**MINIREVIEW**

**Adjuvants in tuberculosis vaccine development**

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**Abstract**

Tuberculosis remains a major public health problem around the world. Because the *Mycobacterium bovis* Bacilli–Calmette–Guerin (BCG) vaccine fails to protect adults from pulmonary tuberculosis, there is an urgent need for improved vaccine formulations. Unlike BCG, recombinant vaccines purified from bacterial expression vectors, as well as naked DNA, require an additional adjuvant. Recent improvements in our understanding of disease immunopathology, together with advances in biochemical and molecular techniques, have permitted the successful development of promising tuberculosis vaccine delivery and adjuvant combinations for human use. Here, we summarize the current state of adjuvant development and its impact on tuberculosis vaccine progress.

**Introduction**

According to the World Health Organization (WHO), one-third of the world’s population is infected with *Mycobacterium tuberculosis* (*Mtb*). Among these latent carriers, around 5–10% will develop clinical tuberculosis, causing 2–3 million deaths and 8–10 million new infections per year (Young & Dye, 2006). In 2007, approximately 9.2 million new cases were reported. Of these, 1.3 million were HIV-positive cases, 1.1 million were reactivation cases and 500,000 cases were multidrug-resistant (MDR-tuberculosis) (WHO, 2009). To date, the only prophylactic available against *Mtb* is the Bacilli–Calmette–Guerin (BCG) vaccine, an attenuated *Mycobacterium bovis* strain that confers protection against several childhood forms of tuberculosis, but fails to prevent pulmonary tuberculosis in adults. Beyond vaccines such as BCG, which are administered before tuberculosis infection, one potential strategy to eliminate or control latent tuberculosis and prevent reactivation consists of postexposure vaccines (Andersen, 2007). In both cases, research efforts are directed towards conferring broad protection against disease and infection, especially by stimulating cellular immune responses involving CD4+ and CD8+ T cells without negative health consequences (Titball, 2008). Thanks to recombinant technology and a growing understanding of the immunopathology of tuberculosis, candidate subunit vaccines have been successfully developed. These vaccines are preferred because of their safety in both normal and immunocompromised patients, although their inherent lack of immunogenicity requires the use of adjuvants capable of inducing a protective T-cell response (Schijns, 2003). In order to be protective against *Mtb*, a candidate vaccine must elicit a specific cell-mediated response, both in immunocompetent and in immunocompromised individuals who are considered a high-risk population for tuberculosis. Consequently, the development of adjuvants to improve tuberculosis vaccines for human use remains a challenge and is equally important to subunit vaccine formulation as antigen discovery (Hoft, 2008). Here, we review the current state of adjuvant development and its impact on tuberculosis vaccine progress.

**Immune response to Mtb**

Protective immune responses against *Mtb* are primarily mediated by the cellular immune system, involving innate
responses in which the major cellular components are neutrophils, macrophages, dendritic cells (DCs) and natural killer cells (NKs), and adaptive responses in which lymphocytes, mainly conventional CD4\(^+\) and CD8\(^+\) αβ T cells, are the major effector cells. These cells produce T-helper type 1 (Th-1) cytokines [interferon (IFN)-γ, interleukin (IL)-2, IL-12] important for the activation of antimycobacterial activities of macrophages (Sable et al., 2007). However, some unconventional T cells (CD4CD8 αβ T-cells, γδ T cells, NK 1.1) have also been implicated in protective immunity to tuberculosis through the recognition of nonprotein mycobacterial antigens including glycolipids (mycolic acids, phosphatidylinositol mannosides, lipoarabinomannan, etc.) and their presentation to a variety of CD1-restricted lymphocytes. These cells also activate antigen-presenting cells (APCs), boost the expression of major histocompatibility complexes (MHCs) and costimulatory molecules and amplify IL-12, IL-18 and IFN-γ production (Doherty & Andersen, 2005).

Recently, the importance of CD8\(^+\) cytotoxic T-lymphocyte (CTL) responses to the generation of an effective vaccine against tuberculosis has also been recognized. Accumulating evidence indicates that the MHC-I pathway is critical to achieve protection (Orme, 2006). Studies with endogenous proteins, such as heat shock protein 65 (HSP65), have shown the superiority of these antigens to stimulate CTLs, which are able to either kill infected macrophages unable to eliminate the bacilli or kill the mycobacteria in the extracellular space directly (Lima et al., 2004).

