Adjuvants for New Anti-Tuberculosis Vaccines

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Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis (Mtb) that sits in the top 10 leading causes of death in the world today and is the current leading cause of death among infectious diseases. Although there is a licensed vaccine against TB, the Mycobacterium bovis bacilli Calmette–Guérin (BCG) vaccine, it has several limitations, namely its high variability of efficacy in the population and low protection against pulmonary tuberculosis. New vaccines for TB are needed. The World Health Organization (WHO) considers the development and implementation of new TB vaccines to be a priority. Subunit vaccines are promising candidates since they can overcome safety concerns and optimize antigen targeting. Nevertheless, these vaccines need adjuvants in their formulation in order to increase immunogenicity, decrease the needed antigen dose, ensure a targeted delivery and optimize the antigens delivery and interaction with the immune cells.

Keywords: vaccines ; adjuvants ; infectious diseases

1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb), that has been included in the top 10 leading causes of death in the world today and is the current leading cause of death among infectious diseases [1][2]. This devastating title came at the expense of 1.4 million lives in 2019 alone [3]. According to the World Health Organization (WHO), a quarter of the world population is infected with Mtb [3] and most of these cases are related to poverty and located in low and middle-income countries [2][3].

Although there is a licensed vaccine against TB, the *Mycobacterium bovis* bacilli Calmette–Guérin (BCG) vaccine, it has several limitations, namely its high variability of efficacy in the population and low protection against pulmonary tuberculosis [4]. This explains the number of Mtb infections worldwide, despite the existence of a vaccine and its availability.

Treatment for TB normally consists of a long antibiotic regimen that decreases the patients' compliance [5]. Nonetheless, the efficacy of this treatment is now compromised by the emergence of multidrug-resistant *Mycobacterium tuberculosis* bacteria against which there is only 50% chance of cure with the available drug treatment [6]. The WHO has set the goal to reduce TB morbidity by 90% and mortality by 95% by 2035 [6].

While drug research and diagnostics need to evolve in order to achieve control over Mtb infections, the discovery of a new and effective vaccine takes a central stage for its cost-effectiveness and ability to prevent or help with the treatment of TB, even of multidrug resistant strains [4][6][7]. By using a vaccine, it is possible to decrease both the transmission of the pathogen and the use of antibiotics to treat it, which means that the vaccine controls the disease and overcomes the antimicrobial resistance (AMR) [7].

Currently, in the TB vaccine pipeline, there are different types of vaccines, including live attenuated, inactive and subunit vaccine candidates [4][8]. Subunit vaccines are not the most advanced at the moment, since the current phase III clinical trials only comprise whole-cell vaccines, however they are promising candidates [9]. This type of vaccines overcomes some of the safety concerns associated with live attenuated or inactive vaccines [10]. Furthermore, as they are designed using specific antigens, they can be targeting different aspects of the infection, optimized as the research progresses and provide additional immune responses for more complex pathogens, such as Mtb [8][11].

While being very safe, subunit vaccines cannot rely solely on the antigen to achieve a desired effect as they are generally poorly immunogenic [10]. Adjuvants are needed in their formulation in order to increase immunogenicity, decrease the antigen dose, ensure a targeted delivery and optimize the antigens interaction with the immune cells [10][11]. Undeniably, adjuvants are an important part of new subunit vaccines design and in general, adjuvants identification, design and

characterization is essential to modern vaccines formulation [12]. Additionally, understanding how adjuvants work plays a determinant role in vaccine development and can be the key factor to make or break the vaccine's chances to succeed [12].

2. The BCG Problem and the Design of New TB Vaccines

BCG is a live attenuated vaccine produced using *Mycobacterium bovis* [13] that has been in use for over 100 years [9][14]. It is an inexpensive, widely available vaccine that is administrated to more than 90% of children in endemic countries [14][15]. The outcomes of this vaccine are very positive when it comes to prevention of childhood meningeal or miliary tuberculosis and overall decrease in child mortality, but the same vaccine fails to protect against adult pulmonary tuberculosis in a homogenous manner in the world population, with highly variable protection ranging from 0% to 80% [7][10][15].

While BCG vaccine has many downsides when pulmonary adult tuberculosis is being analysed, it is still an excellent vaccine that prevents child tuberculosis with low costs and. Therefore, BCG boosting lines of research should also be considered as a good starting point for TB vaccine development [16].

WHO considers the development and implementation of new TB vaccines to be a priority [16]. Accordingly, in 2017, the organization proposed a guideline for the development of these vaccines called "WHO preferred product characteristics" and presented to experts from different branches of the industry, such as scientists, funding agencies and regulators [16].

