Antigen-Specific IFN-γ/IL-17-Co-Producing CD4$^+$ T-Cells are the Determinants for Protective Efficacy of Tuberculosis Subunit Vaccine

Kee Woong Kwon

Institute for Immunology and Immunological Diseases, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul 03722, Korea

Abstract

The antigen-specific Th17 responses in the lungs for improved immunity against *Mycobacterium tuberculosis* infection are incompletely understood. Tuberculosis vaccine candidate HSP90-ESAT-6 (given as a Bacillus Calmette-Guérin prime boost regimen, confers superior long-term protection against the hypervirulent Mtb HN878 infection, compared to BCG or BCG-E6. Taking advantage of protective efficacy lead-out, we found that ESAT-6-specific multifunctional CD4$^+$IFN-γ$^+$IL-17$^+$ T-cells optimally correlated with protection level against Mtb infection both pre-and post-challenge. Macrophages treated with the supernatant of re-stimulated lung cells from HSP90-E6-immunised mice significantly restricted Mtb growth, and this phenomenon was abrogated by neutralising anti-IFN-γ and/or anti-IL-17 antibodies. We identified a previously unrecognised role for IFN-γ/IL-17 synergism in linking anti-mycobacterial phagosomal activity to enhance host control against Mtb infection. The implications of our findings highlight the fundamental rationale for why and how Th17 responses are essential in the control of Mtb, and for the development of novel anti-TB subunit vaccines.

Keywords: ***Mycobacterium tuberculosis***; **BCG-prime boost**; **IFN-γ/IL-17**; **multifunctional T cells**; **phagosome maturation**

Introduction

Tuberculosis an infectious disease caused by *Mycobacterium tuberculosis* is associated with high morbidity and mortality, thus posing a global public health problem. In 2017, TB ranked as one of the top ten causes of death, with an estimated 10 million new cases and 1.6 million deaths [1]. In addition, approximately 1.7 billion people, 23% of the global population, are estimated to have latent TB infection and to be at risk of developing active TB during their lifetime. In addition, the emergence of Mtb strains resistant to TB drugs poses a major growing burden of hard-to-treat infections [2].

Mycobacterium bovis Bacillus Calmette-Guérin currently is the only licensed prophylactic vaccine; however, it provides insufficient protection against TB, and thus, novel effective vaccines are urgently needed [3]. Various types of adjuvants, antigen targets, and vaccine platforms have been developed in an aim to improve Mtb vaccines. These efforts have yielded various results, with some producing positive outcomes in clinical trials. Heterologous prime-boost regimens involving priming with BCG, followed by an adjuvant boost, are a promising vaccination strategy against TB [4], and have a proven high level of efficacy.

Materials and Methods

2.1. Ethics Statement

All animal studies were performed in accordance with Korean Food and Drug Administration guidelines. The experimental protocols used in this study were reviewed and approved by the Ethics Committee and Institutional Animal Care and Use Committee of the Laboratory Animal Research Center at Yonsei University College of Medicine and IACUC of animal care at Chungnam National University .

2.2. Mice

Specific pathogen-free female C57BL/6J mice (6–7 weeks old) were purchased from Japan SLC, Inc. and maintained under barrier conditions in the ABSL-3 facility at the Yonsei University College of Medicine with constant temperature (24 °C ± 1 °C) and humidity (50% ± 5%). The animals were fed a sterile commercial mouse diet with ad libitum access to water under standardized light-controlled conditions (12-h light and 12-h dark periods). The mice were monitored daily, and none of the mice showed any clinical symptoms or illness during this experiment.

Results

3.1. Characterisation of the Immune Responses induced by HSP90-E6

To examine whether HSP90-E6 induces an antigen (Ag)-specific memory T-cell response, we analysed Ag-specific IFN-γ, TNF-α, IL-2, IL-4, IL-10, and IL-17 production in the lungs, spleen, and lymph nodes of mice 4 weeks after the last immunisation and before challenge (**Figure 1**a, green arrow). All Ag-specific Th1 cytokines, except IL-4 and IL-10, were significantly induced in mice immunised with ESAT-6 or HSP90-E6 when compared to mice immunised with BCG alone . When stimulated with purified protein derivative antigen, IFN-γ, TNF-α and IL-2 production in the lungs, spleen, and lymph nodes of mice immunised with HSP90-E6 was significantly higher than that in mice immunised with BCG or ESAT-6 (**Figure 1**b). Notably, upon re-stimulation with ESAT-6, not only Th1 cytokines, but also the Th17-related cytokine IL-17 was significantly produced in all three tissues evaluated in the HSP90-E6 immunised mice, when compared to the other treatment groups (**Figure 1**c). These results suggested that

HSP90-E6/CIA05-boosting establishes Th1/Th17-biased immunity.

Discussion

The present study revealed that the TB vaccine candidate HSP90-E6, given as a BCG-prime boost regimen, confers superior, long-term protection against hypervirulent Mtb HN878 infection when compared to BCG or BCG-E6 alone. Further, elevated E6-specific $CD4^+$ IFN-γ^+IL-17^+ T-cells pre- and post-infection positively correlated with protection against Mtb.

There clearly is an urgent, unmet need for new anti-TB vaccines, and heterologous prime-boost vaccination appears to be a promising strategy. However, despite increased research efforts on this approach in the last two decades, clinical progress has been limited, due to insufficient understandings on protective immune response, targeting antigens, and scheme of boosting. A number of fusion protein-based subunit vaccines are being tested as boosters to BCG. We previously demonstrated that HSP90-E6 formulated with MPL/DDA significantly reduced the bacterial load in mouse lungs after challenge with HN878 [10], but we did not identify an immunologic correlate of protection for this fusion vaccine. Here, we report that HSP90-E6 formulated with CIA05/DDA prolongs BCG-primed boosting and that its protective effect is related to enhancement of Ag-specific-IFN-γ^+IL-17^+ multifunctional T-cells.

The primary rationale for the development of TB vaccines designed to elicit Th1-cell-based immunity is based on evidence from various animal models that a strong IFN-γ-mediated Th1 immune response is the primary protective mechanism of anti-TB immunity .However, an IFN-γ response is not an optimal correlate of protection .and an IFN-γ response alone is not sufficient to control Mtb infection [46].

Conclusions

Collectively, out study demonstrated that IFN-γ-producing T-cells are necessary, but not sufficient for TB defense, and, more importantly, that an increased in the number of cells that produce both IFN-γ and IL-17 is essential for protection. This protective immune determinant related with BCG-primed HSP90-E6 booster vaccination will pave the way for further investigation of a novel strategy to improve BCG-booster vaccines.

KKEEWEE@yuhs.ac

This work is partly presented at Joint Event on 24th World Congress on Pharmacology & 7th World Heart Congress, August19- 20, 2019 at Vienna, Austria