On the other hand, the role of Th-2 cytokines, such as IL-4, IL-5, IL-10 and IL-13, in protective immunity against Mtb remains unclear. It has been suggested that generation of a Th-2 response is associated with a greater risk of progression from Mtb infection to active disease by seriously undermining the efficacy of a Th-1 response to mycobacterial antigens (Doherty & Andersen, 2005). Some authors have also observed a relationship between the presence of concomitant parasite infections and exposure to environmental mycobacteria, with a systemic bias towards Th-2 responses that reduces the efficacy of BCG (Rook et al., 2001).

### Tuberculosis vaccines and adjuvants

In this context, effective tuberculosis vaccine design is based on generating the cellular responses required to kill the bacteria and prevent establishment of infection (against infection and pulmonary disease) or to avoid reactivation or progression toward clinical tuberculosis in the case of latent patients. In the first case, the general strategy involves a prophylactic vaccine able to induce protective immunity, measured in terms of lymphocyte subsets expanded after immunization. In the second case, the strategy focuses on utilizing a postexposure vaccine to eliminate or contain latent tuberculosis and prevent reactivation (Sadoff & Hone, 2005; Sable et al., 2007). Concerns regarding the use of postexposure vaccines and their adverse influences result from the fact that the infected lung has already undergone inflammation, tissue damage and remodeling responses (Orme, 2006). Thus, the effect of vaccination in sensitized and latently infected individuals is a minor concern for mycobacterial vaccines intended to replace BCG, but it is especially relevant for vaccines intended to be given as boosters to individuals already vaccinated with BCG. A vaccine that is safe in a naive recipient may have negative effects in one with pre-existing immunologic memory (Doherty, 2005). Table 1 shows several tuberculosis vaccine candidates that are currently in advanced stages of clinical trials. Of these, subunit tuberculosis vaccines have received special attention because, in spite of their poor immunogenicity, they exhibit a high degree of safety and their production can be standardized.

Currently, such tuberculosis subunit vaccines are prepared from recombinant proteins, purified from bacterial expression vectors or formulated as naked DNA, consisting of recombinant plasmids encoding Mtb antigens under the control of eukaryotic promoters (Doherty & Andersen, 2005; Hof, 2008; Carstens, 2009). They can stimulate T-cell responses against key subunit antigens and are safe even in immunosuppressed individuals. Their main drawback is the limited availability of adjuvants approved for human use to boost their immunogenicity (Hogarth et al., 2003; Mills, 2009). Box 1 provides a short description of adjuvants for human use that have been the result of many years of research and development, including oils and aluminium adjuvants, synthetic adjuvants, second-generation delivery-

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**Table 1. Tuberculosis vaccines in clinical trials**

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Definition and stage of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live vaccines (mycobacterial)</td>
<td></td>
</tr>
<tr>
<td>rBCG30</td>
<td>Entered phase I trials in US during 2004, over expresses Ag85B</td>
</tr>
<tr>
<td>rBCG::ureC-ldo+</td>
<td>Urease deficient mutant, which expresses the Lysteriolysin O gene from <em>Listeria monocytogenes</em></td>
</tr>
<tr>
<td>PanD-Leu — . Auxotroph attenuated strain of Mtb</td>
<td></td>
</tr>
<tr>
<td>Live vaccines (nonmycobacterial)</td>
<td></td>
</tr>
<tr>
<td>MVA-Ag85A</td>
<td>Recombinant, replication deficient vaccinia virus, expressing Ag85A. Entered in Phase I clinical trials in 2005</td>
</tr>
<tr>
<td>Live vaccines (subunit vaccines)</td>
<td></td>
</tr>
<tr>
<td>Nonliving vaccines</td>
<td></td>
</tr>
<tr>
<td>Hybrid 1</td>
<td>Fusion molecule comprised of ESAT-6 and Ag85B. Clinical trials 2004/2005. Proved with LTK63 and IC31 adjuvants</td>
</tr>
<tr>
<td>HyVac4</td>
<td>Fusion protein comprised of TB10.4 and Ag85B. Delivered in IC31 adjuvant. Entered in Phase I clinical trials in 2007</td>
</tr>
</tbody>
</table>

Reproduced from Gupta et al. (2007); Andersen (2007).
Box 1. Adjuvants for human use or in late stage of clinical trials

Adjuvants from the Latin ‘adjuvare’, or ‘to help’, are molecules or macromolecular complexes with carrier/depot or targeting functions and immunomodulant and/or immunomodulatory activities (Guy, 2007; Reed et al., 2009).

