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In Translation

Immunologic goalposts for TB vaccine development

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A limiting factor in identifying effective tuberculosis (TB) vaccines is our incomplete understanding of correlates of protection. In this issue of *Cell Host & Microbe*, Esaulova et al. reveal cell types associated with TB containment and disease progression in non-human primates, which may provide immunologic goalposts for vaccine design.

Tuberculosis (TB) is an ancient and complex human illness that we now know exists as a disease spectrum resulting from a dynamic interaction between the host immune system and the bacilli. Aside from COVID-19, TB is the leading cause of global morbidity and mortality from a single infectious disease agent. *Mycobacterium tuberculosis* (*Mtb*), the etiological agent of TB disease in humans, latently infects approximately one-fourth of the world's population and kills approximately 1.5 million individuals a year (WHO, 2018).

To break the cycle of our ongoing failure to control TB globally, new tools—particularly vaccines—are desperately needed. In stark contrast to the breakneck speed with which three vaccines for COVID-19 were developed and approved in under 1 year, 2021 marks the 100th anniversary of the only existing TB vaccine, BCG—a vaccine that despite near global use for many decades does not alone control TB. The basis of this failure to identify an effective vaccine for TB is our incomplete understanding of immunologic correlates of protection. To proceed rationally, vaccine developers need an improved understanding of what immunity to TB looks like; essentially immunologic goalposts of what to aim for remain urgently needed. In this issue of *Cell Host & Microbe*, Esaulova et al. (2021) offer an exciting dataset on immune correlates during controlled versus active TB.

Most individuals with latent TB infection (LTBI) naturally control the infection and remain asymptomatic; however, 5%–10% of infected individuals later progress to active TB disease. The immune parameters that govern progression from LTBI to active pulmonary TB (PTB) are complex and, due to limitations of conventional

techniques, remain incompletely defined. A way forward has been the increased use of biologically relevant animal models such as non-human primate (NHP) models (Kaushal et al., 2012; Scanga and Flynn, 2014). Coupled with powerful new technologies such as single-cell RNA sequencing (scRNA-seq) and mass cytometry (CyTOF)—which are capable of quantitative and qualitative deep profiling of cells, cell-surface-receptor quantification, extensive mapping of signaling networks in single cells, and establishing cell ontologies on the basis of phenotype and/or function—NHPs are now also being heavily utilized to study pathogenesis and immune correlates (Gubin et al., 2018; Kay et al., 2016).

The advent of single-cell technologies such as scRNA-seq and CyTOF have overcome some previous limitations of conventional techniques to analyze complex interactions between different host immune cell types. These technologies allow for in-depth and unbiased profiling of cell populations in animal models and humans in both healthy and diseased states (Proserpio and Mahata, 2016; Kaushal et al., 2012; Scanga and Flynn, 2014). scRNA-seq can unmask transcriptome heterogeneity of a complete community of cells and assign unbiased identity classification to cell populations, making it suitable for the study of complex inflammatory diseases such as TB (Esaulova et al., 2021). CyTOF allows for longitudinal assessment of cellular protein expression because it simultaneously measures more than 40 cellular parameters at single-cell resolution with over 100 available detection channels (Kay et al., 2016; Atkuri et al., 2015). Combined, these technologies can be used to characterize significant remodeling of immune com-

partments within organs and microenvironments (Gubin et al., 2018).

In the current study, Esaulova and colleagues provide a comprehensive characterization of the lung immune landscape of TB latency and disease in NHPs. The authors used scRNA-seq and CyTOF to survey the immune landscape within the lungs of NHP healthy control (HC), LTBI, and active PTB macaques to identify key immune features associated with protection during TB latency and active TB disease. They validate their finding with traditional techniques such as flow cytometry and immunofluorescence (IF) and extend their finding to clinical samples.