Given the complexity of the disease and to target efficiently the different states of infection, latent or active, TB vaccination should provide several layers of protection, namely by preventing initial infection, reactivation of infection or progression into active disease [14][16]. Moreover, the vaccine should also be suited and safe for administration in immunocompromised patients like HIV positive individuals [14]. While the WHO also describes the need for research on a new newborn/infants TB vaccine [15], this review mainly focuses on the efforts to develop a successful vaccine for pulmonary tuberculosis in adults and adolescents.

A summary of the vaccine's preferred characteristics, for a target population of adolescents and adults, is presented in the Table 1.

Table 1. Summary of a new vaccine's WHO preferred product characteristics [15].

Target Population	**Adolescents and adults**
Outcome Measure and Efficacy	**50% or greater efficacy in preventing confirmed pulmonary TB**
Duration of protection	**Ten years or more**
Safety	**Favourable safety profile, even for high-risk groups as HIV patients**
Schedule	**Less than three doses to achieve primary immunization and booster preferentially after 10 years or more**
Co-administration	**Safe and without interactions with other vaccines administrated to the same population**
Immunogenicity	**Characterization of immune markers and concomitant development of correlate of protection of a TB vaccine**
Programmatic Suitability and Prequalification	**Should meet requirements of WHO suitability of vaccines—vaccine presentation, packaging, thermostability, formulation and disposal**
Value Proposition	**Favourable cost-effectiveness and affordable price**

3. Understanding the Adjuvant's Immune Role by Understanding TB Immunity

After inhalation of infected aerosols, the phagocytosis of the Mtb pathogen by the alveolar macrophages takes place. The block of the lysosome-phagosome fusion leads to the survival of the bacterium within the macrophages with activation of a cell-mediated immune response, such as CD4+ and CD8+ T lymphocytes [17]. From there, the immune response leads to a very important and characteristic cell-mediated consequence which is the formation of a granuloma [17]. This structure composed by macrophages, lymphocytes, stem cells and epithelial cells has the ability to control bacterial replication and induce a latent stage of the disease [17]. Understanding this immune response and the mediators involved is essential for

the development of a vaccine and for choosing an appropriate adjuvant. The main problem with a TB vaccine is that the host immune responses towards Mtb are not yet fully understood and, as said before, there is no defined correlate of protection [18].

Most research lines in the field of TB vaccination focus on T-helper 1 (Th1) type of response, namely polyfunctional CD4+ cells expressing simultaneously IFN-γ, TNF-α, and IL-2 cytokines [13][19][20]. While it seems that investigators are moving towards the discovery and establishment of a correlate of protection, the findings are not clear. Looking at the pathogen's behaviour to understand if these cells are indicative of protection, it is possible to see some ambiguous results since CD4+ cells are related to protection against disease and with immune responses to successful treatments but a high expression of these cells are also related to an increase in the bacterial load during active infection [19]. Moreover, most of the studies already concentrate in finding these cells and thus it is possible that there is some other immune correlate that is not being investigated [19].

Nevertheless, studies on mice and on patients have shown a clear importance of polyfunctional Th1 cells in TB protection and, thus, Th1 is still a desired response in new vaccine candidates [12][19]. In fact, as will be shown in this review, most of these new formulations show protective results and a clear Th1 response.

Th17 cells have also been described as important for immunity against Mtb and they are an immune marker used in some studies [12][17]. It has been previously described, in appropriate mice models, that IL-17 does not play an important role on the early control of the bacteria in the lung, upon infection with Mtb. However, Th17 cells are important for neutrophil recruitment and they are induced by the Mtb infection [20]. The same authors describe that IL-17-producing T-cells may be important for vaccine-mediated protection due to their probable ability to populate the lung and other tissues and, upon infection, start a signal that leads to bacterial load control [20].

In the selection of adjuvants, it is important to categorize the type of response that they are capable to promote, especially when combined with antigens specific for Mtb [18]. It is clear that CD4 T cells are crucial for protection as well as the production of IFN-γ and TNF-α although not enough [18][21]. While other immune mediators are not yet established as essential, their role in Mtb immunity is worth exploring and adjuvant's associated response in new vaccine candidates is an excellent resource for new information regarding these immune responses.

4. Adjuvants in New TB Vaccine Candidates

This section aims to give insights on adjuvants that are being used in innovative TB vaccine candidates. The available data on these new formulations are reported and discussed. The immune response that they trigger will be explored by analysing the available data, which can potentially contribute to the discussion on vaccine formulation. A brief overview of the mechanism of action of the adjuvants described in this review is presented in Figure 1.