Mineral salts. The most widely used adjuvants in human vaccines include aluminium hydroxide, aluminium phosphate and calcium gels (HogenEsch, 2002; Kenney et al., 2002).

Saponins. Qui-A and its purified fraction QS-21 (Stimulon) are triterpene glycosides isolated from the aqueous extract of the bark of the South American tree Quillaja saponaria (Marciani et al., 2003; Sanders et al., 2005; Sun et al., 2009).

Emulsions. Mixture of two immiscible substances in two phases stabilized by one or several surfactants at the interface. Among the oil-in-water (O/W) emulsions are MF59 and Adjuvant Systems. MF59 contains Tween 80 and Span surfactants and the immunostimulant squalene. This emulsion is component of the Influenza vaccine FLUAD (Schultze et al., 2008), and also has been tested with other viruses with promising results (Ansaldi et al., 2008; Mosca et al., 2008). Adjuvant Systems developed by GSK Biologicals are formulations of classical adjuvants mixed with immunomodulators adapted to the antigen (Garçon et al., 2007). AS02A which contains monophosphoryl lipid A (MPL) and QS-21, has been tested with tuberculosis, cancer, malaria and some viruses (Skeiky et al., 2004; Vandepapelière et al., 2008). AS04 with MPL and alum, has successfully passed Phase III trials with vaccines against viral diseases, and is already on the market as a component of the Cervarix® vaccine (Schwarz, 2008). AS01B (a liposomal formulation) and AS03 (with α-tocopherol, squalene and Tween 80) have been developed to induce a stronger T cell responses, especially CTL responses (Garçon et al., 2007; Reed et al., 2009).

Particulate adjuvants. Lipid-based structures, such as liposomes, archeasomes, immune-stimulating complexes (ISCOMs), bio-degradable micro- or nanoparticles, virus-like particles (VLPs), and the AS04 adjuvant system, among others. They are used to deal with mucosal conditions as proteases, denaturation and dilution (Eriksson & Holmgren, 2002; Csaba et al., 2008; Mallapragada & Narasimhan, 2008).

Microbial natural and synthetic derivatives. MPL and some derivatives isolated from Salmonella minnesota R595 lipopolysaccharide (Meraldi et al., 2003; Lahiri et al., 2008); ADP-ribosylating enterotoxins such as, cholera toxin (CT), heat-labile enterotoxin (LT) (Kenney et al., 2002; Freytag & Clements, 2005), and some mutants like LTKE63, with reduced toxicity but significant adjuvanticity (Eriksson & Holmgren, 2002; Eriksson et al., 2004; Freytag & Clements, 2005). Among synthetic microbial adjuvants are the cytosine-phosphate-guanosine ODNs (CpG ODNs), synthetic ODNs containing immunomimulatory CpG motifs which stimulate APCs via TLR9 (Lahiri et al., 2008; Krishnamachari & Salem, 2009; Mutwiri et al., 2009; Vollmer & Krieg, 2009).

Cytokines. Delivered either in protein form or indirectly as DNA (Wang et al., 2002; Mallapragada & Narasimhan, 2008). Candidates include granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1, Th1-inducing cytokines such as IL-12, IL-18, GM-CSF, and some chemokines (Boyaka & McGhee, 2001).

depot systems and receptor-associated adjuvants (Ott & Van Nest, 2007).

Many of these adjuvants have been tested for their efficacy in tuberculosis vaccines, mostly in mouse models in combination with different antigens or fusion proteins. When used alone or in conjunction with BCG in a ‘prime-boost’ strategy or coadjuvanted with cytokines or other molecules, many of these vaccines have been shown to confer protective immunity (Lindblad et al., 1997).

Secreted proteins, HSP, lipoproteins and putative phosphate transport receptors (PstS) have all been evaluated for subcutaneous, oral or intranasal priming vaccination, followed by intradermal or oral BCG vaccination (Doherty et al., 2002; Hogarth et al., 2003; Hoft, 2008). Likewise, emulsions (Haile et al., 2004, 2005), microspheres (Ajardy et al., 2007), toxin derivatives (Takahashi et al., 2006; Badell et al., 2009), cationic lipids (D’Souza et al., 2002) and oligodeoxynucleotides (Kamath et al., 2008) have demonstrated efficacy in inducing strong T-cell responses with high titres of IFN-γ and specific antibodies. Table 2 summarizes several studies evaluating the efficacy of different antigen/adjuvant combinations for tuberculosis vaccination.

