Infectious disease

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Finding antigens for TB vaccines: the good, the bad and the useless

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Prospective, longitudinal clinical studies incorporating high-throughput, single-cell analyses could identify which bacterial antigens to include in TB vaccines — and which to avoid.

The development of effective tuberculosis (TB) vaccines is hindered by a lack of knowledge about the mechanisms of protective immunity and the antigens that induce protective responses. Most TB vaccine efforts have focused on T helper type 1 (T_H1) responses because of their importance for defense against intracellular pathogens, given that *Mycobacterium tuberculosis* (*Mtb*, the causative agent of TB) is a facultative intracellular bacterium; but a vaccine based on that premise provided no protection in clinical trials[1,2]. Subsequent studies have focused on the stimulation of additional T cell effector functions, especially production of interferon-γ (IFN-γ), tumor necrosis factor (TNF), interleukin 2 (IL-2) and IL-17; yet in studies to date, these do not correlate with vaccine protection[1,2].

An effective T cell response to an infectious pathogen depends on multiple features of the T cells (Fig. 1), and understanding these will inform better vaccine development. To this end, a paper from Musvosvi et al.[3] in this issue of *Nature Medicine* sheds welcome new light on the importance of the antigen and epitope (short peptide fragments of antigens) specificity of T cells in immune control of TB. To do this, the authors prospectively collected blood samples from *Mtb*-infected participants who progressed to active TB (progressors) and those who did not (controllers), and carried out T cell receptor (TCR) sequencing and bioinformatic analyses informed by structural studies.

Mtb has a large genome (~4,000 protein-coding genes[4]), so the host T cell repertoire faces the challenge of recognizing many *Mtb* antigens. In addition, TB immunologists face the challenge of identifying which antigens are processed and presented to T cells in humans with diverse human leukocyte antigen (HLA) allotypes, and which of these generate T cells that actually provide protection against TB.

The findings revealed by Musvosvi et al.[3] build on previous efforts using TCR sequences and structural features to infer the antigen specificity of T cells of interest. Advances in sequencing, structure determination and analysis platforms — such as GLIPH2 (Grouping of Lymphocyte Interactions by Paratope Hotspots)[5], DeepTCR[6] and others[7] — have accelerated efforts to identify antigens that can be used as vaccine targets for infectious diseases, or even to identify TCR sequences that predict responses to cancer immunotherapy[7]. Until now, however, there have been no reports of longitudinal studies that relate TCR sampling results to clinical end points — which are vital for diseases such as TB that have variable outcomes.

Leveraging well-characterized TB cohorts in which some individuals progressed to active TB disease and other individuals remained well, Musvosvi et al.[3] used single-cell and bulk TCR sequencing from

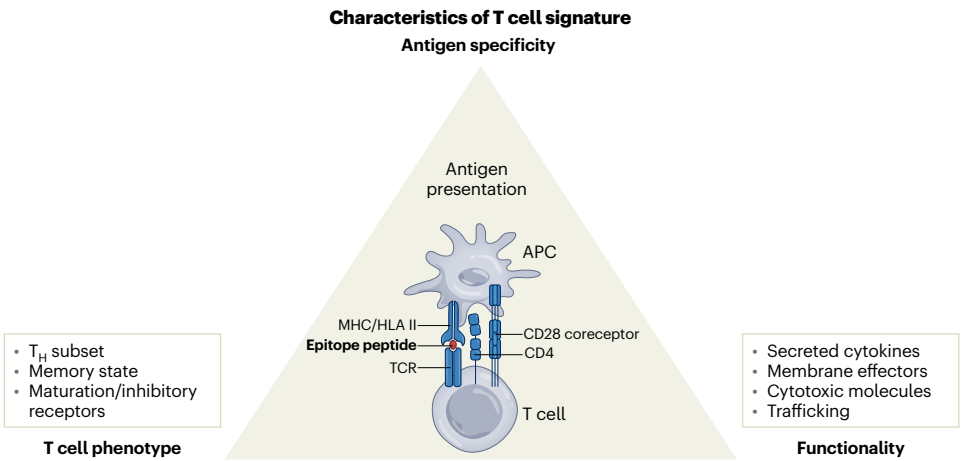

Fig. 1 | Characteristics of the T cell signature. Antigenic peptides are generated through the digestion of pathogen proteins by dendritic cells and other antigen-presenting cells (APCs) and then bound to major histocompabitility complex (MHC; HLA in humans) class II proteins as epitope peptides. Some epitope peptide–MHC complexes are recognized by T cell antigen receptors (TCRs). Signaling through TCRs, co-receptors and cytokines secreted by APCs causes proliferation and differentiation of antigen (epitope)-specific T cells that differentiate to provide distinct phenotypes (T helper or T regulatory cells) and functionality (cytokine secretion and other effectors). Activated antigen-specific T cells traffic to specific tissue sites to perform their effector functions and to mature into memory T cells and acquire inhibitory co-receptor markers of maturation. Central to the process is the recognition of specific peptide–MHC complexes by clonal TCRs.

