Invited Comment - THE LANCET RESPIRATORY MEDICINE

Title: B cells or T cells in TB: a continuing conundrum

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There are already 12 tuberculosis vaccine candidates under phase 1, 2, and 3 development that incorporate vaccine platforms ranging from whole cell vaccines and adjunct proteins to subunit vectors. The promising results published in *The Lancet Respiratory Medicine* of the safety and immunogenicity trial of the new tuberculosis vaccine candidate ID93,1 a fusion protein of four separate *Mycobacterium tuberculosis* antigens given in combination with adjuvant GLA-SE, begs the question, is this just another vaccine candidate or is it novel in some way?

ID93 is a recombinant fusion protein and comprises four antigens associated with virulence (Rv2608, Rv3619, Rv3620) or latency (Rv1813). In the study by Adam Penn-Nicholson and colleagues,1 escalating doses of ID3 plus the adjuvant GLA-SE seemed to be safe and induced robust and sustained antigen-specific CD4 T cell and antibody responses in BCG-immunised, *M tuberculosis*-infected people. While we await further evaluation of this vaccine in phase 2/3 trials, this study sheds further light on two important issues related to vaccine development.

First, the role of antibodies in protective *M tuberculosis* responses has been debated for several decades. IgG subclasses are affected by two main factors: the predominant production of IFN・ leads to IgG2 production, IL-4 and IL-13 switch to IgE, and IgG3 and IL10 switch to IgG1 and IgG3; and the nature of the immunising agent proteins tend to favour IgG1 and IgG3, whereas carbohydrates, including components in adjuvants, induce IgG2.2 Thus, one could argue that IgG measurement presents a relevant biomarker for vaccine immunogenicity and the role of antibodies needs further study.3 However, findings in a non-human primate tuberculosis model suggested a beneficial role of B-cells in *M tuberculosis* infection.4 B lymphocytes can act not only as antigen presenting cells, but also as effector cells,

while antibodies might help to opsonise proteins and further augment T-cell responses.5 Ex-vivo functional analyses of IgG in patients with tuberculosis is required to clarify whether circulating anti-*M tuberculosis* IgG simply is a convenient biomarker for vaccine studies or whether it serves a biological and clinically relevant function.4

The second important issue arising from Penn- Nicholson and colleagues' study is that immune cellsinduced by ID93 plus GLA-SE vaccination in *M tuberculosis* infected

individuals showed greater T-cell differentiation, which is most likely due to existing *M tuberculosis*-specific memory T cells undergoing vaccination-driven expansion.

This finding could be relevant because as differentiated T cells increase in number, less differentiated T cells decrease. These findings shed light on a central issue in vaccinology and immune protection. Adoptive transfer of T cells (in active immunotherapies) show that

protective T-cell responses are associated with a central memory (CD45RA-CCR7-positive) subset that provides long term immunological protection,6 and the same might be true for pathogen-specific cellular immune responses. Do the vaccine-specific differentiated T cells

leave the central memory pool? This question requires answering because circulating T cells represent only 2% of the entire lymphocyte pool and immune-monitoring is based on the assumption that the immune response profile of peripheral T cells might be representative of

the functionality of tissue-resident T cells. This issue could also apply to patients with tuberculosis because immune effector cells are present at sites of disease or inflammation, such as the lymph nodes or in the lung, but not in the peripheral circulation. Results of studies in mice show that the sheer presence of *M tuberculosis*-specific peripheral T cells might be insufficient. Findings of cancer immunology studies have shown that it is difficult to detect circulating antigen-specific

T cells against common mutant tumour antigens. Obtaining access to tissue samples isolated from infection sites in *M tuberculosis*-positive individual is clinically challenging in vaccine studies. However, peripheral T-cell subsets carry markers of recent tissue homing−ie, CXCR3, CCR6, CCR9, and CCR4−which might help to establish whether these cells have been homing to various diseased organs, including the lung, or infected tissue compartments.7 A tissue that is most likely to be differentially present in vaccinees worldwide is fat. Whereas findings of earlier studies showed that adipocytes provided a safe haven for *M tuberculosis*, potentially acting as professional antigen-presenting cells to initiate and sustain cellular immune responses, recent exploratory data in the murine model suggest that adipose tissue can serve as the homing reservoir for longterm memory pathogen-directed immune responses.8

Is there a point of no return if antigen-specific T cellsreside in differentiated T-cell subsets? These T cells might show powerful immune effector functions, defined by cytokine production,9 but without longevity. Although cellular plasticity in organs and tissues of adults is more appreciated, it is also observable in T-cell responses: T cells can revert, even if they are terminally differentiated, to the cradle of long-term immune memory T cells.