Some of the mechanisms by which many of these adjuvants function are (1) generation of long-lasting antigen depots; (2) protection against antigen degradation and elimination; (3) distribution to specific cells; (4) antigen/adjuvant uptake; (5) enhancement of antigen presentation by DCs and (6) induction of CD8+ CTL and/or CD4+ Th-lymphocyte responses (Th-1 or Th-2) (The European Medicines Agency, 2005; Perrie et al., 2008; Reed et al., 2009).

As in any adjuvant design, it is important to consider a number of other factors, such as reduction in antigen titres, the number of immunizations required and efficacy in newborns, the elderly and immunocompromised individuals. Additionally, many potential vaccines consider antigen delivery to mucosal surfaces, an interesting approach to vaccines against pathogens that enter the human body via mucosal surfaces, such as Mtb. The risk of adverse side-effects, molecular stability and industrial constraints and costs must also be considered (Orme, 2006; Aguilar & Rodríguez, 2007).

Trends in adjuvants development for tuberculosis vaccines

Mucosal adjuvants

Most pathogens enter the human body via mucosal surfaces in contact with the surrounding environment, such as those in the nose, lungs and gastrointestinal tract. Mtb is usually transmitted via aerosols and establishes itself in the lungs. Thus, mucosal vaccination at this site can help to prevent pathogen entry and infection (Doherty et al.,...
Mucosal adjuvants for human use have been designed based on bacterial toxins (CT, LT) and their derivatives (CTA1-DD, LT-K63), synthetic CpG-containing DNA, ISCOMs and various cytokines and chemokines with the aim of inducing effective mucosal Th-1 and Th-2 responses (Eriksson et al., 2004; Helgeby et al., 2004; Freytag & Clements, 2005). Examples of these delivery systems include antigen-encapsulating microspheres, various liposome formulations, nanoparticles with surface-adsorbed agents, lipophilic ISCOMs and bacterial products with known adjuvant properties. Such systems enhance the binding, uptake and half-life of antigens and may help to target the vaccine to mucosal surfaces. In addition, based on their mucoadhesive properties, these viscosity-enhancing delivery systems have been designed to slow mucociliary clearance and prolong contact time between the vaccine compound and the nasal tissue (Sajadi-Tabassi et al., 2008; Coucke et al., 2009). The last concept is particularly important, because nonreplicating, and

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### Table 2. Adjuvant formulations used in tuberculosis subunit vaccines

<table>
<thead>
<tr>
<th>Type of adjuvant</th>
<th>Formulation and route of administration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cationic lipids</td>
<td>Ag85A, Ag85B, PstS-3 in VC1052:DpyPE (intramuscular) or in GAP-DLRIE:DOPE (intranasal)</td>
<td>D’Souza et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>BCG lipid encapsulated (L-BCG) (oral)</td>
<td>Aldwell et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>ESAT-6 plus LANAC adjuvant (with TLR3 or TLR9 agonists) (intravenous; subcutaneous; intramuscular)</td>
<td>Zaks et al. (2006)</td>
</tr>
<tr>
<td>Micro or nanoparticles</td>
<td>DNA plasmid encoding eight HLA-A*0201-restricted T-cell epitopes from Mtb formulated in cationic nanoparticles (intramuscular and pulmonary)</td>
<td>Bivas-Benita et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>ESAT-6 encapsulated in PLA microspheres (intranasal).</td>
<td>Carpenter et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>BCG plus sodium alginate microspheres (subcutaneous)</td>
<td>Dobakhhti et al. (2009)</td>
</tr>
<tr>
<td>Toxin derivatives</td>
<td>BCG and PstS-1 antigen plus CT (intranasal)</td>
<td>Falero-Díaz et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Ag85B-ESAT-6 (H1) plus LTK63 (intranasal)</td>
<td>Dietrich et al. (2006)</td>
</tr>
<tr>
<td>CpG DNA</td>
<td>Ag MPT-51 plus CpG DNA (subcutaneous)</td>
<td>de Souza Silva et al. (2009)</td>
</tr>
<tr>
<td>Adjuvant Systems</td>
<td>Mtb72F in AS02A (intramuscular)</td>
<td>Brandt et al. (2004)</td>
</tr>
<tr>
<td>Cytokines</td>
<td>BCG plus AdGM-CSF (subcutaneous)</td>
<td>Wang et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>HSP65 plus IL-12 delivered by HVJ-liposome (intramuscular)</td>
<td>Okada et al. (2009)</td>
</tr>
<tr>
<td>ISCOMs</td>
<td>Ag85B-ESAT-6 (H1) plus CTA1-DD/ISCOMs (intranasal)</td>
<td>Andersen et al. (2007)</td>
</tr>
</tbody>
</table>

