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RESEARCH HIGHLIGHT

Role of antibodies in vaccine-mediated protection against tuberculosis

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The development of an effective prophylactic tuberculosis (TB) vaccine has been the most targeted objective in TB research since the currently used Bacillus Calmette–Guerin (BCG) vaccine is effective only against primary childhood disease but not against the most common form, pulmonary disease. For the past approximately five decades, protective immunity was considered to be mediated by T cells, but the resulting extensive testing of cellular subsets (i.e., CD4, CD8, Th1/2, γδ, and Treg cells) and their cytokine products failed to identify a reliable test that correlated with protection following infection with *Mycobacterium tuberculosis* (*Mtb*) or vaccination. This failure could be attributed to immunoregulatory cytokines and to pathogenic "decoy" immune mechanisms induced by antigens, which have been evolved by the pathogen to evade host resistance [1].

In a study published in the December 2021 issue of *Nature Immunology*, Irvine et al. [2] reported that BCG induces a stronger antibody response following intravenous (i.v.) than intradermal (i.d.) administration in rhesus macaques. IgM antibody levels in the plasma and bronchoalveolar lavage (BAL) fluid were the best indicators of protection against *Mtb* challenge since the *Mtb* burden in the lungs was negatively associated with plasma titers against lipoarabinomannan (LAM) and PstS1 antigens at both 8 and 24 weeks after vaccination. However, the IgG1 and IgA isotype antibody repertoires in the BAL fluid were wider and included Apa antigen specificity. Protected and susceptible macaques were distinguished using a sophisticated partial least-squares discriminant analysis model. The highly balanced cross-validation accuracy was based on three antibody features: BAL fluid anti-HspX/Acr1 IgM at week 4, plasma anti-LAM IgG1 at week 8, and plasma anti-LAM IgM at week 24. The observed antigen-specific and Ig isotype-associated findings contribute to the recent research trend on the involvement of antibodies in protective immunity and the development of vaccination or immunotherapy against TB.

The complexity of T-cell responses in TB, as well as the knowledge that the eminent success of vaccinations against other infectious pathogens (e.g., smallpox, polio, and tetanus) is mediated by antibodies, has encouraged recent research on the role of antibodies in TB vaccination. This approach was supported by the findings in mouse models, whereby depletion of B cells, particularly of IgA producers [3], aggravated TB infection, and by the observation of passive protection with monoclonal IgA antibodies against primary infection as well as relapse from chemotherapy, with a potential application toward the immunotherapy of drug-resistant TB [4–6]. Previously, serological surveys of patients with active TB-associated antigen specificity with organ localization, bacterial load, and HLA association, highlighting the beneficial immunodominance and *Mtb* specificity of the PstS1, Acr1/HspX protein, and LAM antigens for potential serodiagnostic application but without any attribution to vaccination potential [7]. In light of these serological studies in humans, it is surprising that in macaques, elevated IgM antibody levels following intravenous BCG vaccination are most closely associated with reduced pulmonary *Mtb* infection [2].

Previous studies on mucosal or i.v. BCG vaccination in macaques have shown near sterilizing immunity to *Mtb* infection challenge, which correlated with increased *Mtb*-specific IgG, IgA, and IgM antibody levels in the plasma and BAL fluid. In humans, oral administration of BCG in a small subset of volunteers led to increased anti-LAM IgA titers in tears. I.d. BCG vaccination increased LAM-specific IgG serum levels, which correlated with the opsonization and bactericidal action of macrophages and elevated levels of IgG antibodies to *Mtb* glycolipids in vaccinated health care workers (reviewed in [8]).

Despite the above-quoted studies, it is still not clear to what extent the different classes of antibodies contribute to host protection. In the latest study by Irvine et al., IgG and IgM antibodies isolated from BAL fluid from BCG-vaccinated macaques were subsequently shown to opsonize *Mtb* and enhance bacterial uptake by macrophages in vitro [2]. Interestingly, they found that high-affinity LAM-specific IgM antibodies restricted *Mtb* growth in whole blood but not in macrophages alone [2], probably reflecting the contribution of complement activation to opsonization and intracellular killing (Fig. 1).

