Immunobiology of Pediatric Tuberculosis: Lessons Learned and Implications for an Improved TB-Vaccine

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Abstract

Children, especially neonates and young infants, are uniquely vulnerable to tuberculosis (TB) and frequently present with primary progressive pulmonary or disseminated disease. There is an urgent need to understand the unique immunobiology of Mycobacterium tuberculosis (Mtb) in young infants and to identify protective infant immune responses. The existing vaccine against TB, Mycobacterium bovis bacillus Calmette–Guérin (M. bovis BCG), provides a partial protection against TB disease and disseminated forms of TB in infants; however, it is unknown how this partial protection is mediated. To end pediatric TB morbidity and mortality, a fully efficacious next-generation TB-vaccine is needed. Here, we focus on our current understanding of TB immunobiology as it pertains to young infants, and we evaluate what BCG-vaccination, as well as recently trialed novel TB-vaccines, has taught us about the immunobiology of mycobacterial infection in this population.

Keywords
► tuberculosis
► BCG
► vaccine
► Mtb
► infant
► pediatric
► T cell
► Immunity
► immune response

The vast majority of immunocompetent adults exposed to the pathogen Mycobacterium tuberculosis (Mtb) contain or eliminate their infection and never progress to develop tuberculosis (TB) disease. Children, specifically those <5 years old, are uniquely vulnerable to develop TB following Mtb exposure and often present with severe disease phenotypes, such as TB meningitis and miliary TB. Studies from the pre-antibiotic era demonstrate that up to 40% of Mtb-infected infants <12 months old will develop pulmonary TB and up to 20% will experience severe, disseminated disease.1 Although the risk of progression to TB gradually declines during childhood, children <3 years old, and children of any age with underlying human immunodeficiency virus (HIV)-infection or severe malnutrition, are considered at extremely high risk.1,2 Notably, 95% of young children who develop TB do so within 12 months of their first exposure, a phenomenon referred to as progressive primary disease.1 The clear epidemiologic and clinical differences, observed in the risk and severity of TB between young children and adults, support the concept that the immunobiology of Mtb infection in young children differs substantially from that observed in healthy adults.

The existing vaccine against TB, Mycobacterium bovis bacillus Calmette–Guérin (M. bovis BCG), is part of the World Health Organization’s (WHO) Expanded Program on Immunization (EPI) and is routinely administered at birth in nearly all TB-endemic countries. BCG provides partial protection against TB disease and, importantly, disseminated forms of TB during infancy.3,4 However, BCG can induce serious adverse events in HIV-infected infants.5 The WHO has put forth a strategy to end global morbidity and mortality from TB by 2035.6 To end infant TB morbidity and mortality, however, a fully efficacious next-generation TB-vaccine that is safe to use in HIV-infected infants is urgently needed. BCG-induced immune responses have been extensively studied, thereby providing critical insights into the capacity of the infant immune system to respond to mycobacteria. However, to make substantial progress in pediatric TB-vaccine development, a more thorough understanding of the capacity of the human infant to control Mtb infection and of

received March 17, 2017
accepted after revision April 27, 2017
Issue Theme Advances in Pediatric Tuberculosis; Guest Editor: April Palmer, MD.
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ISSN 1305-7707.
BCG-induced immune mechanisms that contribute to its partial efficacy is still needed. In this review, we will focus on our current understanding of TB immunobiology, especially as pertains to young infants. In addition, we will evaluate what BCG-vaccination, as well as recently trialed novel TB-vaccines, has taught us about the immunobiology of mycobacterial infection in this uniquely vulnerable population.

Innate Responses to Mycobacteria in the Young Infant

*Mtb* is an intracellular pathogen, residing within alveolar macrophages (AM) that phagocyte the pathogen using complement and mannose receptors. Pattern recognition receptors (PRR) expressed on AM, including nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and Toll-like receptors (TLRs), serve to activate infected AM and promote production of a variety of proinflammatory cytokines, such as interleukin (IL)-1β, IL-18, and tumor necrosis factor-α (TNF-α), chemokines, such as CXCL10 and CXCL8, antimicrobial molecules, such as cathelicidin, and enzymes that promote autophagy. This inflammatory process recruits a variety of different cell types into the lung, where an organized granuloma is formed in an attempt to restrict bacillary growth.