VC1052:DpyPE, aminopropyl-dimethyl-myristoyloxy-propanaminium bromide-diphytanoylphosphatidyl-ethanolamine; Vaxfectin; LANAC, liposome-Ag-nucleic acid complexes; GAP-DLRIE:DOPE, aminopropyl-dimethylbis-dodecyloxy-propanaminium bromide-dioleoyl phosphatidyl-ethanolamine; PLA, poly-lactide acid; AdGM-CSF, adenooviral granule cytoplasmic-colony stimulating factor; CTA1-DD/ISCOMs, cholera toxin-derived fusion protein/immune-stimulating complexes; HVJ, haemagglutinating virus of Japan.
especially nonparticulate, antigens applied to a mucosal surface must be adjuvanted to induce productive immunity rather than tolerance. Thus, a vaccine with an appropriate adjuvant can induce both mucosal and systemic immune responses, preventing not only infectious disease but also colonization of mucosal surfaces (Davis, 2001).

**Mycobacterial Toll-like receptor (TLR) ligands**

At present, increasing knowledge of the innate immune system, including the identification of ligands and signalling pathways, is providing a new set of targets for the development of novel adjuvants (Schijns & Degen, 2007; Boog, 2008). Pathways specifically involved in the immune response against complex pathogens such as *Mtb* are mediated by receptors expressed on the surface of DCs and macrophages. Engagement of these receptors initiates intracellular signalling pathways, resulting in the activation of immune response genes, including those encoding MHC molecules, costimulatory molecules and inflammatory cytokines. One key receptor class is the TLR family, whose ligands are either presented on the surface of *Mtb* or secreted by the bacterium (Doherty & Andersen, 2005). Mycobacterial TLR ligands include triacylated and diacylated forms of p19, a lipoprotein recognized by TLR 2/1 and TLR 2/6 dimers, respectively. Meanwhile, lipoarabinomannan and phosphatidyl-myco-inositol mannoside are recognized by TLR2, while CpG-containing DNA is recognized by TLR9, which is exclusively present within endosomes and phagosomes. TLR4, the classical receptor for lipopolysaccharide on Gram-negative bacteria, has also been implicated as a sensor for an unidentified, heat-sensitive mycobacterial ligand (Quesniaux et al., 2004; Lahiri et al., 2008). Other important innate immune receptors are the cytosolic nucleotide-binding and oligomerization domain-like receptors or NOD-like receptors (NLRs), which are TLR-related proteins responsible for recognition of intracellular pathogens, including mycobacteria. NOD1 and NOD2 specifically bind diaminopimelic acid and the peptidoglycan breakdown product muramyl dipeptide, triggering the production of proinflammatory cytokines. This suggests a synergistic effect between NLRs and TLR2 in tuberculosis (Korbel et al., 2008).

As mentioned earlier, both TLR and NLR ligands promote inflammation by triggering the release of chemokines and proinflammatory cytokines, expression of adhesion molecules and recruitment of macrophages, DCs and polymorphonuclear neutrophils (Korbel et al., 2008). After antigen processing and expression of epitopes in an MHC-restricted manner, mature DCs can stimulate naive T cells to differentiate into effector cells. Depending on the ligand, the immune response may thus be skewed toward CTL responses or toward a particular Th response (Boog, 2008). Based on increasing evidence for the contribution of CD1-restricted immune responses to protection against tuberculosis, CD1-restricted, nonproteinaceous ligands, such as glycolipids, are also being considered as potential candidates for new tuberculosis vaccines (Hamasur et al., 2003).

