methyl-but-2-enyl pyrophosphate or dimethyl-allyl pyrophosphate) from *M. tuberculosis* and other pathogens. In non-human primate studies, Vγ2/Vδ2T cells expand after initial *M. tuberculosis* infection and demonstrate a recall response in BCG vaccinated animals after *M. tuberculosis* challenge (57, 58). Once activated and expanded, these cells are capable of migrating to the lungs and mucosal surfaces. After infection, these cells can produce IFN-γ, TNF and perforin although their specific function in the lung is unclear (reviewed in (59)). In vitro, Vγ2/Vδ2 T cells are also capable of altering regulatory T cell function in a TH1/IL-2 dependent fashion in co-culture (60). Over the years, these cells have been recognized to interact with and influence the function of many different cell types such as B cells, macrophages, granulocytes, NK cells and dendritic cells (61). The exact contribution of Vγ2/Vδ2 T cells in the host response to *M. tuberculosis* infection is still being examined. Of interest, these cells decrease substantially in HIV infection and do not appear to recover after highly active anti-retroviral therapy (62) that has raised some concerns about their contribution in the host susceptibility to tuberculosis in HIV patient despite immune reconstitution.

Recognition of infected epithelial cells by CD8 T cells and Mucosal-Associated Invariant T cells (MAIT)

M. tuberculosis-infected human airway epithelial cells that express MHC-I molecules have recently been shown to stimulate both classical and non-classical CD8 T cells in vitro (4). CD8 T cells in the airway have been shown to produce both IFN-γ, TNF, and granzymes. Although it is controversial whether epithelial cells are infected in vivo, and if so, how important this is to the pathogenesis of *M. tuberculosis* infection, recognition of these cells by CD8 T cells, and subsequent killing of the intracellular bacteria, could be important in the very earliest phases of infection within the airway (4).

MAIT cells are innate-like CD8 T cells capable of recognizing pathogens via MHC-I related molecules called MR1 molecules that have limited polymorphisms. MAIT cells have been shown to play key roles in the innate responses to intracellular pathogens (e.g., Salmonella) (63) and can produce cytokine such as IFN-γ, TNF, and IL-17. MAITs also have cytotoxic capabilities, and have been shown to be CD107+ and produce granzyme B (64–66). These non-classical CD8 T cells are highly enriched in the human airways and gut. Using *M. tuberculosis*-infected human airway epithelial cells as antigen presenting cells, MAIT cells are able to produce IFN-γ and possibly TNF and granzymes in vitro (39). Although their real contribution to the host response against *M. tuberculosis* infection remains unclear to date, these cells may play an important role in the very early host response in the airways.

Potential regulatory roles of CD8 T cells

In CBA/J mice (which are more susceptible than other mouse strains), CD8 T cells expressing PD-1, Tim-3 or CD122 were able to produce IL10, resulting in greater susceptibility to chronic *M. tuberculosis* infection (67). These data suggest that CD8 may play an important role in the regulating the immune response. Regulatory cytokines such as IL-10 and TGF-β are produced by CD8 T cells in blood from patients with active tuberculosis, and higher levels of CD8 T cells producing these cytokines was associated

with higher bacterial burden (26). The markers that designate regulatory CD8 T cells have not yet been fully defined, but include the following: CD8+ LAG3−FoxP3+CTLA−4+ (68), CD8+ LAB-3− CCL4+ (69) and CD8+CD39+ (70). Joosten et al described a specific type of regulatory CD8 T cell that contained binding motifs and binding affinity for H-LAE. CD4 T cells may have an interaction with regulatory CD8 T cells as CD4 depletion resulted in loss of CXCR3+ CD8 T cells and increased IFN-g in the hilar lymph nodes *of M. tuberculosis* infected cynomolgus macaques. These CXCR3+CD8 T cells expression higher levels of IL10 and a broader polyfunctional T cell profile(7). Whether regulatory CD8 T cells are classically or non-classically restricted is not yet known, and how these cells function to regulate the immune response is not clear. Inflammation in tuberculosis is associated with higher bacterial burden in mice and macaques, and regulation or balancing of inflammation is likely key to resolution of disease. The contribution of CD8 T cells to this immune regulation is not yet known. Importantly, these “regulatory” CD8 T cells have only been identified in peripheral blood of humans and the full extent of their contribution to disease is not yet known.

