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INSIGHTS

Early innate role for CD8αα⁺ cells in tuberculosis

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Cell types that mediate early control of *Mycobacterium tuberculosis* (Mtb) infection are not well understood. Winchell and Nyquist et al. (https://doi.org/10.1084/jem.20230707) show that CD8αα⁺ lymphocytes have a major role in the innate suppression of Mtb growth in the lungs of macaques.

Tuberculosis (TB) is the leading global cause of death due to infectious disease. The current TB vaccine, intradermal Bacille Calmette-Guérin (i.d. BCG), is usually administered near the time of birth and protects infants from severe forms of TB. However, neonatal i.d. BCG vaccination does little to protect against TB in adolescents and adults, the population where most transmission of *Mycobacterium tuberculosis* (Mtb) occurs. New approaches to TB vaccination are sorely needed, but the mechanisms of host protection against Mtb infection are incompletely understood. A better characterization of the key cells and effector functions that mediate control of Mtb infection may provide clues as to the specific types of immunity that should be targeted through vaccination.

CD4 T cells are critical for control of Mtb infection, and an effective TB vaccine will require the generation of robust CD4 T cell immunity. However, to offer early and sustained bacterial control, a new TB vaccine will most likely need to simultaneously engage multiple arms of the immune system. For example, conventional CD8 T cells, donor-unrestricted T cells (DURTs), B cell and antibody responses, as well as long-lived alterations to the function of macrophages though trained innate immunity, may contribute to vaccine-elicited protection against tuberculosis (Lai et al., 2023). Animal models, especially macaques, will be important for formally establishing the roles of various immune cell subsets during tuberculosis.

In the accompanying manuscript, Winchell and Nyquist and colleagues examine the role of CD8⁺ cells in the macaque model of Mtb infection (Winchell et al., 2023). CD8 is expressed on several innate and adaptive lymphocyte subsets, and there are major outstanding issues regarding the precise roles of different CD8⁺ cells during tuberculosis. Firstly, despite years of research, it is still not clear to what extent conventional CD8 T cells can mediate control of Mtb infection. In mice, CD8 deficiency has little impact on the outcome of infection (Mogues et al., 2001), but data from mouse models may underestimate the impact of CD8 T cells in tuberculosis. For example, along with CD4 T cells, CD8 T cells have been found to correlate with protection after primary Mtb infection and challenge of vaccinated monkeys (Darrah et al., 2023; Gideon et al., 2022). Secondly, it is not clear to what extent other CD8⁺ lymphocytes such as natural killer (NK) cells and DURTs contribute to bacterial control during primary infection or challenge of vaccinated hosts.

CD8 is expressed as a dimer. In macaques, NK cells, mucosal-associated invariant T cells (MAITs), some γδ T cells, and NKT cells can express the CD8αα homodimer while conventional CD8 T cells generally express the CD8αβ heterodimer. Thus, CD8α-targeting antibodies preferentially deplete NK cells and DURTs while CD8β-targeting antibodies largely deplete conventional CD8 T cells. A previous study using macaques depleted CD8α⁺ cells after BCG vaccination or after drug-cure of Mtb infection and found increased bacterial replication after subsequent Mtb exposure (Chen et al., 2009). However, the relative contribution of CD8αα⁺ versus CD8αβ⁺ cells to protection against Mtb infection, as well as their role after primary exposure, was still not clear.

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To address these questions, Winchell and Nyquist et al. depleted either CD8α⁺ or CD8β⁺ cells at the time of Mtb infection and euthanized the animals at 6 wk after infection for analysis of the infection and host response, a relatively early time point (see figure). Using 2-deoxy-2-^{18}F-D-deoxyglucose (^{18}FDG)–positron emission tomography/computed tomography imaging, it was found that CD8α depletion resulted in

