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Reprogramming the T cell response to Tuberculosis

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Abstract

Recently, Coscolla, Copin et al. use comparative genomics of *M.tuberculosis* strains to show that most human T cell-recognized epitopes are hyperconserved, but bona fide variable epitopes also exist. This identification of two sets of antigens implies opposing evolutionary processes and has an important impact on Tuberculosis vaccine strategy and design.

Successful pathogens often base their survival strategy on variation of critical antigens targeted by the host immune system. For example, immune evasion through antigen variation results in escape mutants in HIV patients and the need for seasonal flu vaccination. *M. tuberculosis* (Mtb), the causative agent of tuberculosis (TB), has evolved a different evasion strategy that enables long-term co-existence with its human host. Sequence data in recent years has demonstrated a very low genetic diversity among clinical isolates. Mtb survival therefore seems to depend more on its inherent resistance through a very resistant lipid rich cell wall and a refined set of intracellular evasion strategies that, in combination, enable survival of this pathogen under conditions that would eliminate most other bacteria. This results in long-term persistence and the development of immunopathology that forms the basis of cavity formation, TB lung disease and transmission.

In a recent study in *Cell Host and Microbe*, Coscolla, Copin *et al.* present data based on a comparative genomics approach that suggests that evolutionary pressures in the human host drives sequence variation patterns for some Mtb T cell antigens and hyper-conservation for other antigens [1]. The authors compared the sequence variability of 1,226 known Mtb epitopes recognized by human T cells across the genomes of 216 different Mtb strains [1]. Contrary to what might be expected for a pathogen for which cellular immunity controls infection, this analysis confirmed previous findings that human T cell epitopes are hyperconserved across substrains [2, 3]. However, whereas the peptide sequences analyzed were derived from the genome of a single lab strain (H37Rv), T cell recognition was evaluated in TB patients infected with a range of different substrains; this approach may bias the epitopes identified towards conserved sequences. To address this concern, Coscolla, Copin *et al.* examined gene variation in the 216 Mtb genomes, and subsequently analyzed

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these variable genes for the presence of human T cell epitopes. Interestingly, polymorphisms in these selected genes preferentially localized within predicted HLA-binding epitopes. Importantly, screening a set of these variable peptides against TB patients demonstrated that they were *bona fide* human T cell epitopes [1]. Thus, while most known T cell epitopes are under fitness pressure towards sequence hyperconservation, the main finding of the paper is that there exist T cell antigens and derived epitopes that are under an opposing pressure towards variation.

These findings have significant impact on how we think of vaccine design and the concept of protective antigens. If evolution has favored Mtb to conserve sequences recognized by human T cells, then such cells may actually improve Mtb fitness. This may involve the promotion of exaggerated T cell responses, resulting in a terminally differentiated T cell population associated with inflammation, immunopathology and lung cavitation required for efficient transmission (Figure 1A). The implications of this notion are bad news for TB vaccine development efforts that have focused mainly on increasing T cell-derived IFN γ through selection of immunodominant antigens and delivery in strong viral or adjuvant systems optimized to promote maximal Th1 responses. The recent disappointing outcome of one such candidate, MVA85, has triggered a necessary discussion about alternative approaches to TB vaccine development [4].

One interpretation offered by Coscolla, Colin *et al.* is that we should search for vaccine antigens among the relatively few variable antigens [1] – *i.e.* epitope variability reflects that T cells targeting these epitopes are protective against disease. Accordingly, natural T cell responses to these antigens in infected individuals are modest (*i.e.* none of these antigens have previously been reported as immunodominant) and vaccine expansion of these T cells populations could therefore improve control over infection by some as yet undefined protective mechanism (Figure 1B). Indeed, targeting variable influenza antigens via antibodies is an effective vaccine approach, with the caveat that it presents logistical issues towards a broadly protective vaccine. Mtb is not as rapidly mutating as most viral pathogens, and a geographically tailored vaccine to cover local known variants could therefore potentially be useful at least temporarily until the mycobacterial strains adapt. If these antigens are indeed better protective antigens, characterization of variable epitope specific T cells in latently infected versus active/treated TB patients, as well as in animal models of chronic infection, could identify an immune signature greatly needed in vaccine design.