The analysis identified three defining features of PTB in macaque lungs: an influx of type 1 interferon (IFN)-producing plasmacytoid dendritic cells (pDCs), an IFN-responsive macrophage population, and activated T cell responses. In contrast, a cytotoxic CD27⁺ natural killer (NK) cell subset accumulated in the lungs of LTBI macaques. Interestingly, type 1 IFN-producing pDCs and activated T cells found in the lungs of active PTB animals overexpress LAG3, an exhaustion and immunosuppression marker, suggesting that these subset populations contribute to immune failure during active PTB.

Among other findings, scRNA-seq classified three macaque macrophage subsets not previously known to shift extensively in TB, based on *CD163* and *MRC1* expression. The first cluster, *CD163⁺MRC1⁺*, exhibited other classic markers of alveolar-like macrophage (AM-like) and constituted 20%–80% of myeloid cells in lungs. The second cluster, *CD163⁺MRC1⁺TREM2⁺* AM-like macrophages expressed high levels of *TREM2*, *C1Q* genes, and *TREM1276A/B*. Both



CD163⁺MRC1⁺ and *CD163⁺MRC1⁺TREM2⁺* AM-like macrophages were key features of LTBI and HC. In contrast, the third population of *CD163⁺MRC1^{low}* IFN-responsive macrophages were exclusively found in primates with active PTB. This latter population of *CD163⁺MRC1^{low}* IFN-responsive macrophages exhibited a strong signature of response to type 1 and 2 IFNs, high expression of IFN-dependent chemokine, *CXCL9-11*, and expressed *S100A8/9*—proinflammatory proteins previously identified in the serum of active TB patients (Ahmed et al., 2020). These macrophages also express the immunosuppressive checkpoint enzyme indoleamine 2,3-dioxygenase (IDO1), which promotes tryptophan conversion to kynurenine (Esaulova et al., 2021; Gautam et al., 2018). Therefore, these *CD163⁺MRC1^{low}* macrophages are key IFN responders and express suppressive molecules. Interstitial-like *CD161⁺MRC1⁻* macrophages were also identified in HC and LTBI NHPs; however, this population significantly decreased in active PTB.

Interestingly, the authors observed an increase in pDCs in the lungs of macaques with active PTB when compared with LTBI animals. This was reflected by significant depletion of peripheral pDCs in active PTB when compared with the HC or LTBI. The decrease in peripheral pDCs in macaques with active PTB is associated with significantly increased accumulation of peripheral classical monocytes when compared with HC or LTBI macaques. The authors then demonstrate that peripheral pDCs were specifically depleted in PBMCs of individuals with PTB when compared with pDCs in LTBI or HCs. Together, these results describe the distinct accumulation of pDCs in the lung during active PTB disease in both macaques and hu-

mans, likely by recruitment from the periphery.

This work demonstrates that single-cell technologies validated with conventional techniques serve as powerful tools that can elucidate the complex host immune cell interactions often associated with inflammatory diseases like TB. Through this approach, Esaulova and colleagues set immunological goalposts for rational vaccine development through the identification of correlates of protection during LTBI as well as correlates of disease progression in active PTB such as IDO1 and LAG3. The identification of cell types present during contained TB suggests avenues for the development of host-directed therapies. One such avenue is targeting IDO1 or LAG3 to develop host-directed immune therapies via immune checkpoint blockade.

The application of single-cell technologies can lead to unbiased deep insight into a cell's transcriptome, phenotype, physiology, and function, often shifting existing paradigms and expanding our knowledge of complex biological systems. This timely and pivotal study characterizing the lung landscape associated with successful containment versus disease progression during TB significantly expands our understanding of TB immunopathogenesis, and it identifies much-needed immune correlates of protection. The data from this study will provide a valuable roadmap for future TB pathogenesis research and for the rational design of more effective vaccines, immunotherapies, diagnostics, and small molecule inhibitors to control global TB.

DECLARATION OF INTERESTS

P.K.U. and W.R.B. are inventors on patents concerning recombinant BCG vaccines for TB.

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