In conclusion, mycobacterial ligands have great potential as adjuvants due to their ability to activate the innate immune response, ultimately leading to cellular and humoral responses against coadministered antigens (Mills, 2009). In this context, synthetic ligands capable of targeting TLRs more precisely and safely than pathogen-derived ligands are being designed (Guy, 2007). However, a great deal of work is still required, because the success of vaccination is related to the route of administration, the delivery method used and the APC population stimulated by the adjuvant. On the other hand, TLR overstimulation can also generate unwanted toxic effects, and so adjuvant dose and mechanism of action must be carefully considered and potential toxicities should be investigated and characterized (Boog, 2008).

**Promising preliminary formulations**

Despite the limited number of adjuvant systems approved for clinical applications, several vaccine delivery and adjuvant combinations have been evaluated, resulting in promising preliminary formulations. Currently, four leading adjuvants for tuberculosis subunit vaccines are being investigated:

- **CAF01 (LipoVac)**, developed by the Statens Serum Institute, is a novel tuberculosis vaccine adjuvant utilizing 1.3,1.3'-dimethyl-1.3'-dioctadecylammonium (DDA) liposomes with the synthetic mycobacterial immunomodulator α, α'-trehalose 6,6'-dibehenate (TDB) inserted into the lipid bilayer. This adjuvant is formulated using a fusion molecule composed of two immunodominant, secreted proteins from *Mtb*, Ag85B and ESAT-6, a fusion known as Hybrid-1 (H1). In this system, DDA targets the vaccine antigen to APCs while TDB provides proinflammatory stimuli, triggering a Th-1 cytokine response via a TLR-independent pathway (Agger et al., 2008). CAF01 has proven to be highly efficacious, inducing cellular and humoral responses simultaneously in animal models more effectively than the single antigens administered alone. In addition to its priming activity, this vaccine has also been demonstrated to have a BCG booster effect (Doherty et al., 2004; Davidsen et al., 2005).

- **AS01B**, developed by Corixa and GlaxoSmithKline Biologicals, contains the TLR4 ligand MPL and the saponin derivative QS-21 in a liposomal formulation including the fusion molecule Mtb72F. The Mtb72F antigen is comprised of the PPE family member Rv1196 inserted into the middle
of the putative serine protease Rv0125, which is thus present as two fragments (Mtb32C–Mt39–Mtb32N) (Skeiky et al., 2004). In the AS01B or AS02A formulations, this vaccine has also been demonstrated to have priming and BCG booster effects (Brandt et al., 2004).

IC31, also developed by the Statens Serum Institute, consists of a vehicle combining the synthetic antimicrobial peptide KLKLxKLK, which actively loads APCs with antigen, and the immunostimulatory TLR9 ligand ODN1a, with the fusion proteins H1 and Ag85B–TB10.4 (Agger et al., 2006; Lingnau et al., 2007). This vaccine confers protective immunity in murine tuberculosis models and was recently shown to safely induce strong T-cell responses with a mixed Th-1/Th-2 cytokine profile in both neonates and adults (Kamath et al., 2008).

CAF01, AS01B and IC31 are currently undergoing clinical Phase I/II trials. Mtb72F/AS01B is being tested in Lausanne, Switzerland, in individuals previously exposed to BCG or previously treated individuals currently infected with Mtb. H1 in IC31 and CAF01 are being tested in Leiden, the Netherlands, in purified protein derivative (PPD)-negative subjects. These adjuvants share the same basic combination of a delivery vehicle and a Th-1-skewing immunomodulator, conferring more potent protection against tuberculosis infection than single immunomodulators (CpG or MPL) or delivery vehicles lacking immunomodulators (liposomes or niosomes) (Agger et al., 2006).

LTK63, a modified and detoxified heat-labile toxin derived from E. coli, has been combined with the fusion protein H1 for nasal immunization and has passed Phase I clinical trials (in London, UK, with PPD-negative subjects). A strong and sustained Th-1 response mediated by IFN-γ-secreting CD4+ T cells was observed, leading to long-lasting protection against tuberculosis and boosting prior BCG-induced immunity (Dietrich et al., 2006; Badell et al., 2009). Recently, LTK63 has also been shown to induce a marked increase in Ag85B-specific antibody titres, especially the IgG2b isotype (Palma et al., 2008).