The antigen specificity of passive vaccination, investigated with mouse monoclonal antibodies, revealed the effectiveness of anti-Acr1 IgA but not anti-PstS1 antibodies [4]. The suggested mechanisms considered extracellular interaction with *Mtb* followed by FcαR-mediated intracellular bactericidal action within the phagosome of macrophages, possibly involving galectin 3, rather than blockade of the infection. This interpretation is also supported by the observed immunomodulatory influence of cytokines from different subsets of T cells, i.e., the synergistic effects of recombinant Interferon-γ and anti-IL-4 [5]. Notably, passive protection by monoclonal IgA has been found to be confined to the lungs, with no impact on the dissemination of

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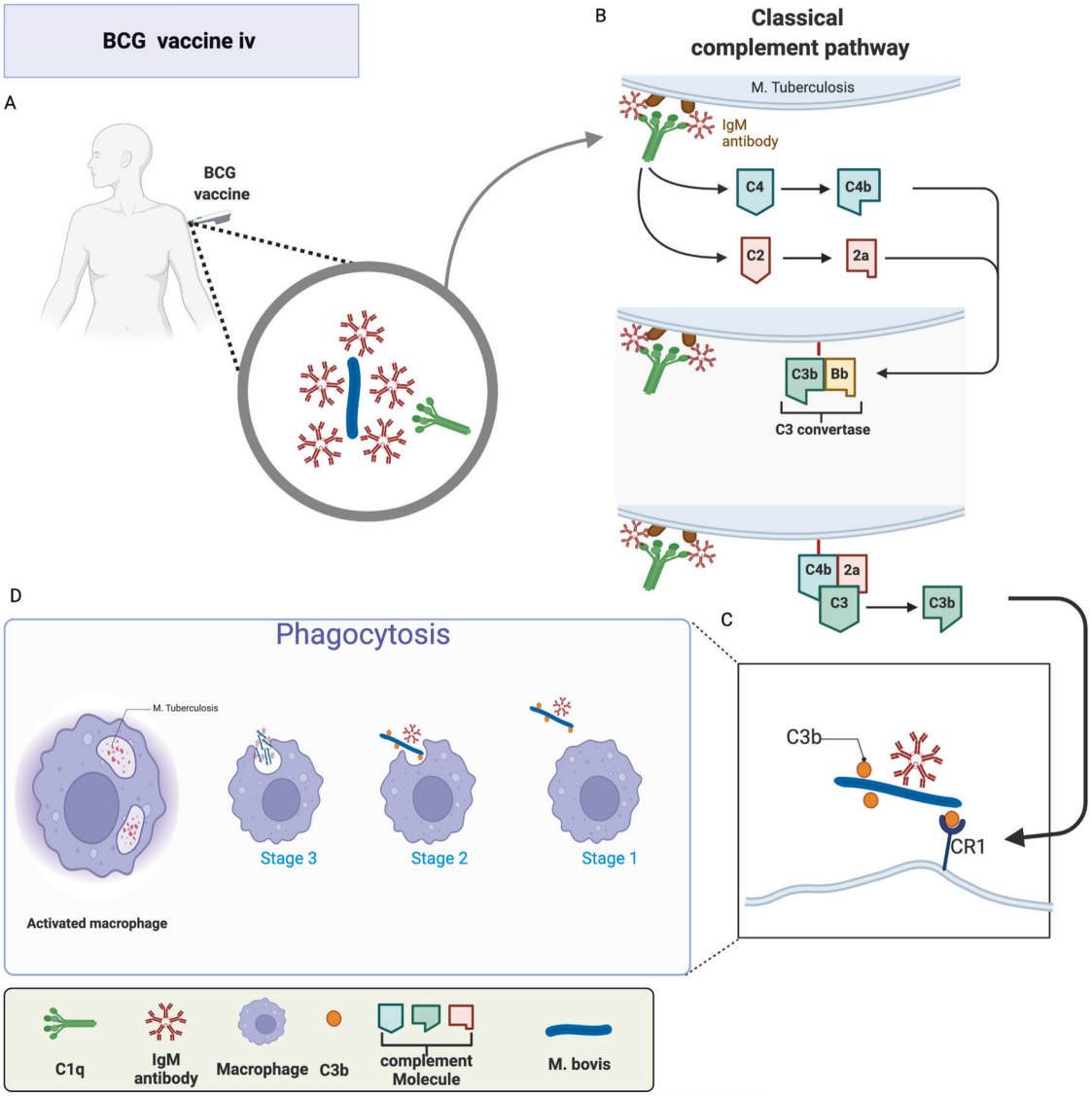

