Immunity
Review

Future Vaccination Strategies against Tuberculosis: Thinking outside the Box

Stefan H.E. Kaufmann[1,*]
[1]Department of Immunology, Max Planck Institute for Infection Biology, Charitéplatz 1, D-10117 Berlin, Germany
*Correspondence: kaufmann@mpiib-berlin.mpg.de
DOI 10.1016/j.immuni.2010.09.015

With almost a dozen vaccine candidates in clinical trials, tuberculosis (TB) research and development is finally reaping the first fruits of its labors. Vaccine candidates in clinical trials may prevent TB disease reactivation by efficiently containing the pathogen *Mycobacterium tuberculosis* (*Mtb*). Future research should target vaccines that achieve sterile eradication of *Mtb* or even prevent stable infection. These are ambitious goals that can be reached only by highly cooperative engagement of basic immunologists, vaccinologists, and clinical researchers—or in other words, by translation from basic immunology to vaccine research and development, as well as reverse translation of insights from clinical trials back to hypothesis-driven research in the basic laboratory. Here, we review current and future strategies toward the rational design of novel vaccines against TB, as well as the progress made thus far, and the hurdles that need to be overcome in the near and distant future.

Introduction

A total of 11 vaccine candidates against tuberculosis (TB) have entered clinical trials within the last several years (Box 1; Kaufmann et al., 2010; StopTB Partnership Working Group on New TB Vaccines, 2009). Many of them look promising. Yet, in none of these cases do we know with certainty whether they will confer robust protection against TB in humans. A widely held view considers infection synonymous with disease, i.e., we consider disease the unequivocal sequelae of infection with a pathogen. The so-called childhood diseases are testament to this view and include measles, mumps, rubella, diphtheria, and tetanus. Moreover, we are used to expecting virtually 100% protection from our experience with vaccines against such diseases, because we consider sterile eradication a prerequisite for cure (Kaufmann, 2009).

For TB, the situation is more complex. Infection with *Mycobacterium tuberculosis* (*Mtb*) does not necessarily lead to active TB disease (Kaufmann, 2006b). Most infected individuals remain healthy. However, they do not achieve sterile eradication of the pathogen and consequently remain latently infected lifelong. It has been estimated that 1/3 of the world population, i.e., somewhere around 2.2 billion individuals on this globe, are latently *Mtb* infected (Figure 1; WHO, 2009; Lönnroth et al., 2010). A vaccine, therefore, is not needed for 90% of individuals if one is satisfied with prevention of TB disease reactivation and accepts latent *Mtb* infection. It is the 10% of individuals at risk of developing disease who represent the targets for novel vaccines against TB. Under these circumstances, future vaccines are satisfactory if they induce an immune response in susceptible individuals comparable to that evoked by natural *Mtb* infection in resistant ones. Alternatively, if one aims at sterile *Mtb* eradication, future vaccines need to perform better than natural immunity in resistant individuals, which only contains infection.

All current pre-exposure TB vaccine candidates in clinical trials at most will achieve prevention of TB reactivation, not sterile eradication (Kaufmann et al., 2010; StopTB Partnership Working Group on New TB Vaccines, 2009). Therefore, long-lasting, if not lifelong, protection is required. This is not a given because both endogenous and exogenous insults can deteriorate vaccine-induced immunity. For example, coinfection with helminths or HIV could reorient or impair the ongoing immune response, respectively (Weir et al., 2006; Azzopardi et al., 2009; Hesseling et al., 2007b). In Sub-Saharan Africa where TB rampages most, virtually everybody is infected by helminths and HIV infections are common (Harries et al., 2010; Hotez and Kamath, 2009). Very little is known about how vaccine-induced immunity deals with coinfections. Equally little is known about the factors that determine whether or not an individual is protected against natural infection with *Mtb* (the 2.2 billion individuals on this globe) or whether someone is at risk of developing TB at a later stage (the 10 million who develop active TB annually).

As a first step in TB vaccine research and development (R&D), we need to understand whether the immune response, which operates in latently infected, resistant individuals, can be induced in susceptible individuals. Deeper insights could be obtained by biomarkers, which distinguish the host response in latently infected healthy individuals, from those who develop TB disease (Parida and Kaufmann, 2010). Comparative transcriptome and metabolome analyses indicate that such biomarkers can be identified (Parida and Kaufmann, 2010). Yet, is it sufficient to be as good as nature or do we need to do better? It is most likely that as a next step in the TB vaccine R&D pipeline and beyond, we need to do better than nature because in a world threatened by HIV, the immune response induced by natural infection or by vaccination is frequently impaired by HIV coinfection, thereby facilitating TB disease reactivation. In fact,

The three phases of clinical trials for TB vaccines

Clinical trials for TB vaccines follow general guidelines with specific modifications (http://clinicaltrials.gov/).

Phase I: Safety and immunogenicity testing in small study groups (10 or more individuals per group). Typically, Phase I trials are first performed in the region where the vaccine candidate was developed and then repeated under similar conditions in a highly endemic area. Eleven TB vaccine candidates have passed Phase I clinical trials.

Phase II: Assessment in larger groups (100 or more individuals per group) of optimal dosage and route of administration; efficacy is based on immune parameters ideally via a biomarker or biosignature as correlate of protection. Currently, no correlate of protection against TB exists that can reliably predict TB disease outbreak. Four vaccine candidates have entered or passed Phase II clinical trials.

Phase III: Assessment of vaccine efficacy against natural infection monitoring of safety in a highly endemic area. Prophylactic TB vaccine trials will need to involve 20,000–50,000 participants and will last several years because even in highly endemic areas, incidences rarely exceed 300/100,000 adults, and incubation time often lasts for years. Moreover, it will be necessary to perform several phase III trials in different regions.

Box 1. The Three Phases of Clinical Trials for TB Vaccines

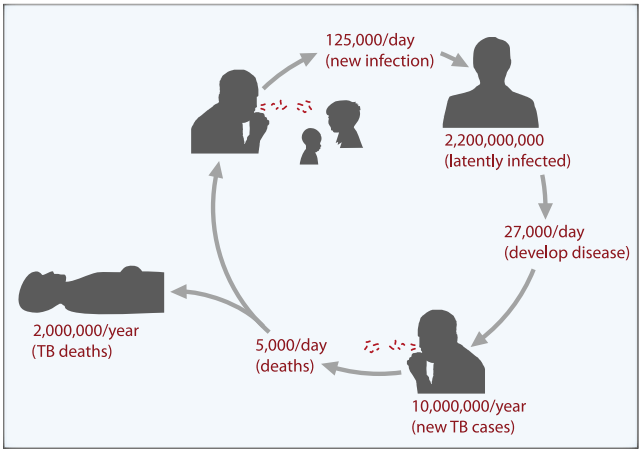

Figure 1. The Vicious Cycle of TB
The figure depicts daily numbers of infections with *Mycobacterium tuberculosis* (*Mtb*), new cases of TB, cases of death because of TB, and numbers of latently *Mtb*-infected individuals, new TB cases annually, and TB deaths annually.

15 million individuals suffer from coinfection with HIV and *Mtb*, of whom annually 1 million develop TB disease leading to half a million deaths (Kaufmann et al., 2010). Complementing this disaster are the increasing incidences of multidrug-resistant (MDR) TB and extremely resistant (XDR) TB (Gandhi et al., 2010).

Fast-Track Overview of the Immune Response in TB

Generally, *Mtb* enters the host within aerosols inhaled to the lungs. Minute droplets of 1–3 μm containing few tubercle bacilli arrive at the deeper alveoli where they are engulfed by alveolar macrophages. Some bacilli are deposited in the lung parenchyma and some are transported to draining lymph nodes where dendritic cells (DCs) prime T lymphocytes. *Mtb* persists in mononuclear phagocytes and DCs where it hides (Russell, 2007). The pathogen accomplishes intracellular survival through several evasion strategies including neutralization of the phagosomal pH and interference with autophagy, which serves as a cell-autonomous defense mechanism (Gutierrez et al., 2004; Deretic, 2006; Russell, 2007). Moreover, *Mtb* can invade the cytosolic compartment (van der Wel et al., 2007). Finally, *Mtb* inhibits apoptosis by production of prostaglandins (Divangahi et al., 2010). During this early stage, *Mtb* may be transported to other sites within infected macrophages (Davis and Ramakrishnan, 2009).

Evidence from a model system suggests benefit for *Mtb* during early stages of granuloma formation (Cosma et al., 2008). Consistent with this, the mycobacterial virulence factor ESAT-6 promotes accumulation of macrophages at the sites of inflammation (Volkman et al., 2010).

Under the influence of specific T lymphocytes, the loose aggregates of mononuclear phagocytes and polymorphonuclear granulocytes transform into solid granulomas composed of macrophages of different activation and maturation stages and different T cell populations in a structured arrangement (Figure 2; Ulrichs and Kaufmann, 2006). Within these solid granulomas *Mtb* is contained but not eradicated. As a corollary, latent infection ensues but disease is prevented. Recent evidence suggests that TB granulomas can acquire lymphoid functions. In the absence of secondary lymphoid organs, granulomas can serve as sites for priming of $CD4^+$ and $CD8^+$ T cells as well as germinal center B cells. Notably, primed T cells are polyfunctional, express markers of central memory T (Tcm) cells, and provide protective immunity against TB in an experimental mouse model (Day et al., 2010).

With the failure of the immune response to contain *Mtb*, necrotic areas may develop and later become caseous. Although it is clear that cavitation is caused by an aberrant immune response, both hypo- and hyperactive immunity can be the cause. Ultimately, *Mtb* grows to enormous numbers in the order of magnitude of 10^{12}–10^{14}. Exuberant damage to the lung, *Mtb* dissemination to other organs, and spread of the contagion in the environment are reflections of active TB disease.