Among PRR involved in innate recognition of *Mtb*, only TLRs have been extensively studied in infants and young children (reviewed in17). Both *Mtb* and *M. bovis* BCG are rich with triacylated lipoproteins recognized by TLR-1/2, and proinflammatory responses to TLR-1/2 ligands are vital in innate recognition and control of *Mtb*. Overall, TLR-induced responses in young infants differ from adults: in cord blood, diminished production of TNF-α by monocytes in response to ligands for TLR-1/2 heterodimers has been demonstrated in comparison to adult monocytes, and this has been linked to high expression of adenosine in the serum of newborns. Furthermore, cytokines produced by monocytes and dendritic cells (DCs) following TLR-stimulation have been shown to skew toward T-helper (Th)-17 and Th-2 differentiation in newborns, a phenotype that persists at least until age 1 year. Specific polymorphisms in the genes for TLR-6, TLR-1, and TLR-2 have also been associated with risk of TB disease among *Mtb*-exposed children from different ethnic backgrounds. In the context of BCG, innate responses were compared among South African newborns, 10-week old, and 36-week old infants: stimulation with a synthetic TLR2-ligand-induced multiple signaling pathways in older infants, while only a singular pathway in neonates. In the same study, in vitro incubation of whole blood with BCG demonstrated an age-dependent increase in expression of the co-stimulatory molecule CD40 on monocytes and myeloid DCs and increasing expression of TNF-α, IL-6, and IL-12p40 in monocytes. Despite this progress in defining unique attributes of TLR responses in monocytes and DCs in young infants, there have been limited studies to investigate the competency of other primary phagocytic responses to mycobacteria in infants. In a study comparing the in vitro capacity of monocyte-derived macrophages (MDM) from BCG-vaccinated infants and adults to ingest and contain *Mtb*, there was no defect observed in uptake of *Mtb* by infant MDM or in containment of *Mtb* growth after 7 days. Overall, there is substantial ex vivo evidence that TLR-mediated responses by cells of monocytes that are involved in the immunobiology of TB are altered during early infancy. It is possible that these unique aspects of the pediatric TLR response contribute to poor containment of infection within the lung, early dissemination of infection, and the progressive primary disease clinical phenotype observed during infancy. Further definition of the competency of primary phagocytic responses to contain mycobacteria and promote effective priming of T cells during early infancy remain research priorities.

Neutrophils are also thought to have a role in the immune response of TB, although whether they contribute toward protection or exacerbation of active disease remains controversial. Neutrophils are abundant in the airways of adults with pulmonary TB, and in some studies, neutrophils have been shown to have mycobactercidal function. Healthy newborns have a reduced pool of neutrophils, and their neutrophils are limited in their capacity to migrate to sites of infection. However, whether or not neonatal neutrophil dysfunction contributes to the increased risk of TB disease during infancy remains unknown.

The Th-1 Axis and Contribution of CD4⁺ and CD8⁺ T Cells to Antimycobacterial Defenses during Infancy

CD4⁺ T cells are key contributors to host defense against TB disease. This has been dramatically illustrated by the HIV epidemic, where the associated decline in CD4⁺ T cell frequency and function is well recognized to significantly increase the risk of TB in adults. Although data regarding rates of TB-HIV co-infection in children continue to be scarce, an increased risk of TB, including severe disseminated forms of disease, is also observed among HIV-infected children. These observations, combined with the well-recognized risk of TB among HIV-infected adults, support the critical role that CD4⁺ T cells play across the age spectrum in host immunity against *Mtb*.

