Humoral immunity in tuberculosis

Tuberculosis (TB) is second only to HIV in the number of deaths caused by infection worldwide, with 9 million new cases and 1.5 million deaths reported in 2013 [1]. The Bacille Calmette-Guérin (BCG) vaccine is an approved vaccination against TB, although its protection is limited to severe childhood disease with no impact on adult pulmonary infection, which drives the spread of the global epidemic [2, 3]. The development of an effective vaccine against TB would be a major step in meeting the Stop TB Partnership's goal of eliminating TB as a public health concern by 2050 (reviewed in [4]). Here we explore a recurrent question in such vaccine design – can antibody-mediated immunity (AMI) contribute to protection against TB?

In the late 19th century, the emergence of serum therapy against pathogens such as *Streptococcus pneumoniae* gave rise to hope that a cure for TB would soon be found [5]. The ability of serum to agglutinate cultures of the tubercle bacillus was demonstrated in the late 1800's, and trials were commenced in an attempt to cure patients with serum taken from horses, cows, or pigs that had been inoculated with varying preparations of mycobacteria. Despite hints of initial success in resolving symptoms, these trials were eventually halted as a result of subsequent equivocal results, inadequate controls and variability in the serum preparations. Albert Calmette, one of the inventors of BCG vaccination, later summarized the disappointing results of serum therapy, "Up to now, therefore, it does not seem that specific

serotherapy has realized the hopes which it aroused" (reviewed in [5]). Subsequently, the lack of correlation between antibody titers and resistance to TB, as well as the scientific obstacle of exactly how antibodies would protect against an intracellular pathogen, directed the search for a TB vaccine away from AMI [2]. The shift in the dichotomy of TB immunity away from AMI towards cell-mediated immunity (CMI) crystallized with the rise of the HIV-TB syndemic, research on which provided empirical evidence that the progressive loss of $CD4^+$ T-cells caused by HIV infection directly relates to increasing susceptibility to developing TB [3].

The current TB vaccine development pipeline reflects this paradigm. The underpinning rationale of the vaccination strategy in the past twenty years has been to induce a T-cell response against dominant *M. tuberculosis (M.tb)* antigens, in the hope that these T cells could rapidly control primary infection and/or prevent progression to active disease [3, 6]. Such an immune response may be induced by recombinant forms of BCG, virally vectored vaccines or avirulent mycobacteria such as *Mycobacterium vaccae* or RUTI (reviewed in [6]). The MVA85A vaccine is the most advanced candidate of this mold, having recently completed a Phase IIb trial in a cohort of 2797 South African infants [7]. Although well tolerated and capable of eliciting a recall $CD4^+$ T-cell response on stimulation with *M.tb* antigen, the MVA85A vaccine failed to confer any protection against infection or disease [7], nor did it prevent disease in HIV-infected patients (Ndiaye *et al*. Lancet Respir Med in press). The setback of a highly immunogenic T-cell vaccine having no efficacy against TB has led to the question of the sufficiency of an isolated

Th1-type response in protection against *M.tb* infection, and to a revival in interest in the contributions of other arms of immunity, such as non-classically restricted T-cells, Th17 cells and antibodies [3, 6]. A vaccine that prevents primary infection would have a large impact on reducing the transmission of TB, and the induction of protective antibodies has the conceptual advantage of being capable of neutralizing or preventing the uptake of the infecting inoculum (reviewed in [8]). The classical strategy of vaccination is to induce AMI, even against intracellular pathogens such as *Salmonella typhi*, with the only exceptions in licensed vaccines being for varicella zoster virus and the partially effective BCG vaccine [2]. The tetanus toxoid vaccine provides a precedent for the induction of "synthetic" immunity; given that antibodies are not produced against tetanus toxin during natural infection, antibodies generated during infection fail to provide protection, however vaccination with the toxoid has been shown to induce antibodies that are highly effective at preventing disease [9]. The renewed attention to AMI converges with evidence on how antibodies could elicit protection against *M.tb,* discussed in this article, as well as with advances in the ability to investigate the difference in efficacy of individual monoclonal antibodies (mAbs) rather than whole serum or polyclonal antibody approaches.[2]