During residence in phagosomes, *Mtb* secretes proteins, which after appropriate degradation can be loaded onto products of the major histocompatibility complex (MHC) class II. This leads to $CD4^+$ T cell stimulation. Mechanisms underlying $CD8^+$ T cell stimulation are not fully understood. This requires peptide presentation by MHC I products, which generally occurs in the cytosol not readily accessed by *Mtb*. Two pathways have been proposed. First, *Mtb* can egress into the cytosol of infected DCs, and this leads to direct loading of MHC I molecules (van der Wel et al., 2007). Second, crosspriming has been observed (Winau et al., 2006). This process comprises apoptosis of macrophages infected with *Mtb*. Resulting vesicles carry mycobacterial antigens, which are taken up by DCs in the vicinity (Winau et al., 2005). These DCs present antigenic peptides with high efficacy both in the context of MHC II and MHC I to $CD4^+$ and $CD8^+$ T cells, respectively. Again, however, *Mtb* can interfere with these processes of antigen presentation. First, *Mtb* inhibits MHC class II processing and thus impairs $CD4^+$ T cell stimulation

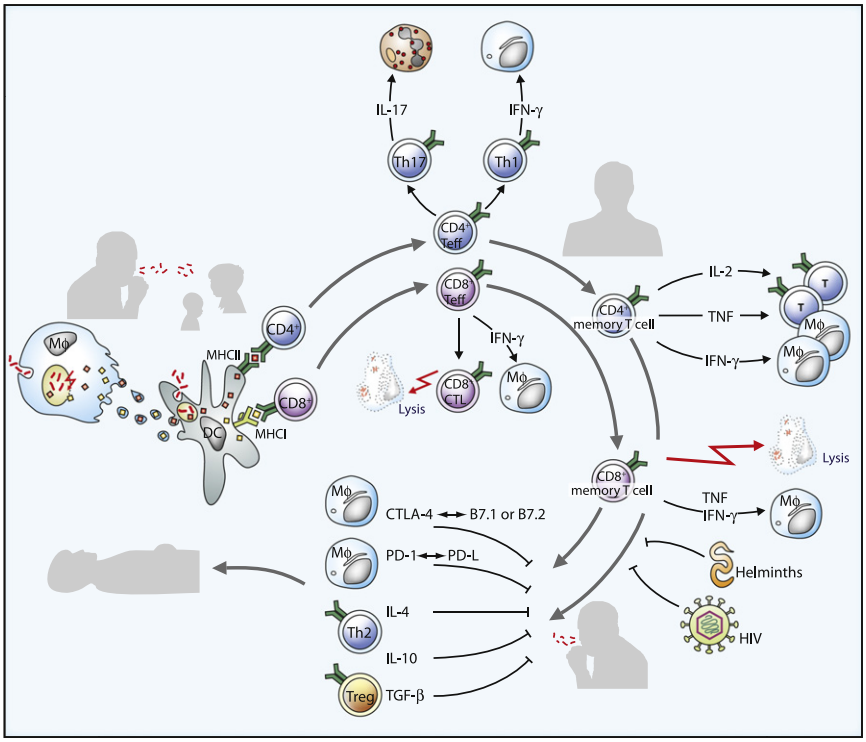

Figure 2. The Pathologic and Cellular Basis of the Host Response in TB

The immune response at the different stages of the vicious cycle of TB. After infection of the host in the lung, macrophages and DCs stimulate specific $CD4^+$ and $CD8^+$ T cells, which are critical for protection against *Mtb*. $CD4^+$ T cells are polarized into Th1 and Th17 cells, which perform effector functions. $CD8^+$ T cells contribute to protection by cytolytic mechanisms and IFN-γ production. As a next step, memory T cells develop. Coexpression of multiple cytokines, notably IL-2, TNF, and IFN-γ, is considered as indicator of protective $CD4^+$ memory T cells, and coexpression of cytolytic molecules, together with IFN-γ and TNF, is considered characteristic for $CD8^+$ memory T cells. Confounding factors include exogenous invaders (e.g., HIV, helminths) as well as endogenous cytokines (IL-4, IL-10, TGF-β) and inhibitory surface molecules (CTLA4, PD-1), which have the potential of disturbing the immune response. Accordingly, the outcome of immunity may vary. Abbreviations: Teff, effector T cell; CTL, cytolytic T lymphocyte; Th, T helper cell; Mφ, macrophage; TNF, tumor necrosis factor; IFN-γ, interferon gamma; TGF-β, transforming growth factor beta; IL, interleukin; MHC, major histocompatibility complex; PNG, polymorphonuclear granulocyte.

(Harding and Boom, 2010). Second, *Mtb* blocks crosspriming for $CD8^+$ T cells through modulating the lipoxygenase pathway (Divangahi et al., 2010).

Stimulation through pattern recognition receptors (PRR) expressed on the surface of DCs and macrophages instructs $CD4^+$ T cell polarization (Akira et al., 2006). These include Toll-like receptors (TLR), C-type lectin receptors (CLR), and others (Dorhoi and Kaufmann, 2009). The TLR2 recognize glycolipids such as certain lipomannan (LM) cognates from *Mtb* and the TLR9 recognize CpG motifs of *Mtb* DNA (Akira et al., 2006). The CLR include DC-SIGN, which binds LM, and Mincle, which binds trehalose dimycolate (TDM) (Schoenen et al., 2010; Ishikawa et al., 2009). Sensing of different molecular patterns through a range of PRR stimulates signaling cascades that are assembled in adaptors including MyD88 and Card9, which then emit signals, which instruct T cells (Dorhoi and Kaufmann, 2009; Dorhoi et al., 2010). Generally, signaling through TLR and Mincle promotes type 1 T helper (Th1) cells, Dectin-1 and Mincle favor Th17 cells, and DC-SIGN induces suppression or exhaustion (Schoenen et al., 2010; Ishikawa et al., 2009; Tailleux et al., 2003; Geijtenbeek et al., 2003; Dennehy et al., 2009; Underhill et al., 1999).

Th1 cells produce a multitude of cytokines, notably, interleukin (IL)-2, interferon-gamma (IFN-γ), and lymphotoxin (LT), and tumor necrosis factor (TNF) and are critical for protection (Figure 2; Cooper, 2009). Although the role of Th17 cells in TB remains to be fully clarified, they seem to play an important role in the early formation of protective immunity in the lung (Khader et al., 2007; Cooper, 2009; Kaufmann, 2006b).

Th2 cells counter-regulate Th1 cells and in this way probably impair protective immunity in TB. Treg cells produce transforming growth factor-beta (TGF-β) and IL-10, which suppress all T cell populations (Belkaid and Tarbell, 2009; Chen et al., 2007; Guyot-Revol et al., 2006; Dlugovitzky et al., 1999). TGF-β is also involved in the formation of the fibrotic wall surrounding granulomas in TB (Toossi and Ellner, 1998).

The MHC class I-restricted $CD8^+$ T cells contribute to protective immunity against TB (Kaufmann, 2006a; Cooper, 2009). These $CD8^+$ T cells secrete perforin and granulysin, which lyse host cells and attack *Mtb* directly (Stenger et al., 1997, 1998). After the first wave of effector T (Teff) cells, the immune response contracts and Teff cells are succeeded by memory T cells (Figure 2; Williams and Bevan, 2007; Sallusto et al., 2004). The memory T cells of Th1 type are polyfunctional, i.e., a single T cell clone produces multiple cytokines, notably IL-2, IFN-γ, and TNF (Foulds et al., 2006). These polyfunctional $CD4^+$ memory T cells are considered crucial mediators of long-term protection against TB (Foulds et al., 2006; Sutherland et al., 2010). Similarly, $CD8^+$ memory T cells develop. The memory T cells further segregate into Tcm and effector memory T cells (Tem), the former being characterized by preferential homing to lymph nodes and the latter by preferential residence in tissue sites such as the *Mtb*-infected lung parenchyma (Figure 2; Sallusto et al., 2004; Mora and von Andrian, 2006).

During containment in the latently infected healthy individual, *Mtb* faces a stress situation notably imposed by hypoxia and nutrient deficiency. In vitro data suggest that such conditions lead to transformation into a dormant stage with low to absent metabolic and replicative activity (Betts et al., 2002; Voskuil et al., 2003; Wayne and Sohaskey, 2001). Accordingly, antigens produced by dormant *Mtb* are called dormancy antigens. Among these are the 48 gene products controlled by DosR,

which senses insults and stress imposed on *Mtb,* e.g., by hypoxia. Dormancy antigens could represent major immune targets during latent infection (Black et al., 2009; Schuck et al., 2009; Leyten et al., 2006; Lin and Ottenhoff, 2008).

The dormant pathogen is highly resistant to immune attack. Eventually, the immune response is downmodulated to avoid collateral damage but still suffices for *Mtb* control. Aberrant immunity, e.g., through exhaustion or suppression of T cells, tips the balance in favor of the pathogen. Two major types of mechanisms have been described in other models. First, cognate interactions between T cells and DCs involving the programmed death (PD)-1-PD-L system or the CTLA-4 and B7.1-B7.2 systems lead to T cell exhaustion (Keir et al., 2008; Chambers et al., 2001; Jurado et al., 2008). Second, Treg cells secrete regulatory cytokines, notably IL-10 and TGF-β, which suppress ongoing immune responses (Belkaid and Tarbell, 2009). Principally, Treg cells express CD25, the high-affinity receptor for IL-2. CD25 can be shed into the circulation where it serves as decoy factor that removes IL-2, resulting in diminished T cell activity because of insufficient IL-2 availability (Shitrit et al., 2006; Rubin et al., 1985; Kursar et al., 2007). Once aberrant immune responses arise, *Mtb* are resuscitated from a dormant to metabolically active stage (Leyten et al., 2006; Kana et al., 2008). This reactivation leads to active TB.