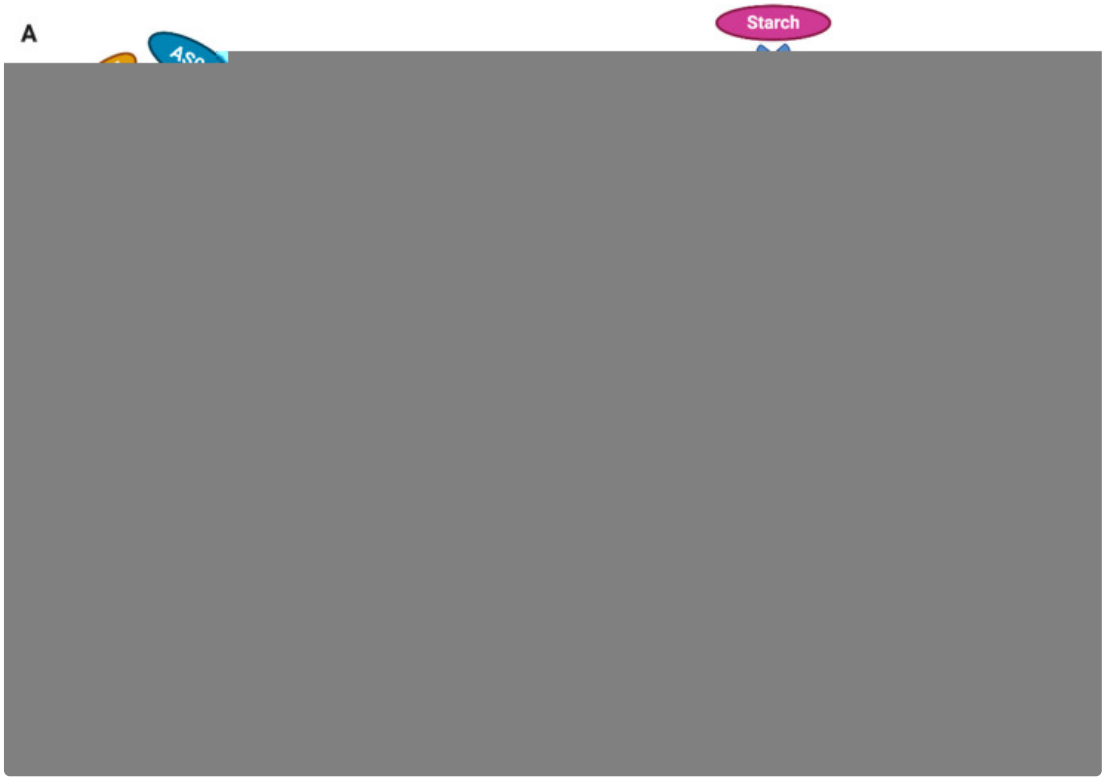

Figure 1. Overview of Proposed Mechanisms of Action of Adjuvants in vaccines. (A) Receptors and Molecule Activation of adjuvants. The active principle of GLA-SE and AS0, the MPLA, activates TLR4 and downstream pathways, namely MyD88 and, after internalization of the CD14/TLR4/MD-2 complex, the "late" TRAM-TRIF pathway. Starch interacts with C-type DC-SIGN receptor. IC31, more specifically, CpG motifs, as well as pDNA present in TMC microparticles activate TLR9 inside the endosome. AS01 and Chitosan activate the inflammasome and promote the subsequent release of pro-inflammatory cytokines. CDNs activate STING receptors in the endoplasmic reticulum. (B) IC31′s KLK peptide increases the permeability of the adjuvant system in the membrane to deliver the antigen and CpG motifs. (C) Cationic Liposome adjuvants, such as CAF01, promote a depot effect on the injection site and release the antigen in a controlled way, stimulating innate immunity.

Table 2. Summary of Adjuvants in TB vaccines Currently in Clinical Stage of Development.

Adjuvant System	Components	Proposed Mechanism of Action	Type of Immune Response	Vaccine Candidate	Immunization Strategy	Adm. Route
IC31	KLK, ODN1a	TLR9 activation (ODN1a) Enhanced delivery of ODN1a to the endosome, enhanced antigen presentation (KLK)	Th1–Polyfunctional T-cells producing IFN-γ, IL-2 and TNF-α	H4:IC31 H56:IC31	Prophylactic Prophylactic, Post-Exposure	I.M. I.M.
GLA-SE	GLA in a Squalene oil-in-water emulsion	TLR4 activation	Th1–Polyfunctional T-cells producing IFN-γ, IL-2 and TNF-α Antigen-specific IgG1 and IgG3 production	ID93: GLA-SE	Prophylactic, BCG booster, Therapeutic	I.M.
AS01	MPL, QS-21	TLR4 activation (MPL) Induction of NLRP3 inflammasome (QS-21)	Th1–Polyfunctional T-cells producing IFN-γ, IL-2 and TNF-α	M72:AS01$_E$	Post-exposure, BCG booster	I.M.
CAF01	DDA, TDB	MINCLE activation Depot Effect Controlled release of the antigen	Th17–T-cells expressing IL-17 Th1 IgA response	H56:CAF01	Prophylactic Homologous Boosting	S.C. I.N.

