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Protection of mice and guinea pigs against tuberculosis induced by immunization with a single *Mycobacterium tuberculosis* recombinant antigen, MTB41

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Abstract

MTB41 is a *Mycobacterium* antigen that is recognized by $CD4^+$ T cells early after experimental infection of mice with *Mycobacterium tuberculosis* and by PBMC from healthy PPD positive individuals. Immunization of mice with plasmid DNA encoding the MTB41 gene sequence results in the development of antigen-specific $CD4^+$ and $CD8^+$ T cells, and protection against challenge with virulent *M. tuberculosis*. In the present studies, in contrast to DNA immunization, we show, that a strong MTB41-specific $CD4^+$ T cell response, but no MHC class I restricted cytotoxic T lymphocyte (CTL) activity is detected in the spleen cells of infected mice. Therefore, this data suggests that the induction of $CD8^+$ T cell response to MTB41 epitopes by DNA immunization may not be relevant to protection because these epitopes are not recognized during the infectious process. We also compared the repertoire of rMTB41 epitope recognition by $CD4^+$ T cells of *M. tuberculosis*-infected mice with the recognition repertoire of mice immunized with the recombinant rMTB41 protein. Both regimens of sensitization lead to the recognition of the same molecular epitope. Coincidentally, immunization with the soluble recombinant protein plus adjuvant, a regimen known to generate primarily $CD4^+$ T cells, resulted in induction of protection comparable to BCG in two well-established animal models of tuberculosis (mice and guinea pigs).

Keywords: *Mycobacterium tuberculosis*; Vaccine; $CD4^+$ T cells; $CD8^+$ T cells; Recombinant antigen

1. Introduction

Approximately one third of the world's population is infected with *Mycobacterium tuberculosis*, and it is estimated that three million deaths due to this disease occur each year [1–3]. Antibiotics provide an effective therapy for tuberculosis, however treatment is long, resulting in inadequate compliancy and in the development of multi-drug resistant strains of *M. tuberculosis* [4–8]. The only tuberculosis vaccine currently available, the live attenuated strain of *Mycobacterium bovis*, bacillus Calmette-Guérin (BCG) while effective in preventing the less common yet severe forms of the disease in young children (meningitis and systemic disease) has minimal effect against adult pulmonary tuberculosis, the most common form of the disease [9–11].

Abbreviations: CFU, colony forming units; PPD, purified protein derivative of *Mycobacterium tuberculosis*; AS02A, adjuvant containing monophosphoryl Lipid A (MPL) and QS-21

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Over the past decade several investigators have concentrated efforts on the development of a better tuberculosis vaccine than BCG. Unfortunately, thus far molecular manipulation of BCG, either by insertion of mammalian genes encoding cytokines (aiming to enhance or modulate the immune response induced by the bacteria) or of *M. tuberculosis* genes encoding unique proteins of the tubercle bacilli, has resulted in mycobacterial variants, which do not appreciably augment the protective efficacy of the original vaccine [12–17]. On the other hand, genetic manipulation of virulent *M. tuberculosis* has generated interesting auxotrophic mutants with low virulence and protective efficacy similar to that induced by BCG in mice challenged with the virulent bacilli [18–21].

Much progress has been made on the development of recombinant subunit tuberculosis vaccines. In particular, bacterial plasmid DNAs containing *M. tuberculosis* genes have been extensively tested [22–27]. One of such genes has been cloned by our group and shown to encode an antigen associated with the early control of infection [28]. This antigen, which we named MTB41 (Rv0915c, TubercuList H37Rv database), belongs to the PE and PPE family of proteins identified in the *M. tuberculosis* genome, and was shown to be recognized by a mouse protective $CD4^+$ T cell line and by *M. tuberculosis* sensitized human PBMC. Moreover, C57BL/6 mice immunized with MTB41-DNA developed both $CD4^+$ (predominantly Th1) and $CD8^+$ antigen-specific T cell responses to rMTB41 protein, and developed protective immunity against infection with *M. tuberculosis* comparable to that induced by BCG.

The initial euphoria regarding the use of plasmid DNA as a means of antigen delivery has been hampered by the findings that protection of mice induced by many different *M. tuberculosis* DNAs could not be reproduced in either the guinea pig [29,30] or monkey models of the disease (Skeiky, Y., Alderson, M., Campos-Neto, A., and Reed, S., unpublished observation). Despite several attempts at optimizing codon usage and alternating prime/boosting with either BCG or virus vectors carrying the *M. tuberculosis* genes, far little improvement has been achieved [31–33]. More recently the results obtained with conventional protein immunization formulated with newer adjuvants capable of both amplification and modulation of the immune response preferentially to the Th1 phenotype have re-instilled optimism about the development of an efficacious tuberculosis subunit vaccine [34,35].

A turning point for the development of subunit vaccines has been the development of novel adjuvant formulations designed to promote strong Th1 immune responses in vaccine recipients. Such adjuvant system containing monophosphoryl lipid A (MPL) and QS-21 (ASO2A, GlaxoSmithKline Biologicals, Rixensart, Belgium) are of particular interest because they have been extensively tested in a number of human malaria and HIV vaccine clinical trials, and shown to modulate preferentially Th1 immune responses [36–38]. More recently these adjuvants have been used experimentally in pre-clinical experiments in association with a tuberculosis polyprotein recombinant vaccine candidate [35].

In the present communication, using conventional immunization with a formulation containing protein/adjuvant, we report that rMTB41 produces protective immunity in both the mouse and guinea pig models of tuberculosis. In addition, we show that the repertoire of rMTB41 epitope recognition by $CD4^+$ T cells obtained from *M. tuberculosis*-infected mice during the remission period of infection is identical to that of cells generated in mice immunized with the recombinant rMTB41 protein.

2. Materials and methods

2.1. Animals

C57BL/6 mice were obtained from Charles River Laboratories (Wilmington, MA). The mice were maintained under specific pathogen-free conditions and used at 8–12 weeks of age. Hartley guinea pigs weighting approximately 250–350 g were from Harlan–Sprague–Dawley Laboratories, North Wilmington, MA.

2.2. Recombinant antigen

The antigen rMTB41 was expressed in *Escherichia coli*, and purified as previously described [28]. The recombinant rMTB41 was assayed for endotoxin contamination using the *Limulus* amebocyte assay (BioWhittaker, Walkersville, MD), and was shown to contain <100 EU/mg of protein.

2.3. Peptides

Overlapping peptides containing 15 amino acids, covering the entire rMTB41 molecule, were synthesized on a Rainin Symphony multiple peptide synthesizer using the *O*-Benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) activation system. Cleavage of the peptides from the solid support was carried out using the following cleavage mixture: trifluoroacetic acid–ethanedithiol–thioanisole–water–phenol (40:1:2:2:3). After cleaving for 2 h, the peptides were precipitated in cold ether. The peptide pellets were then dissolved in 10% (v/v) acetic acid, and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0–60% acetonitrile in water (containing 0.1% TFA) was used to elute the peptides. The purity of the peptides was verified by HPLC and mass spectrometry. Each peptide was purified to >95% before use. The peptides contain an overlapping sequence of 10 amino acids with each other (Fig. 1). A total of 83 peptides were synthesized.

2.4. Immunizations and animal infections

Mice and guinea pigs were immunized intramuscularly, three times, 1 month apart, with 5–8 μg (mice) or 20 μg (guinea pigs) of purified recombinant MTB1 protein mixed with ASO2A adjuvant (GlaxoSmithKline Biologicals, Rix-