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Ag85B–ESAT-6 adjuvanted with IC31® promotes strong and long-lived *Mycobacterium tuberculosis* specific T cell responses in naïve human volunteers

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ABSTRACT

Though widely used, the BCG vaccine has had little apparent effect on rates of adult pulmonary tuberculosis. Moreover, the risk of disseminated BCG disease in immunocompromised individuals means that improved TB vaccines ideally need to be able to efficiently prime mycobacterially-naïve individuals as well as boost individuals previously vaccinated with BCG. Protective immunity against *Mycobacterium tuberculosis* is thought to depend on the generation of a Th1-type cellular immune response characterized by interferon-gamma (IFN-γ) production. In the present study, we monitored safety and IFN-γ responses in healthy TB-naïve humans receiving an entirely novel vaccine, composed of the fusion protein Ag85B–ESAT-6, administered at 0 and 2 months either as recombinant protein alone or combined with two concentrations of the novel adjuvant IC31®. Vaccination did not cause local or systemic adverse effects besides transient soreness at the injection site, but it elicited strong antigen-specific T cell responses against H1 and both the Ag85B and the ESAT-6 components. These strong responses persisted through 2.5 years of follow-up, indicating the induction of a substantial memory response in the vaccine recipients.

1. Introduction

Mycobacterium tuberculosis is one of the world's most devastating pathogens. It is estimated that one third of the world's population is infected with *M. tuberculosis*, leading to an estimated 8–10 million new cases of tuberculosis (TB) disease and 2–3 million deaths annually [1]. At present, the only vaccine against TB is Bacillus Calmette Guérin (BCG), a live attenuated vaccine derived from *Mycobacterium bovis* that has been used since the 1920s. BCG has been shown to protect against TB in early childhood [2,3]. However, the protective efficacy in preventing adult contagious pulmonary TB varies considerably. Though controversial, it has been generally estimated that the effect of BCG vaccination is no longer significant after 10 years [4] and, BCG vaccination has therefore not had the needed impact on the global TB epidemic. BCG is very safe in immunocompetent individuals but as a live replicating vaccine, it represents a risk of disseminated BCG disease in immunocompromised individuals. This was recently demonstrated in human immune deficiency virus (HIV)-infected children in a high endemic setting in South Africa where both of these infections rampage uncontrolled [5,6]. WHO therefore stopped recommending live, attenuated BCG vaccination at birth for HIV-exposed infants, even where there is a risk of TB exposure early in life [7]. This change of what has been routine childhood health practice for the past 40 years is a very serious development in regions where BCG coverage is of key importance to prevent TB in children and emphasizes the urgent need to develop not only more efficacious, but also safer TB vaccination regimes. Therefore, improved TB vaccines that are safe and effective both in naïve individuals, BCG vaccinated as well as latently infected individuals—are urgently needed [8,9].

Various new TB vaccines are currently being developed. Recombinant BCG, and attenuated *M. tuberculosis* are mostly seen as

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replacements for BCG due to these vaccines' ability to prime a powerful response in naïve individuals. Subunit vaccines, based on recombinant DNA, recombinant protein or viral vectors have in contrast, mostly been seen as BCG boosting vaccines [10,11]. As highlighted by the recent change in the BCG vaccination policy in HIV-infected infants, the ideal vaccine should be safe in immunocompromised hosts but also able to prime a strong, long-lived response in BCG-naïve individuals. At Statens Serum Institute (Copenhagen, Denmark) hundreds of antigens from *M. tuberculosis* have been cloned, expressed and screened for both human T cell recognition (*in vitro*) and for protective efficacy in preclinical animal models of TB. Among those studied in more detail, the 6 kDa early secretory antigenic target (ESAT-6) and antigen 85B (Ag85B) appeared particularly promising [12,13]. The Ag85 complex is highly conserved among different species of mycobacteria and consists of multiple secreted components: of these, Ag85A and Ag85B, two highly homologous proteins, are the most immunogenic. Unlike Ag85 complex members, ESAT-6 is a virulence factor, which is largely restricted to the organisms of the TB complex. Of note, both ESAT-6 and Ag85B contain numerous epitopes that are promiscuously recognized by T cells of TB patients with a range of HLA types and these responses are characterized by induction of high numbers of antigen-specific IFN-γ secreting T cells [13,14]. In addition, these antigens are immunodominant during the early phase of *M. tuberculosis* infection, and T cell responses to these antigens were demonstrated to have protective efficacy in animal models of TB [12–14]. These two antigens were fused into a single protein (designated H1) giving a vaccine antigen that in animal models was more effective than either antigen alone [13,15] and which should be broadly recognized in humans.

Host defense against intracellular pathogens such as *M. tuberculosis* depends on effective cell-mediated immunity, in which interactions between T cells and macrophages are crucial [16,17]. A principal effector mechanism of cell-mediated immunity is the activation of infected macrophages by type-1 cytokines, particularly interferon (IFN)-γ, produced by antigen-specific type-1 T cells and natural killer (NK) cells [18]. Therefore, the number of IFN-γ-releasing *M. tuberculosis* antigen-specific T cells and the amount of IFN-γ produced after vaccination have often been used as a surrogate marker of a vaccine's protective efficacy against infection by *M. tuberculosis* [19,20].