In sum, we do not exactly know which types of T cells are needed for containment of *Mtb*: Teff, because "trench warfare" takes place, or memory T cells, because long-term immunity is required. Among Teff, do we need $CD4^+$ Th1 cells, $CD4^+$ Th17 cells, $CD8^+$ CTL, or all of them, with additional help from unconventional CD1-restricted T cells and γδ T cells (Cooper, 2009; O'Shea and Paul, 2010; Brigl and Brenner, 2004; Scotet et al., 2008)? Among memory T cells, do we need Tem cells, which are active at the site of *Mtb* containment, i.e., solid granulomas in the lung, or Tcm cells, residing in draining lymph nodes that constantly generate Teff cell progeny? We need to learn which combinations of T cell subsets and which cytokine "gemisch" are required. We also need to make a register of antigens expressed by *Mtb* during stages of dormancy, resuscitation, and activity. Finally, we need to know whether *Mtb* hides only in macrophages within granulomas or also in mononuclear phagocytes at other tissue sites, or even in other cells, i.e., nonprofessional phagocytes. Thus, even canonical vaccine candidates are surrounded by numerous unknowns. They are, at least in part, "knowable unknowns" and we urgently need to clarify these.

Current Situation

The current vaccine against TB, namely, Bacille Calmette-Guérin (BCG), has been given 4 billion times over the last 90 years (Kaufmann et al., 2010). Yet available evidence suggests that this vaccine does not protect against adult pulmonary TB. It protects against severe disease in infants, and to a lesser extent against lung TB in infants (Kaufmann et al., 2010; Fine, 1995). BCG-induced protection, however, wanes with age. Large studies have shown very limited efficacy of revaccination with BCG in adolescents and adults (Antas and Castello-Branco, 2008). A recent field study has exploited the observation that BCG induces variable protection in infants, by investigating correlates of risk of TB disease after routine BCG vaccination. These studies determined that mycobacteria-specific T cell expression of IFN-γ, or of many other combinations of Th1 cytokines 10 weeks after newborn BCG vaccination, does not predict risk of TB disease (Kagina et al., 2010). These data emphasize that the precise immune mechanisms of protection elicited by BCG remain incompletely understood. In other words, almost 90 years after the first administration of BCG to a baby and more than 100 years since its development, we don't even understand the precise immune mechanisms that protect BCG-vaccinated infants (Calmette, 1927; Fine, 1995; Kaufmann et al., 2010).

Vaccine Candidates in Clinical Trials

Of the 11 vaccine candidates currently in clinical trials, two are killed myobacteria or semipurified mycobacterial components for adjunct therapy of HIV-*Mtb*-coinfected individuals (Kaufmann et al., 2010; StopTB Partnership Working Group on New TB Vaccines, 2009). These will not be discussed further. The remaining nine candidates are preventive pre-exposure vaccines, i.e., given prior to *Mtb* infection (Box 2). The goal of these candidates is the containment of *Mtb* so that latent infection is sustained and TB disease reactivation is prevented or at least delayed. Two of these vaccine candidates should replace conventional BCG. Both are recombinant (r)BCG constructs, which induce superior protection compared to BCG in preclinical animal models. The other seven candidates are subunit booster vaccines to be given on top of the BCG prime. Therefore, they improve or reorient the immune response evoked by BCG. These subunit vaccines comprise a small set of secreted antigens, i.e., proteins secreted by metabolically active *Mtb*. Three candidates harness viral carriers while the other four are protein plus adjuvant formulations. The frequently used antigen 85 (Ag85) cognates, Ag85A or Ag85B, which are most widely used in the current vaccine candidates, are shared by BCG and *Mtb* and hence evoke a true booster response. Additional antigens that are unique to *Mtb* and therefore do not represent true booster antigens are ESAT6 or TB10.4. They are encoded within the region of difference 1 (RD-1) gene cluster, which is present in *Mtb* and absent in BCG (Dietrich et al., 2005).

Subunit Vaccines for Booster of BCG

Currently, two viral carriers are being harnessed for TB vaccines: modified vaccinia virus Ankara (MVA) developed by Oxford University and replication-deficient Adenovirus (Ad) of serotype 5 or 35 (Ad5 created by McMaster University and Ad35 created by Crucell and Aeras) (Sander et al., 2009; Radosevic et al., 2007; McShane et al., 2004; Santosuosso et al., 2006). The MVA and Ad5 virus carriers both express Ag85A whereas the Ad35 coexpresses the antigens Ag85A, Ag85B, and TB10.4. Infections with Ad occur frequently. Accordingly, antibodies exist in many humans (Bangari and Mittal, 2006). This is particularly true for Ad5 but less so for Ad35. Yet, up to 20% of people living in Africa have Ad35-specific antibodies. These pre-existing antibodies could impair Ad-based vaccine efficacy, but evidence arguing against impact of these preexisting antibodies on vaccine efficacy has been obtained from vaccination trial with an Ad5 HIV-1 vector (Hutnick et al., 2009; O'Brien et al., 2009).

New virus carriers in development include simian Ad vectors and lymphocytic choriomeningitis virus (LCMV) (Hill et al., 2010; Flatz et al., 2010). Simian Ad do not infect humans and

Approaches to TB vaccine R&D

Pre-exposure vaccination to delay TB reactivation
Principle: Memory T cells
Pre-exposure: Allow and then contain Mtb infection; prevent disease outbreak
Benefit: Reduce incidence of TB
Risk: Collateral damage by continuous immune response; breakdown (e.g., HIV) or deviation (e.g., helminths) of immunity leading to TB disease reactivation; suppression or exhaustion of immunity leading to disease outbreak

Postexposure vaccination to delay TB reactivation
Principle: Memory T cells
Postexposure: Contain persistent Mtb infection; prevent TB disease reactivation
Benefit: Reduce incidence of TB
Risk: Collateral damage by continuous immune response; breakdown (e.g., HIV) or deviation (e.g., helminths) of immunity leading to TB disease reactivation; suppression or exhaustion of immunity leading to TB disease reactivation

Pre-exposure vaccination to achieve sterile Mtb eradication
Principle: Effector and memory T cells
Pre-exposure: Allow short-term Mtb infection then eradicate Mtb
Benefit: Sterile Mtb eradication cancelling risk of TB reactivation
Risk: Collateral damage by exaggerated immune response

Postexposure vaccination to achieve sterile Mtb eradication
Principle: Effector and memory T cells
Postexposure: Eradicate persistent Mtb
Benefit: Sterile Mtb eradication cancelling risk of TB reactivation
Risk: Collateral damage by exaggerated immune response

Prevention of stable Mtb infection
Principle: Pre-existing antibodies and effector and memory T cells
Pre-exposure: Prevent Mtb infection
Benefit: Sterile Mtb removal cancelling risk of TB reactivation
Risk: Collateral damage by exaggerated immune response; no booster of immunity by natural Mtb infection

Box 2. Approaches to TB Vaccine R&D

therefore risk of blocking preexisting antibodies is probably not an issue (Hill et al., 2010). The LCMV vector is a potent stimulator of Th1 $CD4^+$ T cells and of neutralizing antibodies (Flatz et al., 2010).

Three different types of adjuvants are currently used for protein vaccines. The adjuvant IC31 developed by Intercell has been used in combination with Hybrid1 (H1) by Statens Serum Institut (SSI), which is a fusion protein of Ag85B and ESAT6, or combined with HyVac4 from SSI, which is a fusion protein of Ag85B and TB10.4 (Dietrich et al., 2005; van Dissel et al., 2010). IC31 comprises a cationic antimicrobial peptide ($KLKL_5KLK$) and an oligodeoxynucleotide (ODN), which is not a CpG, yet binds to TLR9 (Lingnau et al., 2007). The cationic peptide increases antigen uptake by APC and provides a depot for slow antigen release. The ODN stimulates both myeloid and plasmacytoid DCs. IC31 induces $CD4^+$ Th1 cells and $CD8^+$ cytolytic T lymphocytes (CTL) as well as antibodies. GlaxoSmithKline (GSK) has designed an adjuvant platform for new vaccines composed of distinct building blocks for different requirements (Reed et al., 2009). AS01 and AS02 have been selected for TB vaccines and currently AS01 is favored for further clinical development (Von Eschen et al., 2009). Both AS cognates contain monophosphoryl lipid A (MPLA), which stimulates Th1 cells and probably Th17 cells through TLR4 signaling and the surface-active compound QS-21, which facilitates $CD8^+$ CTL stimulation. The AS01 is a liposomal formulation whereas AS02 is an oil-in-water emulsion. Both systems form depots for slow antigen release and AS01 apparently is a stronger stimulator of Th1 over Th2 cells whereas AS02 seems to stimulate both T cell populations equally. The antigen in this vaccine (termed M72) is a fusion protein of the antigens Rv1196 and Rv0125 (Von Eschen et al., 2009). Most recently, the CAF01 adjuvant developed by SSI has been formulated with antigen H1, also from SSI. CAF01 is a liposomal formulation composed of dimethyldioctadecyl ammonium bromide (DDA) and trehalose 6,6-di-behenate (TDB), a derivative with lower toxicity of the mycobacterial trehalose dimycolate (TDM), the crucial ingredient of cord factor (Gram et al., 2009). CAF01 allows for slow antigen release from a depot, maturation of APCs, and stimulation of Th1, Th2, and Th17 cells, as well as antibodies and $CD8^+$ CTL. The receptor for TDM and TDB has recently been identified as the C-type lectin, MINCLE (Ishikawa et al., 2009; Schoenen et al., 2010).