The cytokine interferon-gamma (IFN-γ), predominately produced by activated T cells and natural killer (NK) cells, is a key component of a type-1 helper (Th-1) proinflammatory immune response, as are the cytokines IL-2 and TNF-α. Production of IFN-γ has long been considered critical to a successful immune response against *Mtb*, as first observed in murine studies where genetic disruption of IFN-γ production led to disseminated disease. The identification of family members demonstrating vulnerability to disease with non-tuberculous mycobacteria (NTM) species that are considered weakly virulent, termed ‘Mendelian Susceptibility to Mycobacterial Diseases’ (MSMD), confirmed the essential role of the of IFN-γ-signaling pathway to control mycobacterial infections in humans (as reviewed in). Until recently, the contribution of IFN-γ to host defense against mycobacteria was thought to be attributed to the ability of this cytokine to activate infected macrophages, stimulate antimycobacterial
functions, and promote antigen presentation. More recent murine studies suggest, however, that IFN-γ may contribute to host immunity against mycobacteria by inhibiting IL-17 responses and subsequent excessive inflammation.

It has long been recognized that neonatal and infant T cell proinflammatory functions in response to some stimuli are reduced when compared with adult responses. Reduced production of the Th-1 cytokine IFN-γ in response to polyclonal stimulation, mitogen, and oral polio and diphtheria-tetanus-acellular pertussis vaccine has been reported among young infants. However, more recent studies have demonstrated that neonatal T cells do have the capacity to mount robust Th-1 type responses. For example, it was recently reported that neonatal naïve CD4+ T cells generate IFN-γ and IL-2 responses to direct co-stimulation with TLR-1/2 ligands comparable to those produced by naïve CD4+ T cells from adults. In a study of healthy Ugandan children who were TB household contacts, infants <2 years mounted robust IFN-γ responses to Mtb culture filtrate. There was no difference in IFN-γ production by unfractonated peripheral blood mononuclear cells (PBMC) in response to two MTB-specific antigens (ESAT-6/CFP-10) between Ugandan TB household contacts <5 years old, and those between 5–15 years. Thus, even very young children can mount Th-1 responses to Mtb. Among young Brazilian children treated for pulmonary or extrapulmonary TB, there was no defect in the production of IFN-γ or TNF-α in response to a variety of stimulants, including purified-protein derivative (PPD), as compared with healthy tuberculin-skin test (TST)-positive children. Furthermore, BCG-vaccination also clearly induces an adaptive Th-1 response in infants. A direct comparison between CD4+ T cells derived from BCG-vaccinated Gambian neonates and adults demonstrated comparable levels of IFN-γ production by these cells in response to PPD. Moreover, the bias toward Th-2 adaptive immune responses that has been observed among infant CD4+ T cell responses in some circumstances does not appear to be present during Mtb infection, TB disease, or following BCG-vaccination. Thus, across several populations, published findings do not support the hypothesis that an impaired capacity to generate Th-1 type immune responses, or exaggerated Th-2 response, underlies the vulnerability of infants and young children to TB disease. Although the complete absence of a functional Th-1 axis (as observed in MSMD) or impairment in the frequency and function of CD4+ T cells (as observed in HIV-infection) does appear to place young children at increased risk for TB, the presence of Mtb-specific Th-1 T cells alone is not sufficient to provide protection against disease.

In some models of intracellular infection, the presence of pathogen-specific polyfunctional CD4+ T cells has been associated with protection against disease. In the context of Mtb infection and response to BCG-vaccination, however, the contribution of polyfunctional T cells to immune protection against TB disease remains controversial. In Australian neonates, the frequencies of polyfunctional CD4+ T cells (that simultaneously produce IFN-γ IL-2 TNF-α+ ) were similar to adult frequencies following BCG vaccination. Importantly, a vital study that compared cytokine profiles 10 weeks after BCG-vaccination of South African neonates found no difference in any cytokine profile expressed by either CD4+ or CD8+ T cells that correlated with development of TB disease after 2 years of follow-up. In short, the contribution of BCG-induced IFN-γ-producing and polyfunctional T cells in mediating protection against infant TB remains unknown.

CD8+ T cells recognize Mtb-infected cells and are also essential contributors to immune protection against TB (as reviewed in ). Mtb-specific CD8+ T cells have been identified in both adults and children with Mtb-infection and disease, and there is evidence that the presence of Mtb-specific CD8+ T cells that produce IFN-γ may be a highly specific marker for TB disease in young children. Neonatal BCG-vaccination also elicits a CD8+ T cell response, although far reduced from the response observed by CD4+ T cells. As discussed below, targeting a robust Mtb-specific CD8+ T cell response is one of the current approaches toward development of a more efficacious TB-vaccine.

