JEM Journal of Experimental Medicine

INSIGHTS

TB granuloma: CD30 co-stimulation for CD4+ T cell co-operation

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Tuberculosis granuloma T cells express an array of mediators including the CD30 co-stimulatory receptor and its ligand, CD153. CD4 T effector cells require signals through CD30, potentially provided co-operatively by other T cells, to completely differentiate and protect against disease (Foreman et al., 2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20222090).

A new study by Foreman et al. (2023) reveals a novel mechanism by which T cells co-operate through co-stimulation in the granuloma to develop protection against tuberculosis (TB). Adaptive immunity against the intracellular pathogen *Mycobacterium tuberculosis* (Mtb) epitomizes the canonical paradigm of the CD4 T helper 1 (Th1) cell, with IFN-γ help for macrophages at the forefront of its functional armamentarium. But there is still more to understand about precisely how these lymphocytes protect hosts against TB disease, particularly at the site of primary lung infection, in the organized immunologic environment of the granuloma. Foreman et al. (2023) have found that signaling in CD4 T cells through the CD30 co-stimulatory receptor via its ligand CD153, both highly expressed by T cells in the granuloma, is essential for the development of protective T cells in TB (Foreman et al., 2023).

To identify T cell protective mechanisms that function at the site of TB infection, Foreman et al. (2023) explored the transcriptome of bulk CD4 and CD8 T cells isolated from lung granulomas of Mtb-infected rhesus macaques, comparing these cells to T cells in either the blood or bronchoalveolar lavage fluid from the same infected hosts. Granuloma T cells differentially expressed an array of homing receptors, cytokines, chemokines, and transcription factors when compared to T cells from these other two anatomic sites. This included 887 genes in CD4 T cells and 849 in CD8 T cells that were specifically expressed in the granuloma, a finding that offers strong justification for the targeted study of T cells recruited to Mtb-infected tissues across hosts.

Compared to naive T cells in the blood, granuloma CD4 and CD8 effector T cells differentially expressed a large array of co-stimulatory molecules including TNFRSF8 (CD30), TNFRSF4 (OX40), and TNFRSF9 (4-1BB). Interestingly, the ligands for several of these receptors, TNFSF8 (CD30L/CD153) and TNFSF9 (4-1BB-L), were also differentially expressed only in granuloma CD4 T cells. Further data analysis correlating the expression of genes relative to the bacterial burden of individual lung granulomas confirmed that TNFRSF8 (CD30) was one of the most highly expressed genes in both granuloma CD4 and CD8 T cells independent of the potential confounding variable of bacterial load, suggesting that it may play an important role in protection.

A previous publication from the same group found that CD153, the ligand for CD30, is highly expressed by a protective CD4 T cell population in the lung parenchyma of Mtb-infected mice and is required for CD4 T cell–mediated protection in the mouse model of TB (Sallin et al., 2018). The authors further investigated

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the mechanistic role of CD30 in T cell–mediated protection using the mouse model. Similar to CD153$^{-/-}$ mice, CD30$^{-/-}$ mice succumbed earlier to Mtb infection than wild-type mice. However, both mutant strains maintained similar frequencies of Mtb-specific T cells in the lungs, indicating that neither protein is necessary for the generation or initial differentiation of antigen-specific effector cells. Despite this, the authors found that donor CD4 T cells isolated from either CD30$^{-/-}$ or CD153$^{-/-}$ mice failed to protect T cell–deficient recipient mice, suggesting that both elements of this co-stimulatory signaling axis must be expressed by T cells for protection (Foreman et al., 2023).