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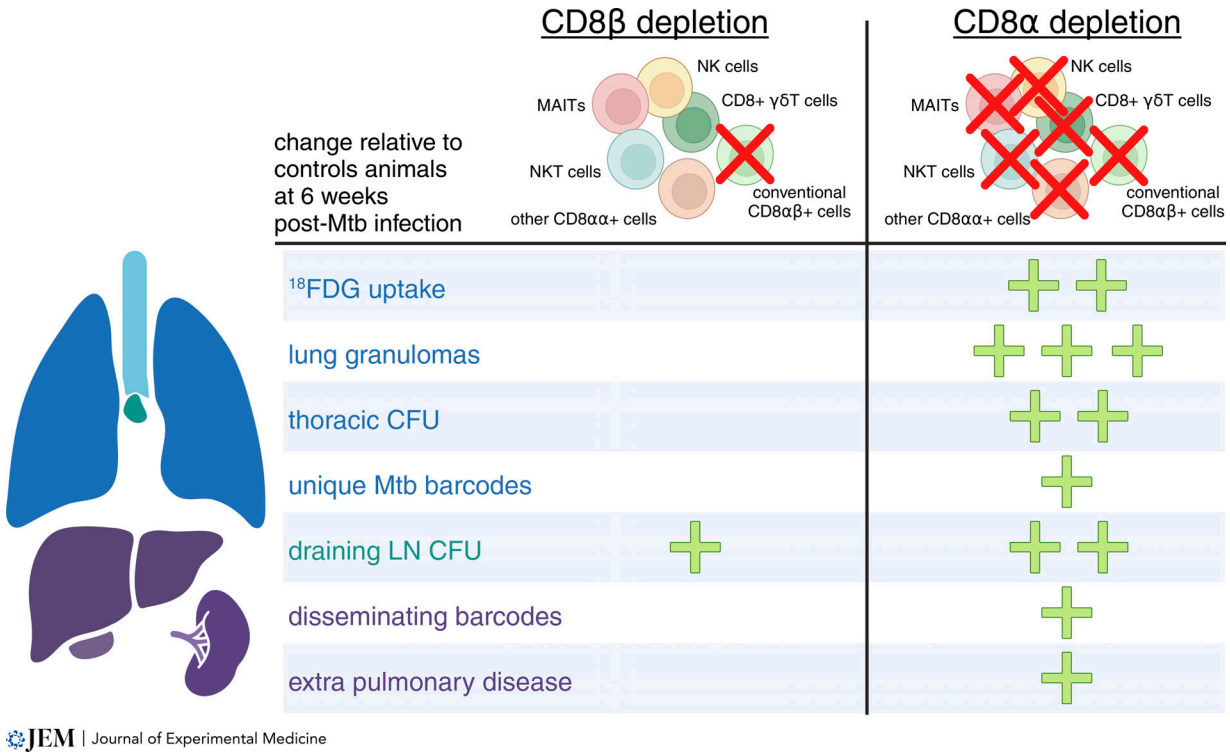

CD8⁺ cells have a major role in the early response to Mtb infection. Depletion of all CD8⁺ cells by administration of CD8α-depleting antibodies prior to Mtb infection results in higher take of the inoculated bacteria, much larger numbers of pulmonary granulomas, higher bacterial loads in the chest, and greater dissemination of bacteria into extrapulmonary tissues. This cannot be due to the activity of conventional CD8 T cells, as preferentially depleting CD8β⁺ cells had much less of an impact on the infection in this time frame. Thus, one or more populations of innate cells or innate-like T cells has a major role in the suppression of Mtb growth at the earliest stages of infection. Figure made with BioRender.

increased overall ^{18}FDG uptake, many more lung granulomas, higher overall numbers of bacteria in the chest, and increased numbers of infected lung lobes, lymph nodes, and extrapulmonary sites. In contrast, CD8β depletion only had significant effects on bacterial loads in thoracic lymph nodes. Previously, using barcoded strains of Mtb, the authors have shown that most granulomas contain a single barcode, indicating that each granuloma is initiated by one bacillus (Martin et al., 2017). The authors again used a barcoded strain of Mtb to infect the animals here, and by quantifying the number of unique barcodes found in the animals the authors were able to estimate the number of bacteria that were able to establish infection after deposition into the lungs. Surprisingly, they found that CD8α depletion resulted in increased numbers of unique barcodes, indicating that CD8α⁺ cells limit the initial "take" of bacteria. They were also able to examine dissemination of the bacteria out of the lungs by quantifying how many barcodes were shared between the lung and other tissues. They found more shared barcodes between the lungs and peripheral tissues of CD8α-depleted animals, indicating that CD8α⁺ cells also prevented the spread of bacteria out of the lungs, not simply the replication of the bacteria after it arrives in a peripheral tissue. In contrast, CD8β depletion did not have a statistically significant impact on bacterial take or dissemination. Collectively, these results show that CD8α⁺ cells act very early to suppress growth and spread of Mtb infection in the lungs. In this time frame, CD8β⁺ cells may suppress bacterial growth in lymph nodes, but they have a more limited role in the lungs.