Fig. 1 Proposed mechanisms of action of *Mtb*-specific IgM antibodies whose production is induced by BCG vaccination. **A** BCG vaccine given i.v. induces the production of IgM antibodies specific for many different *Mtb* antigens, including LAM. **B** *Mtb* antigen-specific IgM antibodies opsonize *Mtb* bacilli and activate the classical complement pathway, resulting in opsonization with C3b. **C** *Mtb* bacilli that are sufficiently coated with these serum-derived ligands bind to CR1 and **D** are subsequently phagocytosed by macrophages in membrane-bound phagosomes for intracellular killing

infection to the spleen, and could not be correlated with its activity in the infection of isolated macrophages. However, in other studies using in vitro infection assays, the passive protection of mice by polyclonal serum antibodies from latently infected health care workers has been associated with distinct glycosylation profiles of antibodies [9]. The possible role of Acr1 antigen-specific antibodies seems implicated also by their selective presence in latent TB [7] and by their elevated levels in i.v. BCG-vaccinated macaques [2].

Since the lungs are the site of entry for *Mtb* infection and for the reactivation of disease in adults, targeting vaccination toward pulmonary mucosal immunity has been a focus of interest. Protection by intranasal (i.n.) vaccination using recombinant gene constructs has been attributed to IgA or IL-10 secreted by Th17 cells [10]. The finding of higher antibody levels in BAL fluid following endobronchial or i.v. vaccine delivery suggested that deep pulmonary rather than intranasal (i.n.) or i.d. localization of antigen delivery is necessary for effective TB vaccination. The lung-localized protective immunity was attributed to Th17 cells following i.n. administration of recombinant DNA constructs or to the recruitment and retention of protective CD8 T cells. Protection by i.n. passive vaccination with monoclonal IgA antibody involved the proinflammatory CD89 IgA receptor on macrophages, which could transmit bactericidal signals against intracellular *Mtb* [11]. Interestingly, however, the bactericidal effect of BAL-derived FcγR2A-binding and NK cell-activating antibodies from i.v. BCG-vaccinated macaques was demonstrable when using BAL fluid-derived but not monocyte-derived *Mtb*-infected macrophages in vitro [2].

A new approach toward antibody immunotherapy of TB has been based on monoclonal single-domain antibody ligands with the T-cell receptor (TCR)-like specificity. Unlike the combined site specificity of conventional antibodies, mimicking the TCR recognition of peptide epitopes complexed with HLA class I molecules targets the surface of *Mtb*-infected cells. Selection of such reagents benefited from prior knowledge of the immunodominant epitopes of the human CD8 T-cell repertoire. Using phage display library technologies, single-domain clones (nanobodies)

were selected, recognizing epitopes on the Acr1 and Ag85B antigens [12–15]. These antibody ligands yet need to be modified into "immunotoxins" following conjugation, e.g., with suitable apoptosis-inducing ligands, to become mycobactericidal, with the potential for being developed as adjuncts for the chemotherapy of TB or for preventing the reactivation of dormant into active TB. Experience with the technologies related to the easy production of these stable, small-size (15-kDa) ligands with low immunogenicity is also available from recent studies in cancer, viral infections, and other diseases.

In conclusion, we consider that future research on human TB needs to address the following two aspects. First, in view of the immunoregulatory influence of cytokines on IgA passive immunotherapy, the effector actions of the same antibody following active vaccination may be negatively modulated by cytokines secreted by Th2 cells to help induce the relevant B-cell response. This constitutes a substantial aspect based on the interpretation of data from studies of passive versus active vaccination. Second, since active pulmonary TB in human adults occurs as reactivation in a minority (~5%) of latently infected subjects, it is necessary to test the efficacy of any vaccine candidates for protection in animal models, evaluating reactivation of dormant infection and not merely the bacterial load of the primary *Mtb* infection. This will represent a substantial increase in the cost, workload, and duration of investigation, which probably contributed to the omission of such testing in the vast majority of past vaccine development studies.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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