Recombinant BCG vaccine expressing multistage antigens of *Mycobacterium tuberculosis* provides long-term immunity against tuberculosis in BALB/c mice

INTRODUCTION

Tuberculosis (TB) caused by Mycobacterium tuberculosis (Mtb) persistently kills nearly 1.5 million lives per year in the world,

Whereas the only licensed TB vaccine BCG exhibits unsatisfactory efficacy in adults.

Taking BCG as a vehicle to express Mtb antigens is a promising way to enhance its efficacy against Mtb infection

Vaccines

- live attenuated vaccines
- Subunit vaccines
- Recombinant BCG vaccines

SEVERAL APPROACHES HAVE BEEN USED TO DESIGN NEW TB VACCINES

CHAPTER 3

MVA85A

the first new generation infant TB vaccine

IL12

TNFα

IL2

IL17

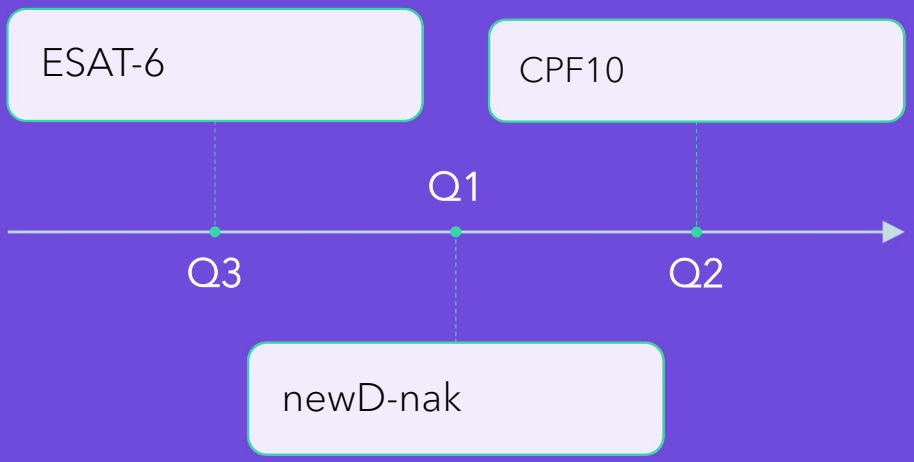
