Available online at www.sciencedirect.com
ScienceDirect
Immunobiology 215 (2010) 133–142
Immunobiology
www.elsevier.de/imbio

Investigation of immunogenic effect of the BCG priming and Ag85A-GM-CSF boosting in Balb/c mice model

Jun Dou[a,*,1], Quan Tang[a,1], Fangliu Yu[a,1], Haitao Yang[b], Fengshu Zhao[a], Weiguo Xu[b], Jing Wang[c], Weihua Hu[a], Kai Hu[c], Chunsheng Liou[a], Xiang Feng He[a], Yaqing Wang[a]

[a]*Department of Pathogenic Biology and Immunology, Southeast University School of Basic Medical Science, Nanjing 210009, China*
[b]*Centers for Disease Control and Prevention of Jiangsu Province, Nanjing 210008, China*
[c]*Department of Gynecology & Obstetrics, Zhongda Hospital, Southeast University, Nanjing 210009, China*

Received 19 February 2009; received in revised form 28 March 2009; accepted 4 April 2009

Abstract

Mycobacterium tuberculosis (*M. tuberculosis*) has once again become a major public health threat owing to the combined effects of a worldwide anti-tuberculosis drug resistance and the emergence of the human immunodeficiency virus pandemic. The Bacille Calmette-Guérin (BCG)-based vaccine has displayed inconsistent efficacy in different trials although it continues to be used to prevent tuberculosis in many countries. In current work, we have developed DNA vaccines expressing *M. tuberculosis* antigen 85A (Ag85A) and cytokine granulocyte macrophage colony stimulating factor (GM-CSF) and aimed to investigate the immune effect in mice based on BCG priming and DNA vaccine boosting and immune protection against *M. tuberculosis* challenge. Our results showed that the activity of cytotoxic T lymphocyte and spleen cell proliferative responses to Ag85A and IFN-γ level as well as the specific antibody titer against Ag85A were significantly increased in mice immunized with prime-boost strategy in comparison with the mice immunized with BCG or DNA vaccine expressing Ag85A and GM-CSF alone. Meanwhile, the immune strategy of BCG-prime and DNA vaccine boost induced mice to generate efficient immune protection against *M. tuberculosis* challenge. Our data demonstrate that BCG-prime and DNA vaccine expressing Ag85A and GM-CSF boost provides a rational strategy for further development of DNA vaccine against *M. tuberculosis* infection.

Keywords: *Mycobacterium tuberculosis*; Antigen 85A; Granulocyte macrophage colony stimulating factor; DNA vaccine; Bacille Calmette-Guérin

Introduction

The *Mycobacterium tuberculosis* (*M. tuberculosis*) is a worldwide public health problem and has afflicted humans for thousands of years. Epidemic Tuberculosis (Tb) has witnessed an escalation recent years due to the spread of the human immunodeficiency virus (HIV) and the increase of multiple drug resistant Tb (Espinal et al. 2001; Fordham and Jenni M. 2002; Hussey et al. 2007) particularly in sub-Saharan Africa and South-East Asia (Claire et al. 2007). In China, Tb remains epidemic with a morbidity of 2 million new infectious cases and death

*Corresponding author.
E-mail address: njdoujun@yahoo.com.cn (J. Dou).
[1]Co-author, equally contributed.

0171-2985/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2009.04.002

of 250 thousands patients with Tb per year (Tang et al. 2007). The current Bacille Calmette-Guérin (BCG) vaccine prevents the invasive complications of childhood Tb, such as meningitis and miliary disease, but it fails to protect against adult pulmonary disease (Espinal et al. 2001; Claire et al. 2007). There is an urgent need for an efficacious vaccine against Tb. Several new Tb vaccines have demonstrated promising results in animal models and a number of vaccine candidates have gone into phase I and phase II clinical trials in humans (Claire et al. 2007; Brennan et al. 2007), however, there is no one vaccine candidate against Tb to replace the current "gold standard" vaccine BCG (Radosevic et al. 2007; von Reyn and Vuola 2002).