New antigens currently assessed for next generation TB subunit vaccines include the heparin-binding hemagglutinin (HBHA), a protein shared by *Mtb* and BCG but differentially methylated (Rouanet et al., 2009). The HBHA is an adhesion molecule for nonphagocytic cells and is involved in extrapulmonary dissemination of *Mtb* (Pethe et al., 2001). Although HBHA is a potent antigen, T cells, surprisingly, are specific for the methylated AA sequences (Locht et al., 2006). These post-translational modifications pose major obstacles for recombinant production by bacteria different from *Mtb*. Recent research for additional vaccine antigens focuses on dormancy and starvation gene products such as the DosR-regulated proteins (Voskuil et al., 2003; Betts et al., 2002; Black et al., 2009; Schuck et al., 2009; Leyten et al., 2006; Lin and Ottenhoff, 2008).

Viable Vaccines to Replace BCG

Strategies to construct vaccines that can replace BCG try to improve upon antigenicity, immunogenicity, or both (Grode et al., 2005; Tullius et al., 2008). The rBCG30 developed by Horwitz and colleagues overexpresses Ag85B, which is shared by BCG and *Mtb* (Tullius et al., 2008). Higher abundance increases its antigenicity. The rBCGΔUreC:Hly vaccine developed by our group has increased immunogenicity compared to BCG (Grode et al., 2005). Hly (listeriolysin from *Listeria monocytogenes*) is a component of this vaccine and perforates membranes but is active only at acidic pH. Because BCG neutralizes phagosomal pH, Hly cannot display its full biological activity there. This required deletion of urease C (ureC), which, together with other factors, is involved in pH neutralization in the phagosome. The original strategem focused on improved antigen release into the cytosol resulting in better $CD8^+$ T cell stimulation. Recent evidence suggests that membrane perforation also increases apoptosis of host cells. This leads to the formation of apoptotic vesicles that carry mycobacterial antigens as cargo. Uptake of these vesicles by DCs allows cross-priming for superior $CD4^+$ and $CD8^+$ T cell stimulation (Winau et al., 2005). More recent evidence suggests that this process can favor stimulation of Th17 cells in addition to Th1 cells (Torchinsky et al., 2009). Although the precise mechanisms underlying superior immunogenicity have not been fully elucidated, this vaccine strain shows better protection in preclinical models not only against the laboratory strain *Mtb* H37Rv but also against a member of the clinically important Beijing genotype family (Grode et al., 2005).

Interestingly, both rBCG vaccines are further attenuated as compared to parental BCG as shown in different animal models such as immunocompromised mice (Grode et al., 2005; Tullius et al., 2008). Hence, they should already be safer in humans than parental BCG. Further strategies to increase safety of such vaccines include introduction of auxotrophy by deletion of genes involved in biosynthesis of essential micronutrients absent in the human host. Auxotrophic *Mtb* and BCG strains include deficiencies in leucine, glutamine, methionine, lysine, and pantothenate (Guleria et al., 1996; Sambandamurthy et al., 2002; Bange et al., 1996). The first attenuated auxotrophic TB vaccine candidates were generated in 1996 but never entered clinical trials (Guleria et al., 1996). Safer vaccines to replace BCG are of great importance. Increased incidence of disseminated BCGosis in HIV^+ newborns leads to a serious disease that can be lethal. This led to the recommendation of the World Health Organization (WHO) against BCG vaccination of newborns with proven HIV^+ status (WHO, 2007).

Viable vaccine candidates in the R&D pipeline from preclinical to Phase 1 clinical development include further modifications of rBCG as well as attenuated r*Mtb* strains. By building on the strategy originally described by the Horwitz group, researchers added either antigens that are absent in BCG (such as TB10.4) or antigens already present in BCG that are being overexpressed (such as Ag85A) (Sun et al., 2009). Our strategem of introducing pore-forming capacities into BCG has been modified by replacing Hly with perfringolysin (pfo) from *Clostridium perfringens* (Sun et al., 2009). Combination of both strategies led to a vaccine combining concomitant expression of pfo and the antigens Ag85A, Ag85B, and TB10.4 (Sun et al., 2009, 2010). This vaccine construct was safe and induced IFN-γ-producing T cells in mice but failed to induce substantially higher protection against TB than parental BCG (Sun et al., 2009, 2010). Vaccines based on *Mtb* attenuation must comprise at least two independent deletions to avoid spontaneous conversion to virulence (Walker et al., 2010). The most advanced candidates are *Mtb* ΔRD1, ΔpanCD, which is devoid of the *Mtb* virulence gene cluster RD1 and auxotrophic because of pantothenate deficiency (Sambandamurthy et al., 2006), and *Mtb* ΔPhoPΔ*fad* (Martin et al., 2006; C. Martin, personal communication). PhoP is part of the two-component system PhoP plus PhoR, which is a transcriptional regulator for numerous virulence factors. Mutation in PhoP has been found critical for attenuation of *Mtb* H37Ra, originally derived from H37Rv (Lee et al., 2008). The *fad* gene product is essential for the synthesis of phthiocerol dimycocerosate (C. Martin, personal communication).

Future strategies to improve efficacy of BCG replacement candidates include introduction of (1) genes encoding dormancy antigens such as the DosR-regulated gene products for post-exposure vaccination of latently infected individuals (Lin et al., 2007); (2) cytokine genes such as those for IL-18 and for IFN-γ for more profound Th1 cell polarization; and (3) deletion of anti-apoptotic genes to facilitate cross-priming.

Is this sufficient or do we need more sophisticated vaccines? A first attempt might be to combine current vaccine candidates to achieve sterile eradication of the pathogen, i.e., prime with the best BCG replacement vaccine followed by a selection of subunit boosters. These vaccines would contain antigens secreted by metabolically active *Mtb* as well as dormancy antigens.

Vaccination Strategies of the Near Future

Pre-exposure vaccination strategies have to induce a memory T cell response that resists exhaustion, suppression, and deviation so that it remains in a stage of alertness, whereby immune mechanisms can be promptly mobilized after encounter with *Mtb*. Novel postexposure vaccines have to restimulate the memory T cells induced by *Mtb*, promote potent Teff cells, and prevent suppressive sequellae.

At stage 1.0, vaccine candidates that contain *Mtb* infection to prevent TB disease outbreak require long-lasting memory T cell responses comprising mostly $CD4^+$ Th1 cells that activate macrophages to control their intracellular predators within solid granulomas. Because *Mtb* switches from a metabolically active to a dormant stage under pressure of the immune response, both dormancy and secreted antigens are needed (Black et al., 2009; Schuck et al., 2009; Leyten et al., 2006; Lin and Ottenhoff, 2008). Even though all current vaccine candidates follow this line of reasoning, the real-life situation is probably more complex. The environmental microbiome of pathogens and commensals may alter the ongoing immune response (Azzopardi et al., 2009; Hesseling et al., 2007a; Mansoor et al., 2009; Wilson et al., 1995; Weir et al., 2006; Hatherill et al., 2009). Humans, particularly in countries with high TB prevalence, are constantly exposed to various microorganisms. Environmental mycobacteria can alter BCG-induced immunity (Wilson et al., 1995; Weir et al., 2006); HIV impairs the $CD4^+$ T cell compartment resulting in increased susceptibility to *Mtb* (Azzopardi et al., 2009; Hesseling et al., 2007a, 2007b; Mansoor et al., 2009), and helminths drive the Th1 cell type response toward a Th2 cell type response, which can impair Th1 cell responses to *Mtb* (Hatherill et al., 2009). Hence, in humans, vaccine-induced immunity, which controls *Mtb*, is continuously exposed to numerous confounding influences from the outside. Uninterrupted fine-tuning is required to maintain protective immunity (Dorhoi and Kaufmann, 2009).

Internal influences may also confound protective immunity. These include suppression by cytokines (IL-10, TGF-β) from Treg cells or by soluble CD25, which acts as a decoy for IL-2, as well as exhaustion through cognate signaling via PD-1-PD-L1 or PD-L2 or CTLA-4-B7.1 or B7.2 (Chambers et al., 2001; Keir et al., 2008; Belkaid and Tarbell, 2009; Rubin et al., 1985; Shitrit et al., 2006; Jurado et al., 2008). Principally, these mechanisms are induced to avoid collateral damage by the ongoing immune response after pathogen eradication. In the case of TB, the pathogen is not eradicated and active TB disease is prevented in the face of persistent *Mtb*. Apparently, this quandary is hard to solve for the immune system. Recent findings in virus systems indicate that IL-21 can counteract exhaustion (Elsaesser et al., 2009; Fröhlich et al., 2009; Yi et al., 2009). Similarly, the immunosuppressive drug rapamycin was recently shown to stabilize memory T cell responses (Araki et al., 2009). Thus, strategies to sustain memory responses should consider blocking of inhibitory cytokines IL-10 and TGF- β or soluble CD25 and inhibitory T cell coreceptors PD-1 and CTLA-4 as well as prevention of inhibitory signals by use of cytokines such as IL-21 or drugs such as rapamycin.

Finally, for efficacious control of latent *Mtb* infection, canonical memory is insufficient (i.e., continuous presence of memory T cells that are reactivated after encounter with the pathogen).

Rather, in TB, the immune response has to contain the pathogen continuously over prolonged periods of time, often lifelong. In sum, although the current vaccination R&D pipeline has come up with several candidates, their ultimate efficacy in the real world still needs to be proven. In fact, the task for these vaccine candidates is not an easy one because they have to induce lifelong immunity, which contains *Mtb* in face of numerous confounding factors such as coinfection, suppression, and exhaustion. Such vaccination strategies therefore need to consider how to fine-tune long-lasting immunity in such a disorienting scenario.