Mtb-reactive T cells (most of which were $CD4^+$) and the GLIPH algorithm to cluster TCRs that recognize the same epitope. Filtering many thousands of these to remove rare similarity groups (more prone to misclustering) yielded 175 TCR similarity groups associated with specific HLA alleles. The majority of these were equally distributed between TB progressors and controllers, while 20 were more frequent in controllers and 10 more frequent in progressors. Further analyses using *Mtb* antigen libraries, artificial antigen-presenting cells and reporter T cell lines led to the identification of epitopes from two *Mtb* proteins, PE13 and CFP-10, recognized by T cell clones that were enriched among controllers. This association between immunologic control of TB and $CD4^+$ T cells that recognize these specific epitopes implies that the antigens containing these epitopes may be good candidates for inclusion in TB vaccines. Further studies will be required to determine whether these associations extend to other HLA alleles, other epitopes in the same antigens and other human populations.

PE13 is a member of a large protein family in the *Mtb* complex. Although the epitope identified by Musvosvi et al.[3] is unique to PE13, the *Mtb* proteome includes nine other peptides in other PE proteins with >80% sequence similarity to this epitope, which prompts the question: might protection be a consequence of cross-recognition of multiple PE13-related proteins expressed by *Mtb*? CFP-10 is notable for its deletion from the existing BCG TB vaccine, and it is secreted as a heterodimer with ESAT-6, which is implicated in *Mtb* virulence. Thus, it stands to reason that CFP-10 would be a valuable vaccine antigen. However, CFP-10 is included (with ESAT-6) as an antigen in tests to detect *Mtb* infection, and those tests do not distinguish between controlled and active TB. Might other CFP-10 epitopes counter the protection associated with the epitope identified in this work? Might T cell functions other than the one measured in the diagnostic test account for protection? Although the authors suggest that PE13 and CFP-10 should be considered for inclusion in subunit vaccines for TB, more work is needed in broader populations before we can decisively conclude that PE13 and CFP-10 are 'good' antigens for TB vaccines.

It is especially noteworthy that the authors identified an epitope recognized by T cell clonotypes enriched in TB progressors, potentially reflecting a 'bad' antigen. This epitope is from *Mtb* EspA, a secreted protein associated with CFP-10: EspA is essential for *Mtb* secretion of CFP-10, and vice versa[8]. The apparently opposite effects of T cell recognition of CFP-10 and EspA highlights the challenges of empirically choosing antigens and emphasizes the need for further systematic analyses incorporating T cell phenotypes, epitopes and TB outcomes.

The authors' finding that a large majority of TCR similarity clusters are found with equal frequencies in TB controllers and TB progressors implies that *Mtb* expresses numerous antigens and epitopes that induce T cells with little impact on TB outcomes — and are therefore useless in the context of infection or vaccine responses. Although this might be considered a neutral effect, generating antigen-specific effector T cells is metabolically demanding, so the effect might actually be detrimental to the host and beneficial to the bacterium — as responses to 'useless' epitopes may limit the expansion, trafficking and functions of T cells that recognize the 'good' epitopes and have potential to eliminate *Mtb*. Another issue to consider is the importance of T cells with functions other than releasing cytokines that promote macrophage killing of *Mtb*: work in other fields has revealed important roles for T cells in mitigating tissue injury and promoting tissue repair[9]. Such effector functions could be tied to antigen/epitope specificity and deserve consideration.

The work by Musvosvi et al. illustrates that with sufficient resources, systematic analyses of T cell responses to pathogens with large proteomes are possible and informative. Many questions remain unanswered, including whether the findings here are generalizable to populations other than the predominantly South African cohort enrolled in this study, whether additional 'bad' epitopes can be identified so they can be avoided in subunit vaccines and deleted from live attenuated vaccines, whether 'useless' epitopes and T cells are really as neutral as they seem to be and, ultimately, whether more work along these lines will result in improved TB vaccines.

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Competing interests

The authors declare no competing interests.