ODN1a–oligodeoxynucleotide (ODN) 1a; TLR–Toll-Like Receptor; MPL–3-O-desacyl-4′-monophosphoryl lipid A; BCG–Mycobacterium bovis bacilli Calmette-Guérin; CAF01–Cationic Adjuvant Formulation 01; DDA–N,N-dimethyl-N,N-dioctadecylammonium; TDB–α,α-trehalose 6,6′-dibehenate; I.M.–Intramuscular; S.C.–Subcutaneous; I.N.-Intranasal.

4.2. Adjuvants in TB Vaccines Currently in Preclinical Studies

Table 3. Summary of Adjuvants in TB vaccines Currently in Preclinical Stage of Development.

Adjuvant System	Components	Proposed Mechanism of Action	Type of Immune Response	Adm. Route
Starch Microparticles		C-type lectin DC specific ICAM-3-grabbing nonintegrin receptor activation Increase in phagocytosis and macrophages activation TLR6 signaling	Th1	I.N.
Chitosan		Inflammasome activation Mucoadhesive, ability to penetrate between cells, controlled release of the antigen, improved cell uptake	Th1 –IFN-γ production, IgG2c Th2 Th17	I.M.
TMC nanoparticles	TMC	DC maturation Increase in antigen's intranasal residence Increase in the antigen's uptake	Th1 Th2 Antigen-specific antibody production	I.N. (TMC-ESAT-6)
TMC Plasmid DNA, Muramyl peptide		DC maturation TLR9 activation (Plasmid DNA) NOD-like receptor 2 activation (muramyl peptide)	Th1 –IFN-γ production, IgG2c	I.M.

Adjuvant System	Components	Proposed Mechanism of Action	Type of Immune Response	Adm. Route
Chitosan-Inulin	**Chitosan Inulin**	**Increase antigen's exposure to immune cells. Decrease in renal clearance and in proteolytic digestion**	**Th1- Polyfunctional T-cells producing IFN-γ, IL-2 and TNF-α Th2–T-cells producing IL4 Antigen-specific antibodies–IgG1 and IgG2b**	**S.C.**
CDN-AddaVax®	**CDNs Addavax® (oil-in-water emulsion)**	**STING activation. (CDNs) Enhanced T-cell and B-cell activation (AddaVax®)**	**Th17 Th1 Th2**	**S.C. I.N.**
Advax®-CpG	**Delta-inulin micropaticles (Advax®) CpG**	**Enhanced phagocytosis and cell recruitment. (AddaVax®) Enhanced T and B cell activation. (AddaVax®) TLR9 activation (CpG)**	**Th1–Polyfunctional T-cells producing IFN-γ, IL-2 and TNF-α**	**I.M. (CysVac2)**
Advax®-CpG-murabutide	**Delta-inulin micropaticles (Advax®) CpG Muramyl dipetide (murabutide)**	**Enhanced phagocytosis and cell recruitment. (AddaVax®) T and B cell activation. (AddaVax®) TLR9 activation (CpG) NOD-like receptor 2 activation (muramyl peptide)**	**Th1–IgG2a and IgG1 production with a IgG2a bias.**	**I.M.**

TLR–Toll-Like Receptor; TMC–Trimethyl Chitosan; DC–Dendritic Cell; CDNs–Cyclic Dinucleotides; STING–Stimulator of interferon genes; I.M.–Intramuscular; S.C.–Subcutaneous; I.N.–Intranasal.

5. Future Perspectives and Conclusion

New TB vaccines are needed, and it is clear that the adjuvants in the formulations can play a determinant role in the success or failure of the vaccine as they help to modulate the immune response and optimize the antigen's presentation through different administration routes.

Due to the natural infection route, mucosal administration has been actively pursued by many researchers since it has the potential to provide physiological and immunological advantages against a Mtb infection, although there are safety concerns associated with adjuvants and this administration that must be taken into account [12][22]. Moreover, the exploration of Th17 responses is quoted by some authors as a new perspective for TB vaccines, since some vaccine candidates selectively inducing just Th1 response failed during the years [23].

As illustrated by some of the described examples, synergistic effect between two or more adjuvants might be further explored in the future, which in turn can stimulate different innate immune pathways (TLRs, NOD receptors, CTL receptors). The formulation of adjuvant systems that take advantage of two or more adjuvants in the vaccine candidate has already been introduced in the TB vaccine pipeline, that is the case of AS01 and IC31, but others have been developed. For example, the novel adjuvant MTOM consists in MPL, trehalose-6.6′-dibehanate, MF59, and heat-killed *Mycobacterium vaccae* and has shown the ability to enhance Th1-type response [24].

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