Recently, attention has been given to CD4+ T cells that produce IL-17 and to the possible role of these Th-17 type T cells in the inflammatory response to TB. Th-17 type cells are a heterogeneous population of cells involved in activation and recruitment of neutrophils, promotion of inflammation and autoimmunity, and antimicrobial activity. Some studies in human disease are consistent with a protective role, while others suggest that presence of these cells correlates with disease pathology or with progression from latent to active disease. IL-17-expressing CD4+ T cells are induced by BCG-vaccination during infancy, consisting principally of IL-17 single cytokine-expressing CD4+ T cells that do not co-express IFN-γ. Currently, it remains unknown whether Th-17 cells are protective or contribute to pathology in pediatric TB specifically. Regulatory T cells (Treg) are a subset of T cells that suppress proinflammatory immunity to protect host tissue from chronic inflammation and prevent autoimmunity; however Treg can suppress immune responses to infection and vaccination, which has been specifically identified in BCG-vaccinated adults. Moreover, there is evidence from murine models that Mtb promotes an environment that drives expansion of Tregs, which delay priming and expansion of effector T cells, and impedes their arrival into the lung (as reviewed ). During the neonatal period, Treg frequency and/or suppressive capacity are uniquely enhanced. It remains unknown whether the exaggerated Treg response present during the neonatal period impairs the immunogenicity of BCG-vaccination during the neonatal period, and this remains a critical area of research.

**Donor-Unrestricted T Cells and their Role in Antimycobacterial Defenses**

Donor-unrestricted T cells (DURT), also known as unconventional T cells, constitute a diverse group of T cells with invariant or semi-invariant T cell receptors (TCRs) that recognize non-conventional ligands, such as lipids or
Renewed Interest in the Contribution of B-Cells to Antimycobacterial Defenses

B-cell and antibody deficiencies have not been found to place individuals at risk for TB, and a protective role for B-cells against this intracellular pathogen has, until recently, not been considered significant. However, B-cells are found within the lung during Mtb infection, and may serve a variety of effector functions including antigen presentation, antibody production, and cytokine secretion (reviewed in 95). A recent study of adults with latent versus active TB demonstrated distinctive Mtb-specific antibody signatures between the two populations and also found that antibodies from individuals with latent Mtb infection could promote NK cell cytotoxic function, as well as promote inflammasome activation within Mtb-infected macrophages and macrophage killing of Mtb.96 In adults, opsonizing BCG with post BCG-vaccination sera augmented phagocytosis and intracellular growth inhibition.97 Although young infants have well-known limitations in antibody production, specifically a restricted capacity to generate antibodies to polysaccharides before age 2 years,98,99 the contribution of antibodies to immune protection in infants has recently been re-examined. A recent case-control analysis, nested within the modified vaccinia Ankara antigen 85A (MVA85A) TB-vaccine candidate trial (discussed in detail in the following sections), reported rising antibody titers to antigen-85A (Ag85A) in infants after BCG-vaccination, and importantly, Ag85A-specific IgG was associated with decreased risk of TB.100 Antibodies may, thus, offer an under-appreciated contribution to vaccine-induced protection against infant TB. Further study will be required to determine whether young infants exhibit a restricted Mtb-specific antibody repertoire, and if so, if this contributes to pediatric immune vulnerability to TB disease and could be overcome through novel vaccination strategies.

