Cloning, expression, and purification of recombinant protein from a single synthetic multivalent construct of Mycobacterium tuberculosis

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Abstract

Tuberculosis remains a major infectious disease with over 8 million new cases and 2 million deaths annually. Therefore, a vaccine more potent than BCG is desperately needed. In this regard, an approximately 800 bp DNA encoding a mycobacterial synthetic gene designated as VacIII (containing ubiquitin gene UbGR and four immunogenic mycobacterial epitopes or genes of ESAT-6, Phos1, Hsp16.3, and Mtb8.4) was sub-cloned into a bacterial expression vector of pRSET-B resulting in a 6×His-VacIII fusion gene construction. This recombinant clone was over expressed in Escherichia coli BL-21 (DE-3). The expressed fusion protein was found almost entirely in the insoluble form (inclusion bodies) in cell lysate. The inclusion bodies were solubilized with 8 M urea and the recombinant protein was purified by Ni–NTA column and dialyzed by urea gradient dialysis. This method produced a relatively high yield of recombinant VacIII protein and the cloned VacIII gene offers the potential development of other vaccine formats such as DNA vaccine and recombinant vaccine. © 2006 Elsevier Inc. All rights reserved.

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Tuberculosis (TB) is one of the world’s major infectious diseases [1]. The current and only available TB vaccine, Bacillus Calmette Guérin (BCG), is a live attenuated strain developed almost a century ago. Based on the results of many clinical trials in developing countries, the efficacy of BCG in eliciting protective immunity has been reported to vary from 0 to 80% [2,3]. In addition to this variable efficacy, BCG, being a live attenuated vaccine, can also potentially cause serious disease in immunocompromised persons. These clearly showed that more effective vaccines other than BCG are needed for the prevention of TB. In addition, the development of new chemotherapy agents besides Isoniazid, Pyrazinamide, Rifampicin, Ethambutol, Streptomycin, and Thioacetazone are important for the treatment for TB. The goals of the treatment are to ensure cure without relapse, to prevent death, to stop transmission, and to prevent the emergence of the drug resistance.

To develop an effective vaccine for tuberculosis, the specific antigens must be identified, and their ability to induce protective immunity must be confirmed. A proper way for antigen presentation also acts as an essential factor in vaccine development [4]. Therefore, immunity to TB and development of a vaccine relies on the identification, formulation, and delivery of protective T cell antigens in a manner that will generate prolonged memory responses of relevant effector cells [3]. Several approaches have been used in the development of TB vaccines, including the development of DNA vaccination, the development of live delivery systems, protein immunogens development [5], and modifying BCG to improve the efficiency of BCG itself [6].