The Distant Future: Vaccines that Achieve Sterile Eradication of *Mtb*

We now move forward to stage 2.0, namely, to vaccines that achieve sterile eradication of the pathogen. Although this is considered an essential requirement for most vaccines in use today, it has not been explored successfully in the realm of TB. Which mechanisms of protective immunity should such vaccination schemes induce?

Let us start our deliberations with post-exposure vaccines, which target the 2.2 billion individuals on this globe who are presumably latently infected with *Mtb*. First, such a vaccine should focus on antigens expressed by dormant *Mtb* in latently infected individuals. Second, this vaccine should induce an aggressive immune response against *Mtb* while minimizing collateral tissue damage from amplification of the immune response. On the positive side, the immune response could be short-lived and wane after elimination of *Mtb*. It is probable that such an immune response would best be achieved by stimulating different T cell populations that attack *Mtb* from multiple angles: first, Th17 cells that attract granulocytes to the site of *Mtb* persistence and create inflammation (Khader et al., 2007); second, Th1 cells that activate macrophages to eradicate their intracellular predators (Kaufmann, 2006b); third, $CD8^+$ CTL that assault intracellular *Mtb* by concerted action of granulysin and perforin (Stenger et al., 1997, 1998). Conventional T cells may be further supported by unconventional T cells, with similar or complementary functions. Thus, animal experiments suggest that in TB, IL-17 is primarily produced by $\gamma\delta$ T cells (Lockhart et al., 2006), and CD1-restricted T cells with specificity for glycolipids of *Mtb* share several effector functions with $CD4^+$ Th1 cells (Brigl and Brenner, 2004). Finally, antibodies could attack *Mtb* released into the extracellular space during host cell destruction.

Thus far, emphasis was laid on elimination of dormant *Mtb,* which is resistant to immune assault. It may be necessary to resuscitate dormant bacteria first to render them more susceptible to immune-mediated clearance. In this case, *Mtb* would need to be attacked after resuscitation, promptly and heavily, by the immune mechanisms described above.

Animal experiments as well as in vitro cell culture experiments with human leukocytes should be performed to elucidate the most potent mechanisms and how they could be combined to achieve synergistic effects. It goes without saying that because of the highly aggressive nature of the immune response, careful risk assessment and monitoring of side effects are essential. Because damage will occur only in infected individuals, safety issues cannot be fully solved in Phase I trials with naive study participants.

As a next step, pre-exposure vaccines that aim at accomplishing the same goals are desirable that can protect naive individuals by rapid elimination of *Mtb* after infection. Although the effector mechanisms are probably very similar, pre-exposure vaccines in addition need to induce a long-lasting memory response, which can be rapidly mobilized upon *Mtb* infection. Moreover, these vaccines should include antigens produced by metabolically active *Mtb* to allow attack promptly after stable infection has been established.

Both scenarios would fail in HIV-coinfected individuals in whom immunity cannot be stimulated at full strength. Finally, confounding issues as described under stage 1.0 remain, notably the risk of T cell exhaustion and the need for fine-tuning in response to coinfection with environmental commensals and pathogens.

The Distant Future: Vaccines that Prevent *Mtb* Infection

We now come to stage 3.0, a strategy of preventing infection by the induction of pre-existing antibodies. This approach has little precedent for TB. Yet, some vaccines (e.g., against human papillomavirus) are based on pre-existing antibodies that eradicate the pathogen before it can hide inside host cells, where it establishes stable infection to cause disease later (Stanley, 2010). Only very few *Mtb* organisms enter the lung that would become targets of antibodies preexisting in the alveolar space. These antibodies should fulfill three tasks at least: first, neutralization of essential functions in *Mtb*, e.g., uptake of iron and other essential micronutrients (Schaible and Kaufmann, 2004); second, stimulation of potent host effector mechanisms, i.e., activation of alveolar macrophages as well as attraction of blood neutrophils and monocytes to the port of entry; third, prevention of *Mtb* uptake by nonprofessional phagocytes, such as lung parenchyma cells, and by professional phagocytes of insufficient competence. Evidence exists that alveolar macrophages cannot be activated to full antibacterial competence (Russi and Crystal, 1997; Geissmann et al., 2010). Hence, their role in TB needs to be determined carefully: do they primarily perform as effectors against or as refuge for *Mtb*? Principally, antibodies bind to cells via cognates of the Fc receptor (FcR) family (Nimmerjahn and Ravetch, 2007). This family includes both inhibitory and stimulatory cognates. Ideally, vaccine-induced antibodies would activate effector cells through activating FcR and be prohibited from binding to inhibitory FcR. Attack of *Mtb* by antibodies should be accompanied by Th17 cells, which attract neutrophils, and by Th1 cells, which in turn activate mononuclear phagocytes (Cooper, 2009). Perhaps further help is required from $CD8^+$ CTL, which assault *Mtb* by the combined coercion of perforin and granulysin (Stenger et al., 1997, 1998). Because this type of aggressive blitzkrieg will ensue only after TB reactivation, monitoring for side effects needs to await Phase III trials, which assess vaccine efficacy against natural *Mtb* infection. Phase I and II trials comprising healthy uninfected or *Mtb* latently infected individuals cannot provide full answers to these safety issues.

Concluding Remarks

After decades of research, the R&D pipeline of TB vaccines is ready to reap the fruits of its efforts. The number of vaccine candidates that have entered the clinical trial pipeline exceeds

the financial resources available for clinical phase III trials (Box 1), so attrition is called for. Although there is hope that within the next decade one or two vaccines with proven protective efficacy and safety may emerge, this by no means signals the end of scientific efforts. All vaccine candidates currently undergoing clinical trials will only delay TB reactivation and hence will depend on efficacious memory capable of controlling *Mtb* in a dormant stage lifelong. Such long-lasting immunity, however, is prone to confounding effects, be they internal such as regulation and exhaustion or external such as coinfection with HIV or helminths. Hence, basic research needs to find alternatives, such as sterile pathogen eradication or prevention of stable infection. Experimental models provide novel information as to how the immune response can be strengthened so that it is superior to the natural immune response against *Mtb* infection, and clinical trials will provide information about immunity evoked by novel vaccine candidates. A full circle of translation of experiments in the wetlab that reveal novel vaccine candidates and reverse translation of findings in the field, which disclose human-specific responses to vaccine candidates, is the best, if not the only, stratagem to move forward to a vaccine that can substantially reduce TB incidences to <1 case per million population as ambitiously proposed by the StopTB Partnership, who seeks elimination of TB by 2050 (Abu-Raddad et al., 2009; Dye and Williams, 2008).

ACKNOWLEDGMENTS

I thank M. Grossman for outstanding editorial support. I also thank D. Schad for her outstanding graphic design of the figures. Many thanks to W. Hanekom of Capetown, South Africa, and C. Martin of Zaragosa, Spain, for helpful comments. I thank the many colleagues and friends with whom I have discussed future vaccination strategies against TB on many occasions, notably members of the Tuberculosis Vaccine Initiative (TBVI) and members of the Bill & Melinda Gates Foundation Grand Challenge 6 Program. Work from my own laboratory on TB vaccine development receives financial support from numerous sources, notably NEWTBVAC FP7 (HEALTH-F3-2009-241745) and Bill & Melinda Gates Foundation Grand Challenges in Global Health (Grant No. 37772). S.H.E.K. is coinventor of the rBCGΔUreC:Hly vaccine candidate and member of the Scientific Advisory Boards of Intercell and of Vaccine Project Management (VPM).

REFERENCES

Abu-Raddad, L.J., Sabatelli, L., Achterberg, J.T., Sugimoto, J.D., Longini, I.M., Jr., Dye, C., and Halloran, M.E. (2009). Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. Proc. Natl. Acad. Sci. USA *106*, 13980–13985.

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Pathogen recognition and innate immunity. Cell *124*, 783–801.

Antas, P.R., and Castello-Branco, L.R. (2008). New vaccines against tuberculosis: Lessons learned from BCG immunisation in Brazil. Trans. R. Soc. Trop. Med. Hyg. *102*, 628–630.

Araki, K., Turner, A.P., Shaffer, V.O., Gangappa, S., Keller, S.A., Bachmann, M.F., Larsen, C.P., and Ahmed, R. (2009). mTOR regulates memory CD8 T-cell differentiation. Nature *460*, 108–112.

Azzopardi, P., Bennett, C.M., Graham, S.M., and Duke, T. (2009). Bacille Calmette-Guérin vaccine-related disease in HIV-infected children: A systematic review. Int. J. Tuberc. Lung Dis. *13*, 1331–1344.

Bangari, D.S., and Mittal, S.K. (2006). Development of nonhuman adenoviruses as vaccine vectors. Vaccine *24*, 849–862.

Bange, F.C., Brown, A.M., and Jacobs, W.R., Jr. (1996). Leucine auxotrophy restricts growth of *Mycobacterium bovis* BCG in macrophages. Infect. Immun. *64*, 1794–1799.

Belkaid, Y., and Tarbell, K. (2009). Regulatory T cells in the control of host-microorganism interactions (*). Annu. Rev. Immunol. *27*, 551–589.

Betts, J.C., Lukey, P.T., Robb, L.C., McAdam, R.A., and Duncan, K. (2002). Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. Mol. Microbiol. *43*, 717–731.

Black, G.F., Thiel, B.A., Ota, M.O., Parida, S.K., Adegbola, R., Boom, W.H., Dockrell, H.M., Franken, K.L., Friggen, A.H., Hill, P.C., et al; GCGH Biomarkers for TB Consortium. (2009). Immunogenicity of novel DosR regulon-encoded candidate antigens of *Mycobacterium tuberculosis* in three high-burden populations in Africa. Clin. Vaccine Immunol. *16*, 1203–1212.