Interestingly, CD4 T cells and γδ T cells in the CD8-depleted animals had several different characteristics compared to their counterparts in control animals. Both cell types displayed increased expression of granzyme B and granulysin as measured by intracellular staining in CD8-depleted animals, indicating that CD8-negative T cells increase their expression of cytotoxic molecules when CD8-positive cells are depleted. Single-cell RNA sequencing (scRNAseq) analysis also showed that CD4 T cells and γδ T cells in the granulomas of CD8α-depleted animals displayed elevated expression of multiple cytotoxic markers compared to those in control granulomas. However, although increased, cytotoxic effector molecules in CD4 T cells and γδ T cells in CD8-depleted animals were still lower than what is found in conventional CD8αβ T cells. The authors performed a sender-receiver analysis of the scRNAseq data and identified IL-15 derived from myeloid cells as a putative driver of the increased cytotoxic gene expression profile of CD4 T cells and γδ T cells in the CD8-depleted animals. This is consistent with previous work showing that IL-15 treatment of macaques preferentially acts on cytotoxic NK cells and CD8 T cells (Mueller et al., 2005; Sneller et al., 2011; Waldmann et al., 2011), as well as work by Okoye et al. (2009) showing that CD8α depletion in macaques results in an IL-15–dependent expansion of memory phenotype CD4 T cells in the blood. Thus, it seems likely that after CD8 depletion, the lack of competition for cytokine leads to an increase in the availability of IL-15 that

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drives compensatory increases in other Mtb granuloma T cell subsets as well as their expression of cytotoxic molecules.

Although CD8β depletion overall had smaller effects compared to CD8α depletion, this study does not formally settle the debate of the relative importance of conventional CD8 T cells during primary Mtb infection. The study endpoint was 6 wk after infection, which is too early in the kinetics of the antigen (Ag)-specific T cell response to have adequately tested the role for conventional CD8 T cells. It will be important in additional experiments to examine the impact of longer-term depletion of $CD8\beta^+$ cells to test if conventional CD8 T cells have a major role in control of primary Mtb infection.

The specific $CD8\alpha^+\beta^-$ innate cells that are responsible for control of Mtb infection are still not clear. The observation that CD8α depletion results in higher numbers of bacteria able to establish infection indicates that these cells may act as a bottleneck at the very earliest stages of Mtb infection. γδT cells, NKT cells, MAIT cells, or NK cells could all contribute. There could also be yet unappreciated $CD8\alpha\alpha^+$ T cell types that play important roles in tuberculosis. For example, a recent report found a $CD8\alpha\alpha^+$ $CD8\beta^{low}$ population of innate T cells in cord blood characterized by CD10 and Helios expression (Billiet et al., 2023). Even more recently, another novel population of unconventional $IL21R^{hi}CD244^{hi}CD11b^{hi}$ CD8αα T cells characterized by high expression of killer-like receptors and granulysin was described (Thomson et al., 2023). It is also not clear what effector functions innate $CD8\alpha^+$ cells employ to suppress early growth and dissemination of Mtb. Many of the $CD8\alpha\alpha^+$ cells in the lungs of uninfected macaques co-express granulysin, granzyme B, and perforin, and it is tempting to speculate that cytotoxic innate-like T cells pre-positioned at the site of Mtb arrival could act immediately to control Mtb infection. Future experiments are needed to identify the exact subpopulations of $CD8\alpha\alpha^+$ cells that mediate this rapid control of Mtb, but this will likely require the generation of new tools that allow for the selective ablation of specific cell types in non-human primates (NHP).

These results have implication for the design of new TB vaccines. Vaccines that target these cells (in addition to conventional CD4 T cells) may enhance very early control of Mtb infection. Since the protective $CD8\alpha^+$ cells are innate or innate-like T cells, it is not clear how they could be targeted with vaccination. For example, it is not known if CD1-restricted T cells generate long-lived memory T cells after in vivo stimulation in NHP. Moreover, our own attempts to stimulate MR1-restricted MAIT cells in macaques by inoculation with 5-OP-RU failed to induce MAIT cell expansion and instead drove MAIT cell dysfunction (Sakai et al., 2021). Thus, innate cells and innate-like T cells may be difficult to target with vaccinations, and more information is needed on how to drive robust and sustained populations of DURT cells in NHP before they can be formally tested as vaccine targets.

On the other hand, it's already known how to generate large populations of long-lived conventional memory CD8 T cells, and Mtb-peptide specific memory CD8 T cells may be able reproduce the protective activity of their innate $CD8\alpha\alpha^+$ counterparts. Indeed, there is a long-standing interest in the potential role of CD8 T cells in vaccine-generated protection against Mtb infection. This study further emphasizes the point that vaccines that elicit high numbers of memory CD8 T cells in the airways should be evaluated for protection against Mtb infection in macaque models. The data hinting that these early-acting, protective $CD8\alpha^+$ cells in the lungs may consume a lot of IL-15 also raise the possibility of utilizing IL-15 as an adjuvant to promote the generation of Mtb-specific T cells with similar activity during vaccination. The ability of CD8 T cells to recognize Mtb-infected macrophages is highly dependent on the particular Ag they recognize (Patankar et al., 2020), and it is likely that CD8 T cells of certain Ag-specificities will display much better in vivo protective capacity than others. The identification of class I–restricted Mtb peptides that elicit protective T cell responses is a critical next step of testing CD8 T cell–targeting TB vaccines (Lewinsohn et al., 2017).

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