Discussion

The most recent and largest vaccine trial against tuberculosis in this decade tested the ability of a viral vector (MVA) expressing a mycobacterial protein (Ag85A) (MVA85-A) to protect against tuberculosis in infants in South Africa. Infants were vaccinated with BCG alone, or boosted with MVA85A. MVA85A targeted *M. tuberculosis* specific polyfunctional CD4 T cells (71), yet showed no protection in this trial. These results underscore the need for improved vaccine strategies. Many have suggested that the induction of CD8 T cells specific for mycobacterial antigens would improve protective responses (reviewed in (72–74)) and newer vaccines are being developed to recruit CD8 T cell function. Classical CD8 T cell recruitment strategies that provide direct or indirect (cross-priming) methods include the use of viral vectors such as adenovirus (75–78), recombinant strains of BCG expressing listeriolysin (79) or overexpression of perfringolysin (80) and newly designed vaccine adjuvants (81). The limited genetic polymorphisms of the MHC-like molecules that restrict non-classical CD8 T cells make these cells an attractive vaccine target. However, much of what is currently known about non-classically restricted CD8 T cells is limited to studies in human airway or blood. While ex vivo studies have shown potentially important roles in the host response to *M. tuberculosis* infection, evidence of their protective roles in the lung and airway in vivo remain unclear. Methods for recruiting non-classical CD8 T cell functions have also been performed (56) but to a limited extent as the protective role of many of these recently described cell types is not yet known and the ability to test these targets is not readily available in small animal models. As pre-clinical vaccine studies are carried out in more human-like animal model systems, and as these candidates move forward into human clinical trials, we will learn more about the efficacy of CD8 T cells in protective vaccines. CD8 T cell function is likely to play a more critical role in the immune response to HIV-tuberculosis co-infection as CD8 T cells have been well recognized to play key roles in the control of HIV infection (reviewed in (82)). Thus, vaccine-induced CD8 T cell function is likely to be important for protection those patients at highest risk for tuberculosis, the HIV-infected population.

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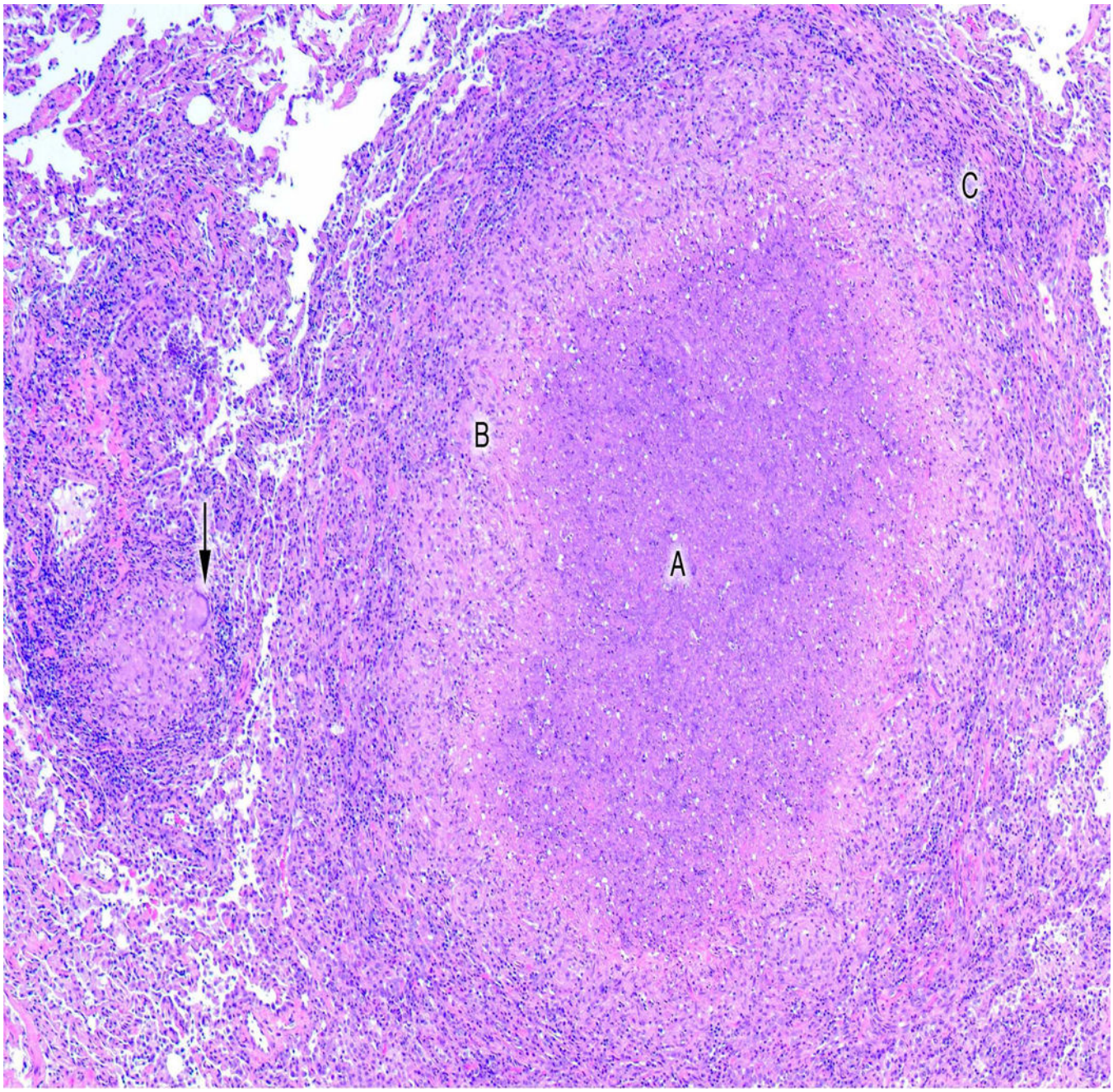