In their previous study focused on CD153, the authors hypothesized that CD153 expressed on CD4 T cells could mediate protection against Mtb by stimulating CD30 on infected macrophages (Sallin et al., 2018). To test this in the present study, the authors used mixed

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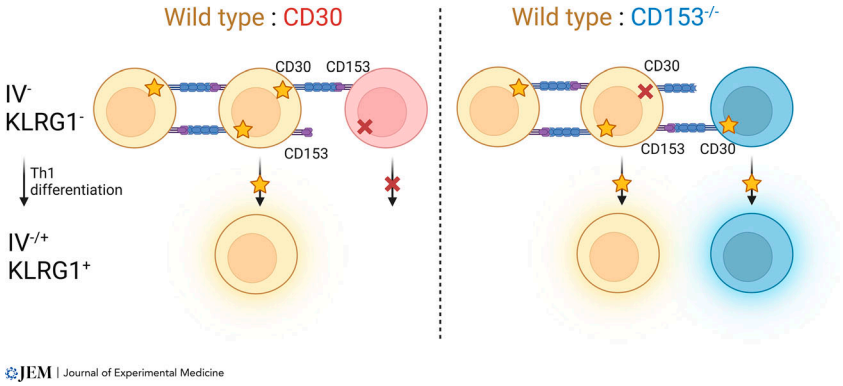

T cells require CD30 expression to undergo Th1 differentiation and mediate protection. Mixed bone marrow chimeras revealed that the protective effect of T cell–derived CD153 in TB is mediated through signals received by other T cells expressing the cognate co-stimulatory receptor CD30. T cells deficient in CD30 therefore fail to undergo Th1 differentiation, even in the context of an infection where wild-type T cells are present. In contrast, CD153-deficient cells can complete Th1 differentiation due to their retained expression of CD30 and the compensatory expression of CD153 by wild-type cells. A star indicates a productive signaling interaction received by CD4 T cells through CD30, which results in Th1 differentiation; an X indicates the absence of such a signal and the failure of complete differentiation.

bone marrow chimeras to determine whether CD30- or CD153-deficient myeloid cells demonstrated a relative impairment in control of intracellular Mtb infection. Contrary to that prior hypothesis, they found that myeloid cells from neither strain had increased frequency of internal bacterial load compared to wild-type cells. These data instead indicated that protective effects of CD30 and CD153 do not result from T cell help provided to infected myeloid cells directly through this co-stimulatory signaling axis. In fact, the authors found no evidence of a protective role for CD30 expression on any hematopoietic cell aside from T cells.

Despite the similar number and frequencies of CD4 effector T cells in CD30$^{-/-}$ mice, the authors noted that CD30$^{-/-}$ CD4 T cells demonstrated a phenotype of stunted Th1 differentiation, with a higher proportion of the T cells being parenchymal IV^{-}, KLRG1^{-} effectors. Using WT:CD30$^{-/-}$ mixed bone marrow chimeras, they found that intra-mouse competition exacerbated this effect and resulted in a smaller proportion of CD30$^{-/-}$ CD44^{+} effector cells, with a profound deficiency in KLRG1-expressing cells (see figure). In stark contrast, WT:CD153$^{-/-}$ chimeras resulted in an equal proportion of CD4 T cells from both genetic backgrounds, indicating that CD153/CD30 signals are received by CD4 T cells expressing CD30. RNA sequencing comparing IV^{-}, KLRG1^{-} CD4 T cells from the WT:CD30$^{-/-}$ chimeras revealed a loss in the expression of genes involved with memory T cell generation, STAT5A signaling, as well as defects in the expression of key effector transcription factors, cytokines, and chemokines among CD30$^{-/-}$ cells. These data indicate that CD30 co-stimulation in CD4 T cells enhances protection against Mtb by enabling Th1 differentiation to proceed to completion.

Data from these two studies (Sallin et al., 2018; Foreman et al., 2023) taken together strongly suggest that protection mediated through CD30/CD153 represents co-stimulatory co-operation between T cells in the granuloma. While the present study indicates that a cell-intrinsic autocrine CD153/CD30 signal may not be required for CD4 T cells to fully differentiate, it also does not absolutely identify the most critical source of protective CD153 signals or fully exclude the possibility that autocrine T cell signaling could contribute in a native infection context. To determine whether CD153 expressed by CD4 T cells may be sufficient to mediate this signal, it would be interesting for future studies to test whether adoptive transfer of wild-type CD4 T cells can restore normal effector Th1 differentiation in CD153$^{-/-}$ recipient mice and rescue their susceptibility to Mtb infection.