Recent research has challenged the long-held notion of a polarization of immunity to either a protective cell-mediated response or a non-functional antibody response. The granuloma, which is characteristic of organized CMI against TB, has been shown to contain B cells – a finding which suggests their involvement in orchestrating the containment of infection [10]. The role of

B cells in the granuloma appears to be immunomodulation through the secretion of cytokines such as IL-10, and the production of immunoglobulin to engage Fcγ antibody receptors (FcγRs) expressed on mononuclear phagocytes (reviewed in [10]. Activation of the FcγR and its downstream pathways by antibodies may play a vital role in controlling TB infection. Mice with loss of function of the FcγR have been shown to have greater lung inflammation and neutrophil infiltration on challenge with TB [11]. Conversely, FcγRIIb$^{-/-}$ mice, which lack the inhibitory subtype of the FcγR, have been shown to produce more IFN-γ, have less lung pathology and reduced mycobacterial burden in the lungs and spleen when challenged with *M.tb* [11]. Antibodies bound to the surface of BCG from vaccinated individuals further demonstrates this synergy between AMI and CMI by augmenting antigen presentation to T cells with a subsequent increase in Th1 cytokine production [2]. In light of this, it is interesting to note that the upregulation of the gene for the high-affinity antibody receptor, FcγR1A, is a consistently strong finding across transcriptomic signatures of active TB patients [12]. The importance of this finding may further be inferred from a small cohort of HIV-TB co-infected patients in Ethiopia, in whom copy-number deletions of the FcγR3B and FcγR2C receptors were associated with greater susceptibility to developing TB [13]. Intriguingly, the intracellular antibody receptor TRIM21, capable of triggering inflammatory signaling via cytosolic recognition of antibody, has been shown to be elevated in TB-infected patients compared with healthy controls – a facet of AMI not previously described in the disease [12]. The beneficial effector functions of the upregulated antibody receptors during

human infection would depend on the specificity of the antibodies that are capable of engaging them during TB infection.

Current vaccines against bacterial pathogens induce neutralizing antibodies against bacillary surface polysaccharides, thus high-affinity antibodies to surface constituents of *M.tb* may be of interest [5, 6]. To this end, efforts to map the targets of the antibody response to TB infection have been advanced by the utilization of protein microarrays. One such study revealed that extracellular proteins secreted by *M.tb*, not surface antigens, are strongly immunodominant in active disease despite representing only 0.5% of the entire proteome [15]. . The total immunoproteome includes almost five hundred *M.tb* proteins, with the group of antigens rarely recognized by patient sera enriched for membrane-associated proteins [15]. Humans do produce antibodies that bind the cell surface of live *M.tb*, but there appears to be a drop in avidity during active infection which may represent antibody class switching or the presentation of alternative antigens by *M.tb* during the course of infection [16]. Several immunogenic surface proteins, such as the PE/PPE family of proteins, exhibit genetic variability suggestive of antigenic variation – an intriguing postulate given that immunodominant T-cell epitopes are hypothesized to be evolutionarily hyper-conserved [16,17]. In line with this concept, murine mAbs against the surface-exposed heparin-binding hemagglutinin (HBHA) protein of *M.tb,* also a target of the human antibody response, have been shown to interfere with bacterial adhesion to macrophages and decrease dissemination of bacilli in murine models [2]. Taken together, these findings lend credence to the possibility that secreted

antigens could divert the antibody response away from surface proteins during active infection because such antibodies may elicit a beneficial host immune response.