Figure 1.

Lung granulomas from *M. tuberculosis*-infected cynomolgus macaques are nearly identical to those from humans. A large caseous granuloma is shown on the right with acellular, eosinophilic staining of caseum (necrosis) (A) surrounded by palisading macrophages (B) along the mantle and lymphocytes (C) along the periphery. A smaller, non-necrotizing granulomas ("satellite granuloma") is seen on the left of the caseous granuloma with a large Langhans giant cell (arrow) within the central area of macrophages with peripherally surrounding lymphocytes. (40× magnification, H&E staining)

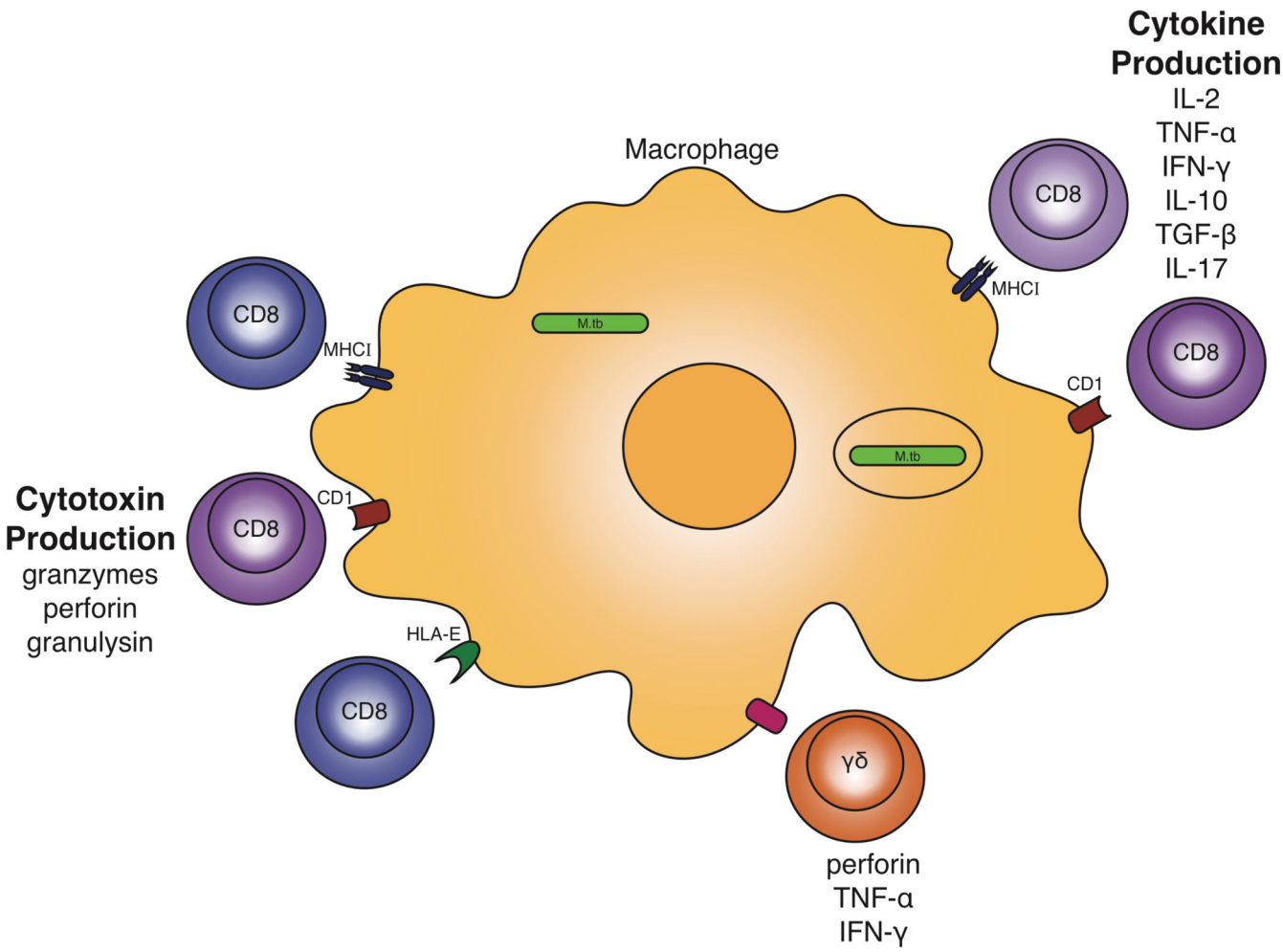

Figure 2.