If future studies support co-operative co-stimulation between T cells, several other important mechanistic questions remain, including whether CD30/CD153 interaction involves T cells of similar or different phenotypes (including between CD4 and CD8 T cells) and antigen specificities, and the temporospatial details of how this occurs. It is tempting to hypothesize that the granuloma may provide an optimal structural environment for co-operative lymphocyte–lymphocyte interactions at the site of infection. Despite the histopathological and immunological differences between granulomas observed in C57BL/6 background mice when compared to macaque or human lung lesions, it is notable that the authors found strong concordance between their mouse and non-human primate studies. Since Mtb typically establishes a chronic infection in mammalian hosts, it will be interesting to assess what role this pathway plays in the ongoing maintenance of protective T cells, and whether loss of the CD30/CD153 interaction during established infection could promote progression to active disease. Finally, given the structural similarity and significant overlap in signaling downstream of different TNF receptor superfamily proteins (Chen and Flies, 2013), future work should determine which other co-stimulatory pathways expressed by granuloma T cells function similarly to CD30/CD153, or in unique ways.

There are several considerations for the real-world application and therapeutic potential of targeting the CD30/CD153 axis. The first is the role of immunomodulatory agents that could positively stimulate this pathway to possibly improve the quality of T cell responses against TB. Unlike other co-stimulatory receptor targets like OX40 and 4-1BB, for which agonist monoclonal antibodies exist, there is no widely available monoclonal antibody that induces signals through human CD30. The development of recombinant forms of CD153 or other agents that can stimulate CD30 on CD4 T cells could be a worthwhile goal for host-directed immunotherapy against TB. Given the findings that CD30 signaling drives the expression of multiple Th1 effector functions known to be protective against TB, as well as gene expression profiles consistent with memory T cell function, it will also be interesting to explore the role of this co-stimulatory pathway in the context of immunization and determine whether Mtb-specific T cells induced by TB vaccine candidates express CD30 in humans. Notably, in macaques immunized with an intravenous dose of Bacillus Calmette-Guérin capable of providing sterilizing immunity against Mtb challenge, CD4 T cells from the bronchoalveolar lavage fluid persistently expressed a gene module including both TNFRSF8/CD30 and TNFSF8/CD153, as well as TNFRSF4/OX-40, TNFRSF9/4-1BB, and ICOS

(Darrah et al., 2020). The induction of T cells expressing these markers may therefore represent an important correlate of protection for future TB vaccine studies.

In contrast, biologic therapies that negatively impact CD30 signaling are already in active clinical use. CD30 is a marker expressed by the Reed-Sternberg cells of Hodgkin's lymphoma and other T cell lymphomas (Castellino et al., 2022). Agents designed to target CD30-expressing cells for destruction include the FDA-approved monoclonal antibody drug conjugate brentuximab vedotin and investigational chimeric antigen receptor T cells with receptors specific for CD30. It is reasonable to predict that agents negatively targeting CD30 could also increase risk for TB and other opportunistic infections normally controlled by CD4 T cells (Maschmeyer et al., 2019).

Overall, this study promotes the idea that T cell co-stimulation may play a larger role in protection against Mtb than widely appreciated. Co-stimulatory pathways may be a viable target for vaccine adjuvants and effective host-directed immunotherapies in TB, as has been the case in cancer. Prior studies have revealed a broad range of outcomes stemming from the normal function and modulation of T cell co-stimulatory and co-inhibitory networks in TB (Bhatt et al., 2009; Snelgrove et al., 2012; Sia et al., 2017; Moguche et al., 2015; Jayaraman et al., 2010; Barber et al., 2011; Phillips et al., 2017). Future work should aim to define the patterns of function of these similar and sometimes overlapping signaling mediators, to determine what their dominant effect on T cell function is in TB, and which offer the greatest potential as beneficial therapeutic targets.

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