The mechanisms by which antibodies could function against TB are thought to be via modulation of host immunity to more efficiently control infection, or through direct anti-mycobacterial properties. In the former case, antibodies may opsonize TB, enhance phagolysome maturation and intracellular killing or, as discussed, stimulate an amplified cell-mediated response [2]. As an example, a human monoclonal IgA against α-crystallin requires the presence of both IFN-γ and the FcαR1/ CD89 antibody receptor to elicit protection in humanized mice [2]. Immunomodulation may also occur by clearance of pro-inflammatory secreted proteins to the lymphatic system, resulting in reduced localized lung inflammation [2]. The existence of mAbs with direct anti-mycobacterial effect in humans is speculative but of great interest. The antibody repertoires of healthcare workers exposed to TB who remained tuberculin skin-test negative, a theoretically resistant population, revealed a preference for a VH3-23, D3-3, JH4 IgA gene recombination which was absent in their colleagues with previous active disease or latent TB [19]. One plausible explanation for this is that resistant individuals produce IgA in the lung mucosa capable of preventing primary infection. The existence of mAbs capable of direct disruption of cell wall division or maintenance has been demonstrated in *C. neoformans*, an intracellular pathogen with a waxy capsule, where protective mAbs alter lipid metabolism gene expression and increase the organism's susceptibility to antifungal drugs [2]. Whether other

functions of antibodies that elicit antibody-dependent cytotoxicity and complement activation can protect against TB remains to be studied, but it is clear from murine models that mAbs against TB can modulate the natural history of infection.

Advances in the ability to produce human mAbs against TB may facilitate the search for functional antibodies in humans against TB of known specificity. New molecular techniques of single B-cell immunoglobulin gene cloning and expression have allowed the rapid production of fully human neutralizing mAbs to a broad range of pathogens such as dengue, influenza, HIV, malaria and streptococcus [20-23]. The pathway of mAb production through to epitope-based immunogen development has been validated in the case of respiratory syncytial virus (RSV), where the epitopes of commercial neutralizing antibodies were mapped and then produced as immunogens that elicited protection against RSV in vaccinated macaques. This technique of producing human mAbs has also led to the discovery of the novel dengue envelope dimer epitope, a target of broadly neutralizing mAbs against live virus and derived directly from dengue patients that is encouraging for future vaccine design [21,23]. The Bill and Melinda Gates Foundation's 2014 revised TB Vaccine Strategy lists the exploration of entirely novel vaccine concepts to exploit greater immunological diversity as a key strategic goal, and the technology here presents one such an opportunity to harness AMI against TB [26]. The original question of whether AMI contributes to immunity against TB is once again compelling, and the impetus to deliver a definitive

answer will surely continue to grow as the search for an effective vaccine continues.

Ashley Jacobs 4 and Robert John Wilkinson 1,2,3,4

1. Clinical Infectious Diseases Research Initiative, Institute of Infectious Disease and Molecular Medicine
2. Department of Medicine, University of Cape Town, South Africa
3. Division of Mycobacterial Research, MRC National Institute for Medical Research, London NW7 1AA
4. Department of Medicine, Imperial College London, United Kingdom, W2 1PG,

Correspondence: Dr. Ashley Jacobs: ashley.jacobs@imperial.ac.uk

References

1. **WHO**, Global Tuberculosis Report 2014, Geneva, Switzerland, 2014.
2. **Glatman-Freedman A**. The role of antibody-mediated immunity in defense against *Mycobacterium tuberculosis*: Advances toward a novel vaccine strategy. *Tuberculosis*. 2006;**86**:191197.DOI: 10.1016/j.tube.2006.01.008.
3. **Delogu G, et al.**. Critical research concepts in tuberculosis vaccine development. *Clin Microbiol Infect*. 2014; **20**:59–65.DOI: 10.1111/1469-0691.12460.