Many studies have revealed that the DNA vaccine is a very powerful and easy method for the induction of strong humoral and cell-mediated immune (CMI) responses in mice. By virtue of its strong capacity to induce cell-mediated T-lymphocyte responses, this vaccine approach is particularly attractive for the prophylaxis of intracellular pathogens, such as Tb and other pathogenic mycobacteria (Huygen 2006). Plasmid DNA encoding the *M. tuberculosis* antigen 85 (Ag85A) has been shown to induce protective and therapeutic immune responses in a murine model of *M. tuberculosis* (Wiker et al. 1986; Nagai et al. 1991; Dou et al. 2005). However, vaccines encoding the single antigen of Mycobacterium did not produce as great a protective effect as BCG and the immunigenicity of the DNA vaccine is lower in primates than in rodents (Renneson et al. 2005; Romano et al. 2006).

It is widely accepted that an acquired CMI response, characterized by the production of type 1 cytokines including IL-12, IFN-γ and TNF-α, is the critical component in host defense against mycobacterial infection (Wakeham et al. 1998; Dietrich et al. 2006). It has been known that the granulocyte macrophage colony stimulating factor (GM-CSF) plays a critical role in inducing the maturation of dendritic cells (DCs) and converting Langerhans cells of the skin to immunostimulatory antigen-presenting cells (APC). GM-CSF also enhances the production of antigen-specific antibody (Sandler et al. 2003; Liu et al. 1998) and will activate helper T lymphocytes type 1 and type 2 and has been utilized as a potent immune adjuvant for vaccine formulations against infectious diseases and cancer (Kamath et al. 1999; Wang et al. 2002; Dou et al. 2006).

In previous work, we successfully developed a novel TB gene vaccine construct expressing Ag85A and GM-CSF along that could induce mice to generate potent immune responses to Ag85A. GM-CSF as an effective adjuvant could increase Ag85A immunogenicity and enhance the activity of cytotoxic T lymphocytes (CTL) in mice immunized with recombinant pI85AGM. However, DNA vaccine expressing Ag85A and GM-CSF had little effect on immune protection from *M. tuberculosis* challenge (Chen et al. 2003; Dou et al. 2004). Thus, both DNA vaccines design and immune adjuvants ways have been sought to enhance DNA efficiency. One such approach is to increase the efficiency of plasmid-based DNA vaccination by a different immune strategy. In this regard, we used BCG for priming and the DNA vaccine expressing Ag85A and GM-CSF for boosting the immune response in the Balb/c mouse model, with the aim to investigate the immune effect and immune protection from *M. tuberculosis* challenge. Our goal was to provide a new strategy for the development of a DNA vaccine based on co-expressing Ag85A and GM-CSF against Tb.

Material and methods

Mice and cell lines

Male Balb/c mice between 4 and 6 weeks of age were used in this study. They were ordered from the Animal Center of Yang Zhou University of China. All mice were housed in the SPF level B animal facility. All animal experiments were conducted following the guidelines of the Animal Research Ethics Board of Southeast University. The SP2/0 cell line (BALB/c mice myeloma cells) and YAC-1 cell line (Moloney leukemia-induced T-cell lymphoma of A/Sn mouse origin) were obtained from Cellular Institute of China in Shanghai and were cultured at 37 °C in 5% CO_2 atmosphere in RPMI 1640 supplemented with 10% fetal bovine serum containing 100 units/ml penicillin G sodium and 100 μg/ml streptomycin sulfate.

Plasmids and primers

Recombinant plasmid pIRES-Ag85A-GM-CSF was constructed by our laboratory (Chen et al. 2003; Dou et al. 2005). The sequence of PCR sense primer for Ag85A gene is 5′TC GCTAGC ATG CAG CTT GTT GAC AGG G3′ and that of anti-sense primer is 5′GGAACGCGT C TAG ATG TTG TGT CTG T3′, which contains restriction endonuclease NheI and MluI sites, respectively. The sequence of PCR sense primer for the mGM-CSF gene is 5′GC TCTAGA AGA TCA CCG GCG AAG GA3′ and that of anti-sense primer is 5′TAT GCGGCCGC TTC CTC ATT TTT GGC C3′, which contains restriction endonuclease XbaI and NotI sites, respectively. The sequence of PCR sense primer for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene is 5′ACCGCAAAGACTGTGGAT GC3′ and that of anti-sense primer is 5′TGA GCTTGACAAA-GTGGTCG3′. The primers were synthesized by the Shenneng Company of Gene and Technology of China in Shanghai.