Brigl, M., and Brenner, M.B. (2004). CD1: Antigen presentation and T cell function. Annu. Rev. Immunol. *22*, 817–890.

Calmette, A. (1927). Sur la vaccination préventive des enfants nouveau-nés contre la tuberculose par le BCG. Annales de l'Institut Pasteur *41*, 201–232.

Chambers, C.A., Kuhns, M.S., Egen, J.G., and Allison, J.P. (2001). CTLA-4-mediated inhibition in regulation of T cell responses: Mechanisms and manipulation in tumor immunotherapy. Annu. Rev. Immunol. *19*, 565–594.

Chen, X., Zhou, B., Li, M., Deng, Q., Wu, X., Le, X., Wu, C., Larmonier, N., Zhang, W., Zhang, H., et al. (2007). CD4(+)CD25(+)FoxP3(+) regulatory T cells suppress *Mycobacterium tuberculosis* immunity in patients with active disease. Clin. Immunol. *123*, 50–59.

Cooper, A.M. (2009). Cell-mediated immune responses in tuberculosis. Annu. Rev. Immunol. *27*, 393–422.

Cosma, C.L., Humbert, O., Sherman, D.R., and Ramakrishnan, L. (2008). Trafficking of superinfecting *Mycobacterium* organisms into established granulomas occurs in mammals and is independent of the Erp and ESX-1 mycobacterial virulence loci. J. Infect. Dis. *198*, 1851–1855.

Davis, J.M., and Ramakrishnan, L. (2009). The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell *136*, 37–49.

Day, T.A., Koch, M., Nouailles, G., Jacobsen, M., Kosmiadi, G.A., Miekley, D., Kuhlmann, S., Jörg, S., Gamradt, P., Mollenkopf, H.J., et al. (2010). Secondary lymphoid organs are dispensable for the development of T-cell-mediated immunity during tuberculosis. Eur. J. Immunol. *40*, 1663–1673.

Dennehy, K.M., Willment, J.A., Williams, D.L., and Brown, G.D. (2009). Reciprocal regulation of IL-23 and IL-12 following co-activation of Dectin-1 and TLR signaling pathways. Eur. J. Immunol. *39*, 1379–1386.

Deretic, V. (2006). Autophagy as an immune defense mechanism. Curr. Opin. Immunol. *18*, 375–382.

Dietrich, J., Aagaard, C., Leah, R., Olsen, A.W., Stryhn, A., Doherty, T.M., and Andersen, P. (2005). Exchanging ESAT6 with TB10.4 in an Ag85B fusion molecule-based tuberculosis subunit vaccine: Efficient protection and ESAT6-based sensitive monitoring of vaccine efficacy. J. Immunol. *174*, 6332–6339.

Divangahi, M., Desjardins, D., Nunes-Alves, C., Remold, H.G., and Behar, S.M. (2010). Eicosanoid pathways regulate adaptive immunity to *Mycobacterium tuberculosis*. Nat. Immunol. *11*, 751–758.

Dlugovitzky, D., Bay, M.L., Rateni, L., Urízar, L., Rondelli, C.F., Largacha, C., Farroni, M.A., Molteni, O., and Bottasso, O.A. (1999). In vitro synthesis of interferon-gamma, interleukin-4, transforming growth factor-beta and interleukin-1 beta by peripheral blood mononuclear cells from tuberculosis patients: Relationship with the severity of pulmonary involvement. Scand. J. Immunol. *49*, 210–217.

Dorhoi, A., and Kaufmann, S.H. (2009). Fine-tuning of T cell responses during infection. Curr. Opin. Immunol. *21*, 367–377.

Dorhoi, A., Desel, C., Yeremeev, V.V., Pradl, L., Brinkmann, V., Mollenkopf, H.-J., Hanke, K., Gross, O., Ruland, J., and Kaufmann, S.H.E. (2010). The adaptor molecule CARD9 is essential for tuberculosis control. J. Exp. Med. *207*, 777–792.

Dye, C., and Williams, B.G. (2008). Eliminating human tuberculosis in the twenty-first century. J. R. Soc. Interface *5*, 653–662.

Elsaesser, H., Sauer, K., and Brooks, D.G. (2009). IL-21 is required to control chronic viral infection. Science *324*, 1569–1572.

Fine, P.E. (1995). Variation in protection by BCG: Implications of and for heterologous immunity. Lancet *346*, 1339–1345.

Flatz, L., Hegazy, A.N., Bergthaler, A., Verschoor, A., Claus, C., Fernandez, M., Gattinoni, L., Johnson, S., Kreppel, F., Kochanek, S., et al. (2010). Development of replication-defective lymphocytic choriomeningitis virus vectors for the induction of potent CD8+ T cell immunity. Nat. Med. *16*, 339–345.

Foulds, K.E., Wu, C.Y., and Seder, R.A. (2006). Th1 memory: Implications for vaccine development. Immunol. Rev. *211*, 58–66.

Fröhlich, A., Kisielow, J., Schmitz, I., Freigang, S., Shamshiev, A.T., Weber, J., Marsland, B.J., Oxenius, A., and Kopf, M. (2009). IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. Science *324*, 1576–1580.

Gandhi, N.R., Nunn, P., Dheda, K., Schaaf, H.S., Zignol, M., van Soolingen, D., Jensen, P., and Bayona, J. (2010). Multidrug-resistant and extensively drug-resistant tuberculosis: A threat to global control of tuberculosis. Lancet *375*, 1830–1843.

Geijtenbeek, T.B., Van Vliet, S.J., Koppel, E.A., Sanchez-Hernandez, M., Vandenbroucke-Grauls, C.M., Appelmelk, B., and Van Kooyk, Y. (2003). Mycobacteria target DC-SIGN to suppress dendritic cell function. J. Exp. Med. *197*, 7–17.

Geissmann, F., Manz, M.G., Jung, S., Sieweke, M.H., Merad, M., and Ley, K. (2010). Development of monocytes, macrophages, and dendritic cells. Science *327*, 656–661.

Gram, G.J., Karlsson, I., Agger, E.M., Andersen, P., and Fomsgaard, A. (2009). A novel liposome-based adjuvant CAF01 for induction of CD8(+) cytotoxic T-lymphocytes (CTL) to HIV-1 minimal CTL peptides in HLA-A*0201 transgenic mice. PLoS ONE *4*, e6950.

Grode, L., Seiler, P., Baumann, S., Hess, J., Brinkmann, V., Nasser Eddine, A., Mann, P., Goosmann, C., Bandermann, S., Smith, D., et al. (2005). Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille Calmette-Guérin mutants that secrete listeriolysin. J. Clin. Invest. *115*, 2472–2479.

Guleria, I., Teitelbaum, R., McAdam, R.A., Kalpana, G., Jacobs, W.R., Jr., and Bloom, B.R. (1996). Auxotrophic vaccines for tuberculosis. Nat. Med. *2*, 334–337.

Gutierrez, M.G., Master, S.S., Singh, S.B., Taylor, G.A., Colombo, M.I., and Deretic, V. (2004). Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. Cell *119*, 753–766.

Guyot-Revol, V., Innes, J.A., Hackforth, S., Hinks, T., and Lalvani, A. (2006). Regulatory T cells are expanded in blood and disease sites in patients with tuberculosis. Am. J. Respir. Crit. Care Med. *173*, 803–810.

Harding, C.V., and Boom, W.H. (2010). Regulation of antigen presentation by *Mycobacterium tuberculosis*: A role for Toll-like receptors. Nat. Rev. Microbiol. *8*, 296–307.

Harries, A.D., Zachariah, R., Corbett, E.L., Lawn, S.D., Santos-Filho, E.T., Chimzizi, R., Harrington, M., Maher, D., Williams, B.G., and De Cock, K.M. (2010). The HIV-associated tuberculosis epidemic—When will we act? Lancet *375*, 1906–1919.

Hatherill, M., Adams, V., Hughes, J., De Kock, M., Mavakla, W., Pienaar, B., Mahomed, H., Hussey, G., and Hanekom, W.A. (2009). The potential impact of helminth infection on trials of novel tuberculosis vaccines. Vaccine *27*, 4743–4744.

Hesseling, A.C., Cotton, M.F., Marais, B.J., Gie, R.P., Schaaf, H.S., Beyers, N., Fine, P.E., Abrams, E.J., Godfrey-Faussett, P., and Kuhn, L. (2007a). BCG and HIV reconsidered: Moving the research agenda forward. Vaccine *25*, 6565–6568.

Hesseling, A.C., Marais, B.J., Gie, R.P., Schaaf, H.S., Fine, P.E., Godfrey-Faussett, P., and Beyers, N. (2007b). The risk of disseminated Bacille Calmette-Guerin (BCG) disease in HIV-infected children. Vaccine *25*, 14–18.

Hill, A.V., Reyes-Sandoval, A., O'Hara, G., Ewer, K., Lawrie, A., Goodman, A., Nicosia, A., Folgori, A., Colloca, S., Cortese, R., et al. (2010). Prime-boost vectored malaria vaccines: Progress and prospects. Hum. Vaccin. *6*, 78–83.

Hotez, P.J., and Kamath, A. (2009). Neglected tropical diseases in sub-saharan Africa: Review of their prevalence, distribution, and disease burden. PLoS Negl. Trop. Dis. *3*, e412.

Hutnick, N.A., Carnathan, D.G., Dubey, S.A., Makedonas, G., Cox, K.S., Kierstead, L., Ratcliffe, S.J., Robertson, M.N., Casimiro, D.R., Ertl, H.C., and Betts, M.R. (2009). Baseline Ad5 serostatus does not predict Ad5 HIV vaccine-induced expansion of adenovirus-specific CD4+ T cells. Nat. Med. *15*, 876–878.

