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Review

Therapeutic vaccines for tuberculosis—A systematic review

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ABSTRACT

For eradication of tuberculosis (TB) by 2050, the declared aim of the Stop TB Partnership, novel treatment strategies are indispensable. The emerging epidemic of multi-drug resistant (MDR) TB has fuelled the debate about TB vaccines, as increasing numbers of patients can no longer be cured by pharmacotherapy. Of several proposed modalities, TB vaccines administered in therapeutic manner represents a promising alternative, despite the controversial history due to the occurrence of exacerbated immune response. A modified concept of immunotherapy is required in order to justify further exploration. In this paper we systematically reviewed the most advanced therapeutic vaccines for TB. We address the rationale of immunotherapeutic vaccination combined with optimized pharmacotherapy in active TB. We summarize preclinical and patient data regarding the five most advanced therapeutic vaccines currently in the pipeline. Of the five products that have been tested in animal models and in humans during active or latent TB, the quality of the published clinical reports of two of these products justify further studies in patients with active TB. This systematic review fuels further clinical evaluation eventually including head-to-head comparative studies.

1. Introduction

With 1.3 million deaths annually, tuberculosis (TB) has remained a tremendous infectious threat around the world [1]. Following the identification of *Mycobacterium tuberculosis* (*Mtb*) as a causative agent of TB in 1884, and the development of a highly effective treatment with multi-drug short-course therapy the battle seemed to be won until hopes were shattered with the emergence of drug-resistant TB [2]. Currently, an estimated 630,000 TB cases worldwide are multi-drug resistant (MDR), with 84 countries reporting at least one case of extensively-drug resistant (XDR)-TB [3]. The paucity of novel therapeutic agents is an important set-back to fight TB [4].

Powdered sputum was used as a remedy for haemoptysis in China in the 16th century [5]. Despite lack of a detailed description, this is the earliest record of immunotherapy in TB. Robert Koch was the first to inoculate TB patients with semi-purified culture supernatants of *Mtb* – the old tuberculin – as a therapeutic vaccination [6]. The exacerbated immune response that subsequently occurred has continued to fuel the discussion about the safety and efficacy of TB immunotherapy [7]. Although the adverse events of the old tuberculin have been widely publicized, very little published evidence is available to substantiate the secondary literature [8,9]. Over 50 years ago, South African researchers used anti-TB drugs in combination with tuberculin [10]. Although their study had low sample size and many drop-outs and was underpowered to detect a difference in survival, sputum culture conversion at six months tended to be better in the immunotherapy group compared to the group receiving standard care alone, with no major adverse events detected.

Current TB immunotherapy modulates immunity, tipping the balance between T-helper (Th)-2 and Th-1 to a Th-1 response, or targeting dormant, persister, slowly replicating *Mtb* bacilli [11]. TB disease results in a pathological immune response, and reversing this provides an important asset and might be regarded as a novel approach [12,13]. Decreasing inflammation by inhibiting LPS biosynthesis leads to an increased survival in *Acinetobacter baumannii* infected mice, suggesting a survival benefit from immune

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intervention [14]. A similar notion was also observed in Koch's tenth experiment, when rats were fed with TB-infected meat, protecting the animals against subsequent *Mtb* challenges [12]. These data together provide experimental evidence of a potential benefit of immune therapy in TB.

Several novel promising TB immunotherapeutic vaccine candidates are in the pipeline.

RUTI vaccine is composed of detoxified *Mtb* cellular fragments expressing a wide range of latency antigens with proven safety and immunogenicity [15]. Heat-killed *Mycobacterium vaccae* is an inactivated environmental mycobacterium with completed phase III trials [16]. Two other NTM – *Mycobacterium smegmatis* and *Mycobacterium indicus pranii* – and V5 have been studied in animal and human models. The immunotherapeutic potential of several TB vaccines, such as DNA vaccines, has been demonstrated although these compounds were initially designed for prevention of primary infection [17–20]. In contrast, attempts using a viral-vectored TB vaccine for therapeutic purpose failed in a mouse model due to toxicity, probably reflecting an exacerbated immune response [19]. Here, we discuss the most advanced TB vaccines specifically designed for therapeutic application and we systematically analyse the relevant studies of the available candidate therapeutic vaccine products.

2. Methods

2.1. Search strategy and selection criteria

We searched PubMed and EMBASE databases in September 2013 to identify relevant non-clinical as well as clinical studies for TB vaccines intended for therapeutic use. We identified five candidates, namely RUTI, *M. vaccae*, V5, *M. smegmatis*, and *M. indicus pranii*. Additionally, we searched the national database of CNKI (Chinese National Knowledge Infrastructure) to detect relevant studies on *M. smegmatis*. We consulted the World Health Organization International Clinical Trials Registry Platform (ICTRP) for additional clinical studies. Key words for database search included "Tuberculosis" OR "TB" OR "*Mycobacterium tuberculosis*". We used the vaccine product name and "immunotherapy" OR "therapeutic vaccine". The search strategy was supplemented by hand searching reference lists of all relevant articles. Other vaccine candidates with potential therapeutic use, not originally designed for therapeutic applications, were considered beyond the scope of this review.