Of interest is also the recent finding that Th17 cells, unlike Th1 cells, do not exhibit immune senescence,10 although their role in tuberculosis is controversial and can depend on the stage of disease. Equally important in assessing vaccine immunogenicity in individuals harbouring *M tuberculosis* infection is its epigenetic imprint on the overall dynamicity of immune responses**.**

Ineffective immune responses can be ‘unlearned’ by several ways−ie, by the vaccine plus the adjuvant itself, as recently shown for BCG vaccination11 and therefore

contribute to T-cell fitness and reprogramming. A different mechanism of vaccination might be required, especially in the case of vaccination of individuals previously exposed to *M tuberculosis*. The vaccine target could expand T-cell populations, not yet activated during infection.

Smart adjuvants increase target-specific immune responses and antigen-saving−a vaccine formulation with less antigen content but with an excellent adjuvant can work as effectively as one with a high antigen content (which affects production costs). In addition, the

endogenous microbiome substantially affects antigen specific immune responses. Patients with cancer who harbour certain intestinal bacteria, such as *Akkermansia muciniphila*,12 exhibit better clinical responses to immune checkpoint inhibition. With this in mind, vaccine studies in *M tuberculosis*-infected individuals receiving standard anti-tuberculosis therapy would also require microbiome profiling, considering that antibiotic treatment has longlasting

effects on microbiota.13 Although seemingly complex as first glance, a holistic analytical approach is required to outsmart *M tuberculosis*, an infectious agent that has co-evolved with humankind throughout history.

While safety and clinical efficacy are important in the assessment of new vaccine candidates, the novel insights provided by the vaccine into mechanisms of protection and clinically relevant markers of vaccine take and protection are also important. In this regard, the vaccine

candidate described by Penn-Nicholson and coworkers1 is not just another vaccine−it is novel, focusing attention on the continuing conundrum of long-term immune memory T-cell responses in tuberculosis.

References

1 Penn-Nicholson A, Tameris M, Smit E, et al. Dose definition of the novel tuberculosis vaccine ID93 + GLA-SE for tuberculosis endemic countries: a randomised, double-blind, controlled phase 1 clinical trial. *Lancet Respir Med* 2018

2 Germann T, Bongartz M, Dlugonska H, et al. Interleukin-12 profoundly up-regulates the synthesis of antigen-specific complement-fixing IgG2a, IgG2b and IgG3 antibody subclasses in vivo. *Eur J Immunol* 1995; **25:** 823‑29.

3 Boggiano C, Eichelberg K, Ramachandra L, et al. The impact of *Mycobacterium tuberculosis* immune evasion on protective immunity: implications for TB vaccine design - Meeting report. *Vaccine* 2017; **35:** 3433‑40.

4 Jeyanathan M, Yao Y, Afkhami S, Smaill F, Xing Z. New tuberculosis vaccine strategies: taking aim at un-natural immunity. *Trends Immunol* 2018; published online Feb 9. https://doi.org/10.1016/j.it.2018.01.006.

5 Rao M, Valentini D, Poiret T, et al. B in TB: B cells as mediators of clinically relevant immune responses in tuberculosis. *Clin Infect Dis* 2015; **61** (suppl 3)**:** S225‑34.

6 Maus MV, Fraietta JA, Levine BL, Kalos M, Zhao Y, June CH. Adoptive immunotherapy for cancer or viruses. *Annu Rev Immunol* 2014; **32:** 189‑225.

7 Jacquelot N, Enot DP, Flament C, et al. Chemokine receptor patterns in lymphocytes mirror metastatic spreading in melanoma. *J Clin Invest* 2016; **126:** 921‑37.

8 Han SJ, Glatman Zaretsky A, Andrade-Oliveira V, et al. White adipose tissue is a reservoir for memory t cells and promotes protective memory responses to infection. *Immunity* 2017; **47:** 1154‑68.

9 Becattini S, Latorre D, Mele F, et al. Functional heterogeneity of human memory CD4(+) T cell clones primed by pathogens or vaccines. *Science* 2015; **347:** 400‑06.

10 Bowers JS, Nelson MH, Majchrzak K, et al. Th17 cells are refractory to senescence and retain robust antitumor activity after long-term ex vivo expansion. *JCI insight* 2017; **2:** e90772.

11 Arts RJ, Moorlag SJ, Novakovic B, et al. BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. *Cell host & Microbe* 2018; **23:** 89‑100.

12 Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018; **359:** 91‑97.

13 Wipperman MF, Fitzgerald DW, Juste MA, et al. Antibiotic treatment for Tuberculosis induces a profound dysbiosis of the microbiome that persists long after therapy is completed. *Sci Rep* 2017; **7:** 10767.