Advances in the Development of Next Generation Infant TB-Vaccines

In addition to the partial protection against disseminated TB that BCG-vaccination provides to young infants, BCG is recognized to enhance immune responses to the subsequent vaccines given during infancy and to provide an overall decrease in all-cause mortality during early childhood.101–103 Given the protective benefits offered by BCG-vaccination during infancy, it remains unethical to withhold BCG-vaccination in current vaccine trials, and vaccine candidates are being evaluated by their ability to enhance BCG’s efficacy in a prime-boost strategy.105 Understanding how BCG mediates its beneficial effects is thus critical to efforts to develop novel TB-vaccines that could ultimately replace BCG. One explanation for the non-specific protective benefit of BCG vaccination is the phenomenon of ‘trained immunity’: a heightened, prolonged activation state of monocytes to secondary infections (and possibly to other vaccinations) following immunization with a live vaccine.106,107 This prolonged innate responsiveness is related to epigenetic modifications that impact cell metabolism, autophagy, and intracellular signaling.108–111 Of note, trained immunity was also recently demonstrated in NK cells,112 a finding that could be highly relevant to host protection against Mtb, since following BCG-vaccination NK cells become major producers of IFN-γ in neonates and infants.94 Enhanced, prolonged innate responsiveness could contribute not only to the partial, but crucial effect induced by BCG-vaccination in protecting infants from disseminated TB, but also to beneficial heterologous effects observed following BCG-vaccination, such as decreased morbidity from other infectious diseases.101,113 It is clear that novel pediatric TB-vaccines will need to be evaluated in the context of the non-specific beneficial effects mediated by BCG.114

Multiple TB-vaccine candidates are in trial, classified as either live whole-cell vaccines, adjuvanted proteins, virally vectored proteins, or mycobacterial lysates.8 The only efficacy trial of a TB-vaccine candidate performed to date utilized the MVA85A vaccine, as a TB-booster vaccine following neonatal BCG-vaccination. Unfortunately, although polyfunctional CD4+ and IFN-γ+ CD8+ T cells were induced by this vaccine, no enhanced protection against TB was observed in BCG-vaccinated infants following MVA85A-booster vaccination compared with booster vaccination with a placebo.115 Although this lack of efficacy is clearly a disappointment,
this important trial has provided critical insights into unique aspects of the response of young infants to vaccination and potential correlates of TB immunity and vulnerability, specific to this age group. Among infants randomized to receive BCG-vaccine followed by placebo booster in this trial, an increased frequency of CD4\(^+\) HLA-DR\(^+\) T cells was correlated with an increased risk of TB disease during the 2-year follow-up period, suggesting that T cell activation either places infants at risk for TB disease or is a predictor of poor vaccine response.\(^{100}\) In an earlier MVA85A dose-finding study performed in BCG-vaccinated South African infants, no relation between the MVA85A earlier MVA85A dose-boosted-responding infants and the MVA85A CD8\(^+\) Ag85A-specific T cell responses exhibited mixed central and effector cytokine responses, inclusion of additional immunodominant CD4\(^+\) and CD8\(^+\) T cells were detectable, this response was lower compared with that in South African adolescents and adults in the same study.\(^{116}\) These findings raise two conflicting hypotheses that vaccine-induced polyfunctional T cells are simply not correlates of protective immunity in infants, or that boosting with MVA85A, at least of IFN-\(\gamma\)-responses, was simply inadequate in eliciting a sufficient number of IFN-\(\gamma\) T cells to offer protection against TB disease. In addition to cytokine repertoire, the capacity of a vaccine to induce T cells with a central memory phenotype and function could also contribute to vaccine efficacy.\(^{117}\) Central memory T cells are characterized by C-chemokine receptor type 7 (CCR7) expression and provide heightened responses upon secondary challenge.\(^{118}\) In a comparative analysis of memory induction among different age groups following vaccination with MVA85A, MVA85A-induced T cell responses exhibited mixed central and effector memory phenotypes in older age groups, whereas in infants Ag85A-specific CD4\(^+\) T cells were mostly effector memory.\(^{119}\) Finally, the detailed immune studies and systems immunology approach to data analysis performed within the context of this infant TB efficacy trial have suggested several avenues to pursue to improve infant TB vaccines. These include further study of both vaccine-induced and passively acquired antibody responses, inclusion of additional immunodominant CD4\(^+\) and CD8\(^+\) T cell antigens to increase the magnitude of T cell responses, and consideration of targeting cell types not previously studied in infants, such as DURT cells.