Modified Vaccinia Ankara (MVA) adenovirus, a recombinant-vector vaccine expressing the secreted mycobacterial antigens Ag85A and 85B, has been studied as a subunit vaccine, either as a prime vaccine or as a BCG-boosted vaccine (Williams et al., 2005; Santosuosso et al., 2006). Although this system has a potent adjuvant effect and can deliver vaccine antigens through mucosal tissues to induce strong T-cell stimulation, its drawbacks include increased reactogenicity and pre-existing immunity induced by exposure to natural antigens that are cross-reactive with vector components (McShane et al., 2005; Hoft, 2008). Phase I/II clinical trials have been completed for MVA-Ag85A in Oxford, UK, and Gambia to assess vaccine safety, immunogenicity and dosage in individuals previously exposed to mycobacterial antigens.

**Final considerations**

Tuberculosis vaccine development has been progressing empirically for many years. Currently, increased understanding of the immune system and the development of advanced delivery and adjuvant systems are enabling the design of improved prophylactic vaccines. As a result, in the last 10 years, the international research community has developed more than 200 tuberculosis vaccine candidates currently being tested in mouse, guinea-pig and human primate models. These approaches are aimed at achieving a more potent and prolonged immunological memory, a goal of great global importance, given the rise of MDR-tuberculosis worldwide and the poor efficacy of the BCG vaccine against adult pulmonary tuberculosis.

Despite a lack of relevant animal models that correlate with protection in humans and the lack of markers capable of demonstrating the efficacy of an antigen/adjuvant combination (needed for a faster acceptance of new adjuvants), promising vaccines from the Fifth Framework Program FP5 (Mtb72F/AS01B, H1 in IC31 and CAF01; MVA-Ag85A) have been developed and tested in preclinical and clinical trials, and the optimized formulations and adjuvant combinations have been produced using good manufacturing practices. Further improvement of these adjuvants through Box 2. Adjuvant/vaccine development, some guidance

- Preclinical and appropriate toxicology studies need to be designed to evaluate the risk assessment and safety profile of the adjuvant and adjuvant/vaccine combination in question. In addition to a deeper understanding of the basic biology of the immune response, knowledge of the genetic heterogeneity of the population is required to give rise to the concept of genetically determined individual responsiveness and susceptibility (Sesardic, 2006).
- Because the adjuvant is not the active ingredient in a vaccine and the action of the vaccine/adjuvant combination is the result of multiple factors, immune responses obtained with one antigen/adjuvant cannot be extrapolated to other antigens or even to the same combination given by different routes (Sesardic & Dobbelaar, 2004).
- A highly efficient and cost-effective method for comparison of adjuvants with a new antigen is to conduct multiplex small-scale, Phase I comparative studies in humans with a new antigen, using adjuvants previously found to be safe when used with other antigens in human trials (Alving, 2002).
- Preclinical safety studies are necessary to identify possible causes of toxicity before undertaking clinical studies. Such studies might be designed to demonstrate the safety and efficacy of the vaccine components and the absence of immunotoxicity (Brennan & Dougan, 2005).
- The Aeras Global TB Vaccine Foundation is the most visible nonprofit organization currently working to take new tuberculosis vaccines from the preclinical stage through Phase III testing in humans. The newly created Vaccine Expert Groups (VEG) are in charge of compiling and updating currently available recommendations concerning adjuvants.
combination with other delivery systems or recently identified mycobacterial immunomodulators is underway in the context of FP7 (from 2007 to 2013).

It is clear that more research is required on adjuvants’ effects on antigen presentation, APC activation, long-lived memory T-cell induction and Th-1/Th-2 cell polarization to avoid undesirable effects. Efforts directed toward the development of postexposure vaccines against latent tuberculosis are also needed. Thus, the development of new adjuvants and delivery methods is as important as the search for antigens that allow discrimination between latent and active disease. Also, special attention to several candidate nonprotein antigens (sulphaltoglobin lipids, phosphoantigens, etc.) is required, due to their potential usefulness in subunit vaccines and/or adjuvants capable of stimulating CD1-restricted γ-δ or NKT cells. Only a multidisciplinary approach will allow an effective balance between risks and benefits in the selection of vaccine antigens, adjuvants and delivery systems, leading to new and improved vaccines against Mtb, one of the most devastating human pathogens. Box 2 summarizes some relevant recommendations to improve adjuvant development.

References


Adjuvants in tuberculosis vaccine development