On the other hand, it is still unclear what evolutionary pressures drive hyperconservation of human T cell epitopes in Mtb. Notably, genes encoding highly conserved human T cell epitopes are also highly conserved in Non-Tuberculosis Mycobacteria (NTM) and in long-term *in vitro* cultured BCG; one would not expect selective pressure by the immune system in either of these cases [3, 5]. The alternative interpretation of the data is therefore that hyperconserved sequences represent strong selective pressure for required protein function, marking them as important antigens to target by vaccination. It is important to bear in mind that the vaccines developed and evaluated so far target hyperconserved antigens and are still demonstrably protective in animal models, confirming the vaccine value of these antigenic targets [4]. Conversely, the Mtb-induced T cell response does not reliably clear Mtb

infection, leading to primary and reactivation disease in ~10% of infected individuals. Animal models suggest that ongoing Mtb infection drives T cells to a less protective terminally differentiated state marked by KLRG1-expression, reduced proliferative capacity and altered T cell homing [6]. Thus, vaccine-induced cells targeting the same conserved antigens as are targeted in natural infection can protect – even when administered in infected mice (on top of the endogenous response to the same antigens) [7], by offering functional capacities lacking from the endogenous response (Figure 1C). Compared to the strongly Th1 focused and fully differentiated response found during infection, a vaccine response may contribute functional diversity and enable both early and sustained recruitment of T cells to the site of infection, *e.g.* through Th17 and/or Tfh-like cells [6, 8], and resistance to T cell terminal differentiation by increasing IL-2 secreting memory T cells [9]. Therefore, instead of avoiding the conserved antigens in TB vaccine design, a vaccine that reprograms the response profile against conserved epitopes through improved quality seems a valid strategy (Figure 1C).

If the pathogen has evolved to push host responses towards differentiation and exhaustion, then an intriguing angle to this discussion is to target cryptic or subdominant epitopes that are not (or only weakly) recognized during infection (Figure 1D). Vaccine immune responses against cryptic epitopes in ESAT-6 (a conserved virulence factor), which are not targeted during natural infection can improve control of Mtb infection in mice [10]. Relevant for this discussion, these cryptic epitope-specific T cells are more refractive to the negative influence of chronic infection and showed more limited terminal differentiation and better maintenance of vaccine-imprinted memory than the infection-promoted dominant epitopes from the same molecule.

Overall, the immune response induced by natural infection is insufficient in the 8-9 million people that acquire active TB each year, and TB vaccinology is still searching for a signature of a protective T cell to control disease. Regaining control of immunity by reprogramming the specificity and/or quality the infection-manipulated response may represent a remedy for improved TB control and shift the balance in favor of the host.

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Figure 1.

Vaccine strategies against *M.tuberculosis* (Mtb) antigens. (A) Coscolla, Copin et al. identify both hyperconserved and variable human T cell epitopes within the Mtb genome during natural infection. Variable epitopes may reflect an ability of T cells targeting these epitopes to restrict Mtb infection/transmission. Hyperconserved epitopes may reflect that T cells targeting these epitopes actually benefit Mtb fitness, e.g. by infection-driven terminal differentiation to promote immunopathology for bacterial transmission. This model has implication for vaccine strategies and optimal immune control Mtb strain coverage. (B) If variable epitopes reflect Mtb evasion from T cells that recognize these epitopes, then a successful vaccine could target and expand these protective T cells. (C) If hyperconserved epitopes reflect that Mtb-driven T cells targeting these epitopes benefit bacterial fitness, then a successful vaccine could imprint these T cells with functions associated with improved control of infection (e.g. IL-2 or IL-17 secretion and improved homing to the lung).

(D) Vaccines targeting conserved cryptic/subdominant epitopes, which are not/weakly targeted during infection, could add a vaccine-imprinted T cell response refractive to Mtb-driven terminal differentiation.