Ishikawa, E., Ishikawa, T., Morita, Y.S., Toyonaga, K., Yamada, H., Takeuchi, O., Kinoshita, T., Akira, S., Yoshikai, Y., and Yamasaki, S. (2009). Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J. Exp. Med. *206*, 2879–2888.

Jurado, J.O., Alvarez, I.B., Pasquinelli, V., Martínez, G.J., Quiroga, M.F., Abbate, E., Musella, R.M., Chuluyan, H.E., and García, V.E. (2008). Programmed death (PD)-1:PD-ligand 1/PD-ligand 2 pathway inhibits T cell effector functions during human tuberculosis. J. Immunol. *181*, 116–125.

Kagina, M.N., Abel, B., Scriba, T.J., Hughes, E.J., Keyser, A., Soares, A., Gamieldien, H., Sidibana, M., Hatherill, M., Gelderbloem, S., Mahomed, H., Hawkridge, A., Hussey, G., Kaplan, G., and Hanekom, M., and other members of SATVI. (2010). Specific T cell frequency and cytokine expression profile do not correlate with protection against tuberculosis, following BCG vaccination of newborns. Am. J. Respir. Crit. Care Med., in press. Published online June 17, 2010. 10.1164/rccm.201003-0334OC.

Kana, B.D., Gordhan, B.G., Downing, K.J., Sung, N., Vostroktunova, G., Machowski, E.E., Tsenova, L., Young, M., Kaprelyants, A., Kaplan, G., and Mizrahi, V. (2008). The resuscitation-promoting factors of *Mycobacterium tuberculosis* are required for virulence and resuscitation from dormancy but are collectively dispensable for growth in vitro. Mol. Microbiol. *67*, 672–684.

Kaufmann, S.H.E. (2006a). Envisioning future strategies for vaccination against tuberculosis. Nat. Rev. Immunol. *6*, 699–704.

Kaufmann, S.H.E. (2006b). Tuberculosis: Back on the immunologists' agenda. Immunity *24*, 351–357.

Kaufmann, S.H.E. (2009). The New Plagues: Pandemics and Poverty in a Globalized World (London: Haus Publishing).

Kaufmann, S.H., Hussey, G., and Lambert, P.H. (2010). New vaccines for tuberculosis. Lancet *375*, 2110–2119.

Keir, M.E., Butte, M.J., Freeman, G.J., and Sharpe, A.H. (2008). PD-1 and its ligands in tolerance and immunity. Annu. Rev. Immunol. *26*, 677–704.

Khader, S.A., Bell, G.K., Pearl, J.E., Fountain, J.J., Rangel-Moreno, J., Cilley, G.E., Shen, F., Eaton, S.M., Gaffen, S.L., Swain, S.L., et al. (2007). IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. Nat. Immunol. *8*, 369–377.

Kursar, M., Koch, M., Mittrücker, H.W., Nouailles, G., Bonhagen, K., Kamradt, T., and Kaufmann, S.H. (2007). Cutting Edge: Regulatory T cells prevent efficient clearance of *Mycobacterium tuberculosis*. J. Immunol. *178*, 2661–2665.

Lee, J.S., Krause, R., Schreiber, J., Mollenkopf, H.J., Kowall, J., Stein, R., Jeon, B.Y., Kwak, J.Y., Song, M.K., Patron, J.P., et al. (2008). Mutation in the transcriptional regulator PhoP contributes to avirulence of *Mycobacterium tuberculosis* H37Ra strain. Cell Host Microbe *3*, 97–103.

Leyten, E.M., Lin, M.Y., Franken, K.L., Friggen, A.H., Prins, C., van Meijgaarden, K.E., Voskuil, M.I., Weldingh, K., Andersen, P., Schoolnik, G.K., et al. (2006). Human T-cell responses to 25 novel antigens encoded by genes of the dormancy regulon of *Mycobacterium tuberculosis*. Microbes Infect. *8*, 2052–2060.

Lin, M.Y., and Ottenhoff, T.H. (2008). Not to wake a sleeping giant: New insights into host-pathogen interactions identify new targets for vaccination against latent *Mycobacterium tuberculosis* infection. Biol. Chem. *389*, 497–511.

Lin, M.Y., Geluk, A., Smith, S.G., Stewart, A.L., Friggen, A.H., Franken, K.L., Verduyn, M.J., van Meijgaarden, K.E., Voskuil, M.I., Dockrell, H.M., et al. (2007). Lack of immune responses to *Mycobacterium tuberculosis* DosR regulon proteins following *Mycobacterium bovis* BCG vaccination. Infect. Immun. *75*, 3523–3530.

Lingnau, K., Riedl, K., and von Gabain, A. (2007). IC31 and IC30, novel types of vaccine adjuvant based on peptide delivery systems. Expert Rev. Vaccines *6*, 741–746.

Locht, C., Hougardy, J.M., Rouanet, C., Place, S., and Mascart, F. (2006). Heparin-binding hemagglutinin, from an extrapulmonary dissemination factor to a powerful diagnostic and protective antigen against tuberculosis. Tuberculosis (Edinb.) *86*, 303–309.

Lockhart, E., Green, A.M., and Flynn, J.L. (2006). IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. J. Immunol. *177*, 4662–4669.

Lönnroth, K., Castro, K.G., Chakaya, J.M., Chauhan, L.S., Floyd, K., Glaziou, P., and Raviglione, M.C. (2010). Tuberculosis control and elimination 2010-50: Cure, care, and social development. Lancet *375*, 1814–1829.

Mansoor, N., Scriba, T.J., de Kock, M., Tameris, M., Abel, B., Keyser, A., Little, F., Soares, A., Gelderbloem, S., Mlenjeni, S., et al. (2009). HIV-1 infection in infants severely impairs the immune response induced by Bacille Calmette-Guérin vaccine. J. Infect. Dis. *199*, 982–990.

Martin, C., Williams, A., Hernandez-Pando, R., Cardona, P.J., Gormley, E., Bordat, Y., Soto, C.Y., Clark, S.O., Hatch, G.J., Aguilar, D., et al. (2006). The live *Mycobacterium tuberculosis* phoP mutant strain is more attenuated than BCG and confers protective immunity against tuberculosis in mice and guinea pigs. Vaccine *24*, 3408–3419.

McShane, H., Pathan, A.A., Sander, C.R., Keating, S.M., Gilbert, S.C., Huygen, K., Fletcher, H.A., and Hill, A.V. (2004). Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. Nat. Med. *10*, 1240–1244.

Mora, J.R., and von Andrian, U.H. (2006). T-cell homing specificity and plasticity: New concepts and future challenges. Trends Immunol. *27*, 235–243.

Nimmerjahn, F., and Ravetch, J.V. (2007). Fc-receptors as regulators of immunity. Adv. Immunol. *96*, 179–204.

O'Brien, K.L., Liu, J., King, S.L., Sun, Y.H., Schmitz, J.E., Lifton, M.A., Hutnick, N.A., Betts, M.R., Dubey, S.A., Goudsmit, J., et al. (2009). Adenovirus-specific immunity after immunization with an Ad5 HIV-1 vaccine candidate in humans. Nat. Med. *15*, 873–875.

O'Shea, J.J., and Paul, W.E. (2010). Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science *327*, 1098–1102.

Parida, S.K., and Kaufmann, S.H. (2010). The quest for biomarkers in tuberculosis. Drug Discov. Today *15*, 148–157.

Pethe, K., Alonso, S., Biet, F., Delogu, G., Brennan, M.J., Locht, C., and Menozzi, F.D. (2001). The heparin-binding haemagglutinin of *M. tuberculosis* is required for extrapulmonary dissemination. Nature *412*, 190–194.

Radosevic, K., Wieland, C.W., Rodriguez, A., Weverling, G.J., Mintardjo, R., Gillissen, G., Vogels, R., Skeiky, Y.A., Hone, D.M., Sadoff, J.C., et al. (2007). Protective immune responses to a recombinant adenovirus type 35 tuberculosis vaccine in two mouse strains: CD4 and CD8 T-cell epitope mapping and role of gamma interferon. Infect. Immun. *75*, 4105–4115.

Reed, S.G., Bertholet, S., Coler, R.N., and Friede, M. (2009). New horizons in adjuvants for vaccine development. Trends Immunol. *30*, 23–32.

Rouanet, C., Debrie, A.S., Lecher, S., and Locht, C. (2009). Subcutaneous boosting with heparin binding haemagglutinin increases BCG-induced protection against tuberculosis. Microbes Infect. *11*, 995–1001.

Rubin, L.A., Kurman, C.C., Fritz, M.E., Biddison, W.E., Boutin, B., Yarchoan, R., and Nelson, D.L. (1985). Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. J. Immunol. *135*, 3172–3177.

Russell, D.G. (2007). Who puts the tubercle in tuberculosis? Nat. Rev. Microbiol. *5*, 39–47.

Russi, T.J., and Crystal, R.G. (1997). Use of bronchoalvaeolar lavage and airway brushing to investigate the human lung. In The Lung: Scientific Foundations, R.G. Crystal, J.B. West, P.J. Barnes, and E. Weibel, eds. (Philadelphia: Lippincott-Raven Publishers), pp. 371–382.

Sallusto, F., Geginat, J., and Lanzavecchia, A. (2004). Central memory and effector memory T cell subsets: Function, generation, and maintenance. Annu. Rev. Immunol. *22*, 745–763.

Sambandamurthy, V.K., Wang, X., Chen, B., Russell, R.G., Derrick, S., Collins, F.M., Morris, S.L., and Jacobs, W.R., Jr. (2002). A pantothenate auxotroph of *Mycobacterium tuberculosis* is highly attenuated and protects mice against tuberculosis. Nat. Med. *8*, 1171–1174.