Antigen presenting cells facilitate presentation of *M. tuberculosis* specific antigens to a variety of different CD8 T cell types using a range of MHC and MHC-like molecules. Antigens are presented to conventional CD8 T cells via MHC I and these cells have either produce cytokines or have cytotoxic function. Non-conventional CD8 T cells recognize antigens through other MHC-like molecules (e.g., HLA-E, CD1) to facilitate host immune responses.

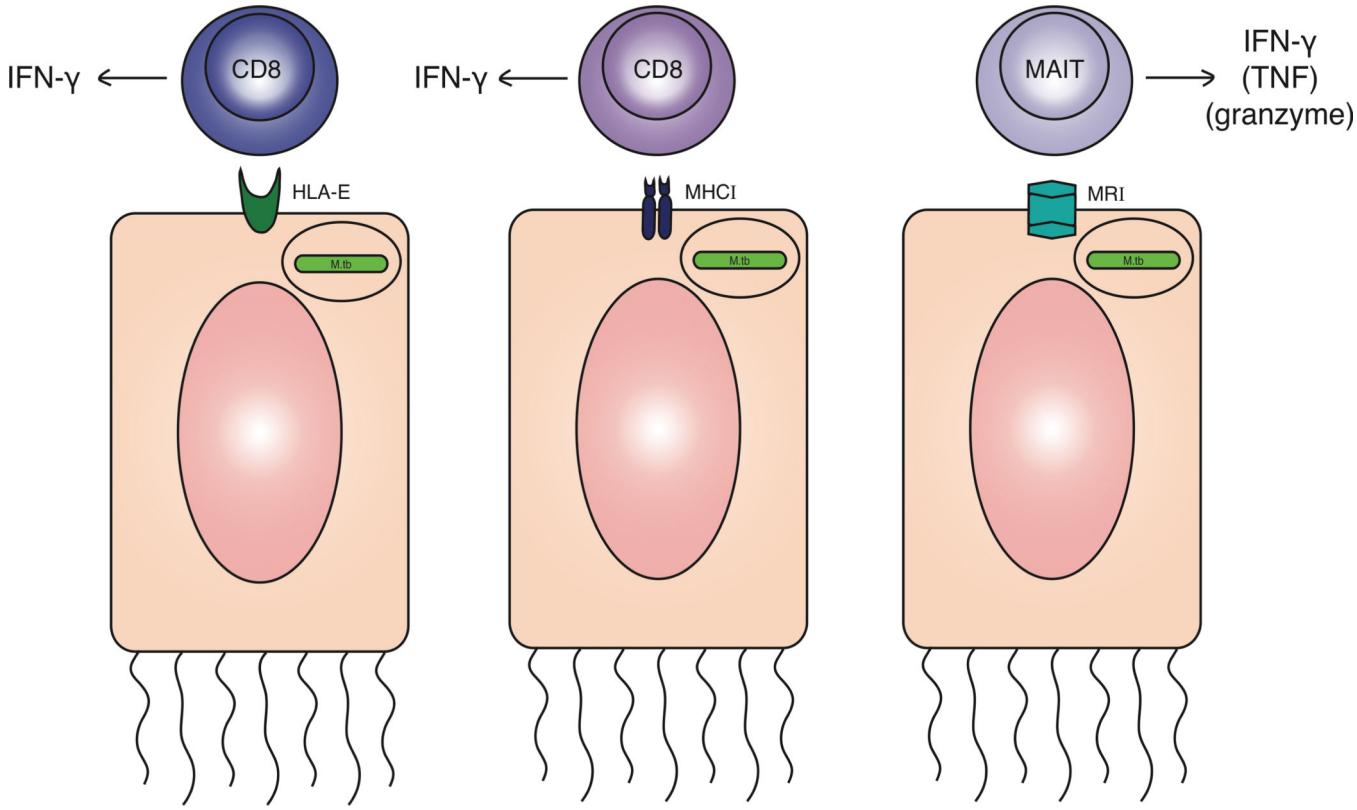

Figure 3.

Airway epithelial cells can function as antigen presenting cells to both conventional and nonconventional CD8 T cells including MAIT cells.