4. **Dye C, et al.**. Prospects for Tuberculosis Elimination. *Annu. Rev. Public. Health.* 2013;**34**:271–286.DOI: 10.1146/annurev-publhealth-031912-114431.
5. **Glatman-Freedman A and Casadevall A**. Serum therapy for tuberculosis revisited: Reappraisal of the role of antibody-mediated immunity against *Mycobacterium tuberculosis*. *Clin. Microbiol. Rev.* 1998; **11**:514–532.
6. **Andersen P and Woodworth JS**. Tuberculosis vaccines - rethinking the current paradigm. *Trends Immunol.* 2014; **35**:387–395. DOI:10.1016/j.it.2014.04.006.
7. **Tameris MD, *et al.*** Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet*. 2013; **381**:1021–8. DOI: 10.1016/S0140-6736(13)60177-4.
8. **Hawn T, *et al.*** Tuberculosis Vaccines and Prevention of Infection.*Microbiology and Molecular Biology Reviews*. 2014;**78**:650671.DOI: 10.1128/MMBR.00021-14.
9. **Johnson S**. Antibody responses to clostridial infection in humans. *Clin. Infect. Dis.* 1997; **25 Suppl 2**:S173–S177.
10. **O'Garra A, et al**. The Immune Response in Tuberculosis. *Annu. Rev. Immunol.* 2013; **31**:475–527.DOI: 10.1146/annurev-immunol-032712-095939.
11. **Maglione PJ, et al**. Fc Receptors Regulate Immune Activation and Susceptibility during *Mycobacterium tuberculosis* Infection. *J. Immunol.* 2008; **180**:3329–3338. DOI: 10.4049/jimmunol.180.5.3329.

12. **Joosten S, et al**. A Helicopter Perspective on TB Biomarkers: Pathway and Process Based Analysis of Gene Expression Data Provides New Insight into TB Pathogenesis. *PLoS ONE*. 2013; **8**.DOI: 10.1371/journal.pone.0073230.
13. **Machado LR, *et al.*** Copy number variation of Fc gamma receptor genes in HIV-infected and HIV-tuberculosis co-infected individuals in sub-saharan Africa. *PloS one*. 2013; **8**:e78165. DOI: 10.1371/journal.pone.0078165
14. **Kunnath-Velayudhan S, *et al.*** Dynamic antibody responses to the *Mycobacterium tuberculosis* proteome. *Proc. Natl. Acad. Sci. U.S.A.* 2010; **107**:14703–14708.DOI: 10.1073/pnas.1009080107.
15. **Perley C, et al**. The Human Antibody Response to the Surface of *Mycobacterium tuberculosis*. *PLoS ONE*. 2014; **9**.DOI: 10.1371/journal.pone.0098938.
16. **Talarico S, et al**. Variation of the *Mycobacterium tuberculo*sis PE_PGRS33 Gene among Clinical Isolates. *Journal of Clinical Microbiology*. 2005;**43**:49544960.DOI: 10.1128/JCM.43.10.4954-4960.2005.
17. **Comas I, *et al.*** Human T cell epitopes of *Mycobacterium tuberculosis* are evolutionarily hyperconserved.*Nat. Genet.* 2010; **42**:498–503.DOI: 10.1038/ng.590.
18. **Smith K, et al**. Rapid generation of fully human monoclonal antibodies specific to a vaccinating antigen. *Nat Protoc*. 2009; **4**:372–384.DOI: 10.1038/nprot.2009.3.

19. **Wilson P and Andrews S**. Tools to therapeutically harness the human antibody response. *Nat. Rev. Immunol.* 2012; **12**:709–719.DOI: 10.1038/nri3285.
20. **Muellenbeck M, *et al.*** Atypical and classical memory B cells produce *Plasmodium falciparum* neutralizing antibodies. *J. Exp. Med.* 2013; **210**:389–399.DOI: 10.1084/jem.20121970.
21. **Dejnirattisai W, *et al.*** A new class of highly potent, broadly neutralizing antibodies isolated from viremic patients infected with dengue virus. *Nat. Immunol.* 2014.DOI: 10.1038/ni.3058.
22. **Correia BE, *et al.*** Proof of principle for epitope-focused vaccine design. *Nature*. 2014; **507**:201–6. DOI:10.1038/nature12966
23. **Hanekom W**. Revision of the Bill and Melinda Gates Foundation TB Vaccine Strategy, 2014. Presentation at Keystone symposium on host response to tuberculosis, Santa Fe, NM, 24th January 2015