2.2. Data acquisition

Two investigators (MIG and SAP) independently reviewed the title and abstract of all publications identified by the search strategy. The full text of the relevant papers was reviewed using predetermined criteria for further quality assessment of all clinical studies with reported randomized, controlled trials in which subjects received immunotherapy and/or chemotherapy. The study subjects were defined as TB patients, irrespective of drug susceptibility of Mtb isolates, with or without co-infection. With no language restrictions, we included all clinical trials in humans and excluded open-label and self-reporting studies.

2.3. Quality assessment

For quality assessment we used the Jadad scoring system [21]. Points were awarded as follows: study described as randomized, 1 point; additional point for mentioning the appropriate method, 1 point; inappropriate randomization method, deduct 1 point; study described as double-blind, 1 point; appropriate method of blinding, 1 point; inappropriate method of blinding, deduct 1 point, and description of withdrawals and dropouts, 1 point; maximum score, 5 points; 3–5 scores reflecting high quality.

3. Results and discussion

3.1. The RUTI vaccine

The RUTI candidate vaccine has been designed at the Hospital Universitari Germans Trias i Pujol in Catalonia, Spain [22]. It is composed of detoxified and liposomal cellular fragments of *Mtb* bacilli from the company Archivel Farma in Badalona, Catalonia, Spain. It is cultured under stress conditions (intra-granulomatous conditions) to induce latency antigens which would normally be hidden from the immune system [23,24]. It is detoxified to decrease the risk of the exacerbated immune response and fragmented to facilitate processing and presentation of cell wall antigens. The cell wall antigen preparation has an average size of 0.1 μm and exerts adjuvant properties [23]. RUTI contains very low lipoarabinomannan (LAM), an endotoxin-like molecule, which has been implicated in intra-granulomatous necrosis. RUTI is delivered in liposomes to warrant the homogeneity of the preparation, and probably promoting access to the intracellular compartment, resulting in Major Histocompatibility Complex (MHC) class I presentation to $CD8^+$ T cells [24]. RUTI vaccine expresses a wide range of latency antigens. As RUTI does not decrease the bacterial load directly, it needs to be given subsequent to previous chemotherapy [22,23]. The immune response to RUTI has been studied in mice, guinea pigs and healthy volunteers and is characterized by a poly-antigenic, mixed Th1/Th2/Th3 response [22]. Its main immunotherapeutic effect however is induction of Th1 response not only against growth-related antigens but also structural antigens as shown in a murine model [24]. The role of the Th3 induction has been less obvious but it might be involved in the disease chronicity as shown in murine model of TB [22].

In the experimental animals immunized with RUTI, no elevated Immunoglobulin (Ig)E levels were observed and histology revealed no eosinophilia, necrosis, or granulomatous infiltration, and allergic or hypersensitivity reactions have not been observed [22]. In murine models, RUTI triggered a Th1/Th2 response as well as IgG1, IgG2a and IgG3 antibodies against some 13 *Mtb* antigens, reflecting its broad immunogenicity [22]. The Th1 response was enhanced as shown by increased interferon (IFN)-γ expression compared to controls under chemotherapy alone. Further, RUTI increased lung $CD8^+$ T cells, considered relevant to control latent TB infection (LTBI) [23]. In guinea pigs, RUTI elicited a 10-fold increase in IFN-γ production by $CD8^+$ T cells [25]. When LTBI was induced, RUTI reduced relapses and induced splenic T-cells [26]. RUTI-treated mice showed less pulmonary granulomatous infiltration than mice with BCG treatment [24]. Also, RUTI stimulates stronger IFN-γ secretion by $CD4^+$ cells compared to BCG against early secretory antigen target (ESAT)-6, Ag85B, and purified protein derivatives (PPD) and it induces an immune response against structural antigens Ag16 kDa and Ag38 kDa. The mRNA expression of Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-12, inducible Nitric Oxide (NO) synthase, and 'regulated upon activation, normal T-cell expressed and secreted' (RANTES; or Chemokine Ligand 5: CCL5) in lung tissue were all increased [24]. RUTI was at least as potent as BCG in reducing bacillary load. Combining BCG prime and RUTI boost enhanced this effect [27].

In goats infected with *M. caprae*, an experimental animal model for TB vaccine trials [28], RUTI was combined with isoniazid therapy and compared to an untreated control group and a group that received only isoniazid. Only the RUTI plus isoniazid-treated animals showed significantly increased IFN-γ release [29]. Safety issues were negligible; a mild transient increase in body

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