While they have the advantages that they can be produced cheaply in bulk, at a very high level of purity, recombinant proteins are generally poorly immunogenic when administered alone. To improve immune responses to subunit vaccines, it is usually necessary to administer them together with an adjuvant. One of the stumbling blocks to developing anti-bacterial vaccines has been the lack of adjuvants effective in stimulating cell-mediated immunity, that are safe enough to be used in humans. Aluminum salts, the AS03/04 and MF59 are the only vaccine adjuvants approved for human use today, and primarily promote a Th2 or humoral response [21–23]. Most of these adjuvants have proven useless for TB vaccines—or worse than useless, inducing immune responses which were less effective than those seen in unvaccinated animals [9,22]. Intercell AG (Vienna, Austria) has developed a new adjuvant, called IC31® which contains two components: a cationic polyaminoacid KLK, and the oligodeoxynucleotide ODN1a, combined at a molar ratio of KLK:ODN1a of 25:1. IC31® composed of KLK and ODN1a has been shown to induce potent and sustained cellular as well as humoral responses to a variety of peptides and antigens [24,25] and to be effective in animal models of TB [24,26]. Its mode of action appears to be mainly mediated by a vaccine depot formation at injection site, the enhanced uptake of antigens into APC, activation of APC and TLR9 signaling [29,30]. Repeated dose toxicity studies in animals with the Ag85B–ESAT-6 fusion protein administered together with IC31® (dosage: 50 μg Ag + 500 nmol KLK + 20 nmol ODN1a), have caused no safety concerns, and preclinical vaccine studies in mice and guinea pigs indicated that it was highly immunogenic and efficacious [24,26,27]. Here we report the first human vaccine trials for TB of a recombinant protein antigen, and the first human trials of IC31, a new adjuvant for stimulating strong cell-mediated responses. The results from a clinical phase 1 study show that the vaccine is well tolerated, highly immunogenic in naïve individuals and induces strong Th1 responses that persist for more than 2.5 years after vaccination. These results represent a new paradigm for TB vaccines and demonstrate that priming with a safe subunit vaccine in naïve individuals is a safe and feasible alternative to BCG or novel attenuated TB vaccines.

2. Materials and methods

2.1. Ethics statement

All subjects gave informed consent for blood sampling, X-rays and tuberculin skin testing after verbal and written information was provided. The study protocol (EUDRACT no.: TEST-001599-14, LUMC protocol no: 05.075), the Investigator's Brochure and the Investigational Medicinal Product Dossier were approved by the accredited Ethical Review Board of LUMC.

2.2. The investigational products

Ag85B–ESAT-6 is the recombinant fusion protein of Ag85B and ESAT-6, developed and manufactured by Statens Serum Institute (Copenhagen, Denmark). IC31® is a two-component adjuvant system developed by Intercell AG (Vienna, Austria), composed of the cationic polyaminoacid KLK and the oligodeoxynucleotide ODN1a in specific molar ratio of 25:1 KLK to ODN1a. KLK is composed of the amino acids lysine (K) and leucine (L). ODN1a is a single-stranded oligodeoxynucleotide based on alternating sequences of the nucleic acids inosine and cytidine. The final products were manufactured by Statens Serum Institute, in an accredited GMP facility and supplied to the study site as a sterile suspension for injection with a pH of 7.4. The injected volume was 0.5 ml. The vaccine was analyzed and QA released according to specifications before shipment to the clinical site. For H1 a GLP compliant repeated dose toxicity study in rabbits was conducted (LAB Scantox, study nr 55926) in accordance with the CPMP Note for Guidance on preclinical pharmacological and toxicological testing of vaccines for human use (CPMP/SWP/465/95) providing guidance in the EU as of June 1998. Furthermore non-GLP repeated dose toxicity studies were conducted in rhesus and cynomolgus monkeys.

2.3. Study design and objectives

The study was an open label, single-centre, non-randomized, exploratory phase I trial in healthy mycobacterially-naive male subjects. The primary objective of the trial was to evaluate safety of the adjuvanted TB subunit vaccine (H1) administered intramuscularly in different adjuvant formulations, at 0 and 2 months. The primary endpoints comprised sequential monitoring of a standard set of laboratory safety parameters (such as differential white blood cells, platelets, alanine aminotransferase, creatinine, etc.), active solicitation for local and systemic adverse events following vaccine injections, and passive surveillance for solicited and unsolicited local and systemic adverse events by scheduled telephone interviews and self-reporting in a diary for up to 8 months after the first vaccination.

The secondary objective was to evaluate the immunogenicity profile of the vaccine. Cell-mediated responses were measured through detection of IFN-γ spot-forming cells by ELISPOT and of IFN-γ production in supernatants of peripheral blood mononuclear