Sambandamurthy, V.K., Derrick, S.C., Hsu, T., Chen, B., Larsen, M.H., Jalapathy, K.V., Chen, M., Kim, J., Porcelli, S.A., Chan, J., et al. (2006). *Mycobacterium tuberculosis* DeltaRD1 DeltapanCD: A safe and limited replicating mutant strain that protects immunocompetent and immunocompromised mice against experimental tuberculosis. Vaccine *24*, 6309–6320.

Sander, C.R., Pathan, A.A., Beveridge, N.E., Poulton, I., Minassian, A., Alder, N., Van Wijgerden, J., Hill, A.V., Gleeson, F.V., Davies, R.J., et al. (2009). Safety and immunogenicity of a new tuberculosis vaccine, MVA85A, in *Mycobacterium tuberculosis*-infected individuals. Am. J. Respir. Crit. Care Med. *179*, 724–733.

Santosuosso, M., McCormick, S., Zhang, X., Zganiacz, A., and Xing, Z. (2006). Intranasal boosting with an adenovirus-vectored vaccine markedly enhances protection by parenteral *Mycobacterium bovis* BCG immunization against pulmonary tuberculosis. Infect. Immun. *74*, 4634–4643.

Schaible, U.E., and Kaufmann, S.H.E. (2004). Iron and microbial infection. Nat. Rev. Microbiol. *2*, 946–953.

Schoenen, H., Bodendorfer, B., Hitchens, K., Manzanero, S., Werninghaus, K., Nimmerjahn, F., Agger, E.M., Stenger, S., Andersen, P., Ruland, J., et al. (2010). Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. J. Immunol. *184*, 2756–2760.

Schuck, S.D., Mueller, H., Kunitz, F., Neher, A., Hoffmann, H., Franken, K.L., Repsilber, D., Ottenhoff, T.H., Kaufmann, S.H., and Jacobsen, M. (2009). Identification of T-cell antigens specific for latent *mycobacterium tuberculosis* infection. PLoS ONE *4*, e5590.

Scotet, E., Nedellec, S., Devilder, M.C., Allain, S., and Bonneville, M. (2008). Bridging innate and adaptive immunity through gammadelta T-dendritic cell crosstalk. Front. Biosci. *13*, 6872–6885.

Shitrit, D., Izbicki, G., Bar-Gil Shitrit, A., Raz, M., Sulkes, J., and Kramer, M.R. (2006). Role of soluble interleukin-2 receptor levels in patients with latent tuberculosis. Lung *184*, 21–24.

Stanley, M. (2010). Prospects for new human papillomavirus vaccines. Curr. Opin. Infect. Dis. *23*, 70–75.

Stenger, S., Mazzaccaro, R.J., Uyemura, K., Cho, S., Barnes, P.F., Rosat, J.P., Sette, A., Brenner, M.B., Porcelli, S.A., Bloom, B.R., and Modlin, R.L. (1997). Differential effects of cytolytic T cell subsets on intracellular infection. Science *276*, 1684–1687.

Stenger, S., Hanson, D.A., Teitelbaum, R., Dewan, P., Niazi, K.R., Froelich, C.J., Ganz, T., Thoma-Uszynski, S., Melián, A., Bogdan, C., et al. (1998). An antimicrobial activity of cytolytic T cells mediated by granulysin. Science *282*, 121–125.

StopTB Partnership Working Group on New TB Vaccines. (2009). StopTB Tuberculosis Vaccine Pipeline 2009. http://www.stoptb.org/wg/new_vaccines/assets/documents/TB%20Vaccine%20Pipeline%202009.pdf.

Sun, R., Skeiky, Y.A., Izzo, A., Dheenadhayalan, V., Imam, Z., Penn, E., Stagliano, K., Haddock, S., Mueller, S., Fulkerson, J., et al. (2009). Novel recombinant BCG expressing perfringolysin O and the over-expression of key immunodominant antigens; pre-clinical characterization, safety and protection against challenge with *Mycobacterium tuberculosis*. Vaccine *27*, 4412–4423.

Sun, R., Hone, D. M., and Sadoff, J. C. February 2010.Recombinant BCG strains with enhanced ability to escape the endosome. U.S. patent 7,666,656.

Sutherland, J.S., Young, J.M., Peterson, K.L., Sanneh, B., Whittle, H.C., Rowland-Jones, S.L., Adegbola, R.A., Jaye, A., and Ota, M.O. (2010). Polyfunctional CD4(+) and CD8(+) T cell responses to tuberculosis antigens in HIV-1-infected patients before and after anti-retroviral treatment. J. Immunol. *184*, 6537–6544.

Tailleux, L., Schwartz, O., Herrmann, J.L., Pivert, E., Jackson, M., Amara, A., Legres, L., Dreher, D., Nicod, L.P., Gluckman, J.C., et al. (2003). DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. J. Exp. Med. *197*, 121–127.

Toossi, Z., and Ellner, J.J. (1998). Host response to *Mycobacterium tuberculosis*. Front. Biosci. *3*, e133–e140.

Torchinsky, M.B., Garaude, J., Martin, A.P., and Blander, J.M. (2009). Innate immune recognition of infected apoptotic cells directs T(H)17 cell differentiation. Nature *458*, 78–82.

Tullius, M.V., Harth, G., Maslesa-Galic, S., Dillon, B.J., and Horwitz, M.A. (2008). A replication-limited recombinant *Mycobacterium bovis* BCG vaccine against tuberculosis designed for human immunodeficiency virus-positive persons is safer and more efficacious than BCG. Infect. Immun. *76*, 5200–5214.

Ulrichs, T., and Kaufmann, S.H.E. (2006). New insights into the function of granulomas in human tuberculosis. J. Pathol. *208*, 261–269.

Underhill, D.M., Ozinsky, A., Smith, K.D., and Aderem, A. (1999). Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. Proc. Natl. Acad. Sci. USA *96*, 14459–14463.

van der Wel, N., Hava, D., Houben, D., Fluitsma, D., van Zon, M., Pierson, J., Brenner, M., and Peters, P.J. (2007). *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. Cell *129*, 1287–1298.

van Dissel, J.T., Arend, S.M., Prins, C., Bang, P., Tingskov, P.N., Lingnau, K., Nouta, J., Klein, M.R., Rosenkrands, I., Ottenhoff, T.H., et al. (2010). Ag85B-ESAT-6 adjuvanted with IC31 promotes strong and long-lived *Mycobacterium tuberculosis* specific T cell responses in naïve human volunteers. Vaccine *28*, 3571–3581.

Volkman, H.E., Pozos, T.C., Zheng, J., Davis, J.M., Rawls, J.F., and Ramakrishnan, L. (2010). Tuberculous granuloma induction via interaction of a bacterial secreted protein with host epithelium. Science *327*, 466–469.

Von Eschen, K., Morrison, R., Braun, M., Ofori-Anyinam, O., De Kock, E., Pavithran, P., Koutsoukos, M., Moris, P., Cain, D., Dubois, M.-C., et al. (2009). The candidate tuberculosis vaccine Mtb72F/AS02A: Tolerability and immunogenicity in humans. Hum. Vaccin. *5*, 475–482.

Voskuil, M.I., Schnappinger, D., Visconti, K.C., Harrell, M.I., Dolganov, G.M., Sherman, D.R., and Schoolnik, G.K. (2003). Inhibition of respiration by nitric oxide induces a *Mycobacterium tuberculosis* dormancy program. J. Exp. Med. *198*, 705–713.

Walker, K.B., Brennan, M.J., Ho, M.M., Eskola, J., Thiry, G., Sadoff, J., Dobbelaer, R., Grode, L., Liu, M.A., Fruth, U., and Lambert, P.H. (2010). The second Geneva Consensus: Recommendations for novel live TB vaccines. Vaccine *28*, 2259–2270.

Wayne, L.G., and Sohaskey, C.D. (2001). Nonreplicating persistence of *mycobacterium tuberculosis*. Annu. Rev. Microbiol. *55*, 139–163.

Weir, R.E., Black, G.F., Nazareth, B., Floyd, S., Stenson, S., Stanley, C., Branson, K., Sichali, L., Chaguluka, S.D., Donovan, L., et al. (2006). The influence of previous exposure to environmental mycobacteria on the interferon-gamma response to bacille Calmette-Guérin vaccination in southern England and northern Malawi. Clin. Exp. Immunol. *146*, 390–399.

WHO. (2007). Revised BCG vaccination guidelines for infants at risk for HIV infection. Wkly. Epidemiol. Rec. *82*, 193–196.

WHO. (2009). Global Tuberculosis Control: Epidemiology, Strategy, Financing. WHO Report 2009 (Geneva: World Health Organization).

Williams, M.A., and Bevan, M.J. (2007). Effector and memory CTL differentiation. Annu. Rev. Immunol. *25*, 171–192.

Wilson, M.E., Fineberg, H.V., and Colditz, G.A. (1995). Geographic latitude and the efficacy of bacillus Calmette-Guérin vaccine. Clin. Infect. Dis. *20*, 982–991.

Winau, F., Hegasy, G., Kaufmann, S.H.E., and Schaible, U.E. (2005). No life without death—Apoptosis as prerequisite for T cell activation. Apoptosis *10*, 707–715.

Winau, F., Weber, S., Sad, S., de Diego, J., Hoops, S.L., Breiden, B., Sandhoff, K., Brinkmann, V., Kaufmann, S.H.E., and Schaible, U.E. (2006). Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. Immunity *24*, 105–117.

Yi, J.S., Du, M., and Zajac, A.J. (2009). A vital role for interleukin-21 in the control of a chronic viral infection. Science *324*, 1572–1576.