Several other novel pediatric TB-vaccine candidates are currently under study. A dose-finding trial of AERAS-402 (Cruell Ad35; a virus-vectored vaccine expressing a fusion protein of the antigens Ag85A, Ag85B, and TB10.4) was performed as a booster-vaccine candidate in BCG-vaccinated infants in Sub-Saharan Africa, but reported low CD4\(^+\) T cell responses at all dose levels.\(^{120}\) In a recent immunogenicity study of M72/AS01 (an adjuvanted recombinant fusion protein M72, derived from mycobacterial antigens Mtb32A and Mtb39A) in Gambian infants, low levels of polyfunctional CD4\(^+\) T cells were induced when M72/AS01 was given as a booster following BCG-vaccination, though these increased mildly following a second M72/AS01-dose.\(^{121}\) Moreover, vaccine specific CD8\(^+\) T cells were not induced in infants. The potential for enhancing protection by magnifying the vaccine-induced CD8\(^+\) T cell response in infants has been further explored in the TB vaccine candidate VPM1002. This recombinant M. bovis BCG-vaccine expresses listeriolysin (Hly) from Listeria monocytogenes, perturbing the phagosome to facilitate antigen translocation into the cytoplasm, thus encouraging induction of CD8\(^+\) T cells.\(^{122}\) In a recent head-to-head comparison between VPM1002 and BCG in South African infants, however, similar levels of CD4\(^+\) and CD8\(^+\) T cell responses were demonstrated following either BCG- or VPM1002-vaccination.\(^{122}\) The development of candidate TB-vaccines that elicit strong CD8\(^+\) T cell responses continues to be an important research priority.

Environmental Influences on Infant Immune Responses to TB and TB-Vaccines

Although BCG is the most widely used vaccine in the world, it has long been recognized to have variable efficacy in different populations.\(^{123}\) Although not completely understood, increased exposure to NTM, as encountered in tropical settings, has long been thought to interfere with studies of BCG’s efficacy.\(^{124}\) Specifically, it has been postulated that NTM exposure could impart partial protection against TB such that BCG-vaccination cannot provide additional protective benefit.\(^{123–125}\) However, infants receive BCG-vaccine prior to significant NTM exposure, suggesting that during infancy, environmental factors other than NTM exposure must also influence the immunogenicity of BCG. For example, using populations of infants from the UK and Malawi who received BCG-vaccination within the first few weeks of life and had very limited pre-vaccination exposure to NTM, Malawian infants had a substantially reduced BCG-induced immune response.\(^{126,127}\) Induction of IFN-\(\gamma\) immune responses following MVA85A-vaccination was also noted to be lower in South African compared with UK infants.\(^{115,128}\) Other environment considerations that could impact vaccine-induced protection in young infants are prenatal factors. For example, as priming of the immune system can occur in utero,\(^{129}\) prenatal exposure to maternal infection may impact the young infant’s immune response to infectious disease and vaccination. In a murine model, in utero priming of the fetal immune system with mycobacterial antigens resulted in enhanced protection against BCG-challenge.\(^{130}\) However, the impact of latent maternal Mtb infection on the subsequent risk of TB during infancy has not yet been reported. Additional important contributors to vaccine-induced protection and vulnerability to TB in young infants are the presence of malnutrition and vitamin D deficiency.\(^{131,132}\) The impact of co-infections, such as malaria, helminths, and cytomegalovirus (CMV), on risk of infant TB and TB-vaccine responses, especially in settings where these are co-endemic, remains an active and important area of investigation. Thus, factors that are specific to infancy, as well as the environment of the study population, should be further included in design of infant TB-susceptibility studies and vaccine trials.

Concluding Remarks

Global efforts to eliminate suffering from TB, with emphasis on the development of an improved vaccine, have highlighted
the importance of understanding the unique immunobiology of Mtb in target populations, such as young infants, as well as an urgent need to identify correlates of protective immunity that can be utilized to gauge vaccine efficacy. To date, the immune vulnerability of infants and young children remains poorly understood and most investigations have examined one or a limited number of immunologic parameters in isolation. Success will likely require investigations that employ unbiased systems-based approaches that can examine multiple, interacting immune effectors rather than focusing on a limited number of immunologic outputs. The contribution of B-cells and DURT cells to the infant immune response to TB and the possibility of boosting these responses with novel vaccines must continue to be studied. Researchers and funding agencies must welcome the challenge of performing ethical translational studies enrolling infants and young children in TB endemic environments and consider that the immunobiology of Mtb and correlates of protective immunity against this pathogen may differ across different age groups and populations.

Conflict of Interests

None.

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