GM-CSF

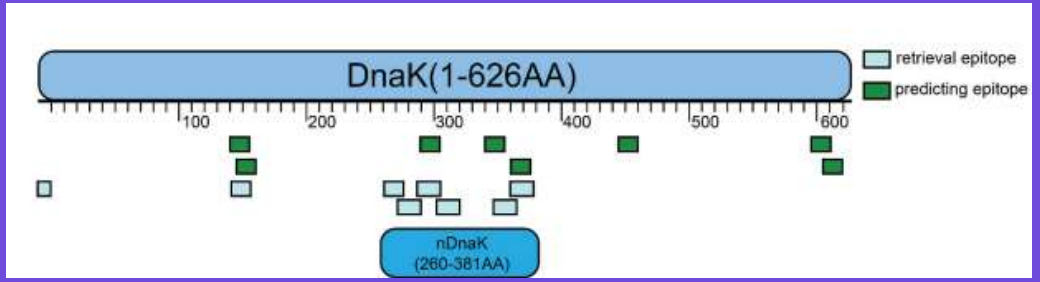
IFNɣ

Antibody response

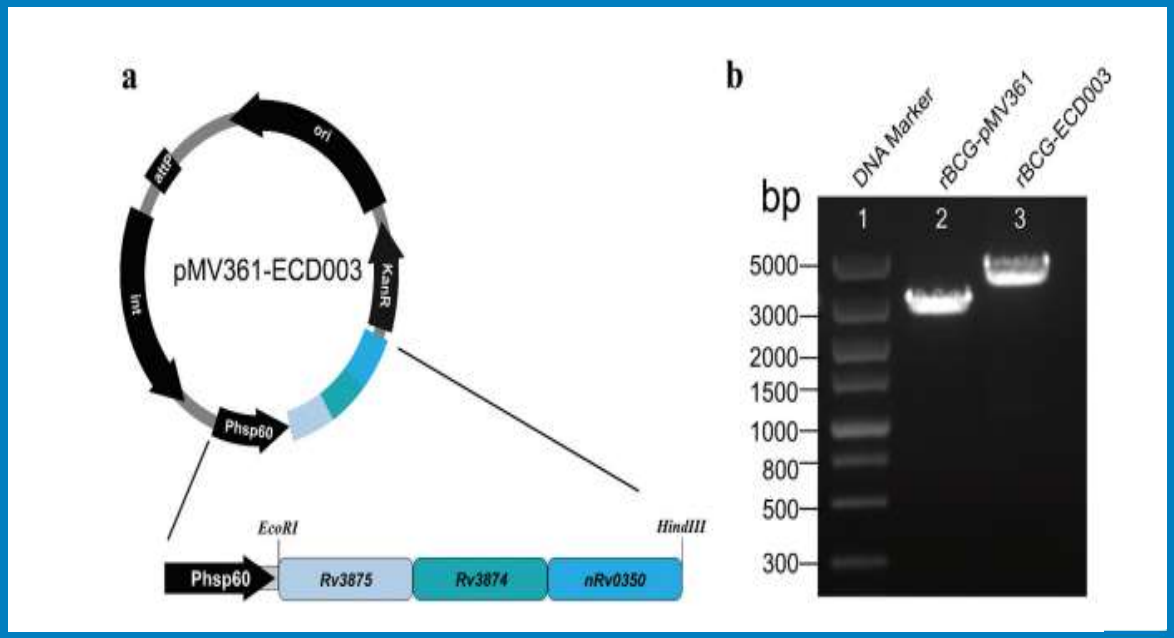
rBCG-ECD003

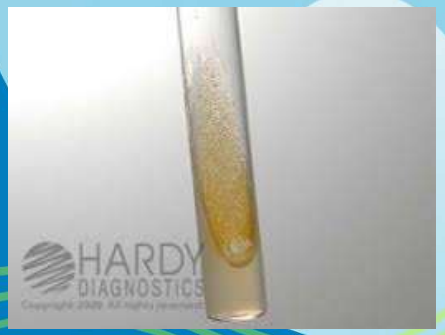
FUSION PROTEIN





Construction of recombinant BCG strain rBCG-ECD003





IMMUNIZATION AND SAMPLE COLLECTION

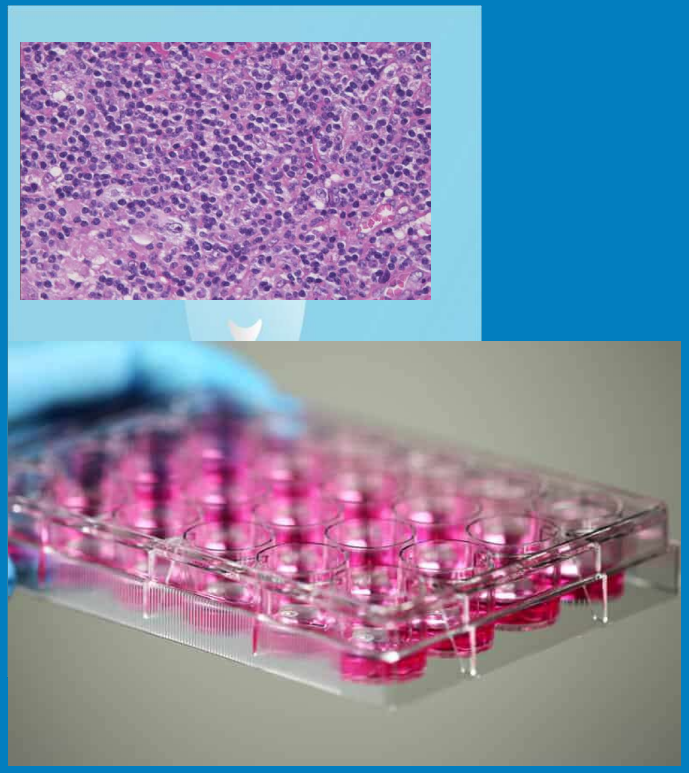
At each time point (4th and 12th week), 6 mice in each group were euthanized to collect spleen, liver, and lung samples sterilely to analyze. cell concentration of lymphocyte suspension was1*106 cells/ml

The serum were collected at the 4th, 8th, and 12th week, and stored at −80°C

(12 mice in each group)

1(BCG group)	2 (r BCG-ECD003 group)	3 (control group)
1*106 BCG cfu/200μlPBS	1*106 rBCG-ECD003 cfu/200μlPBS	200μl PBS

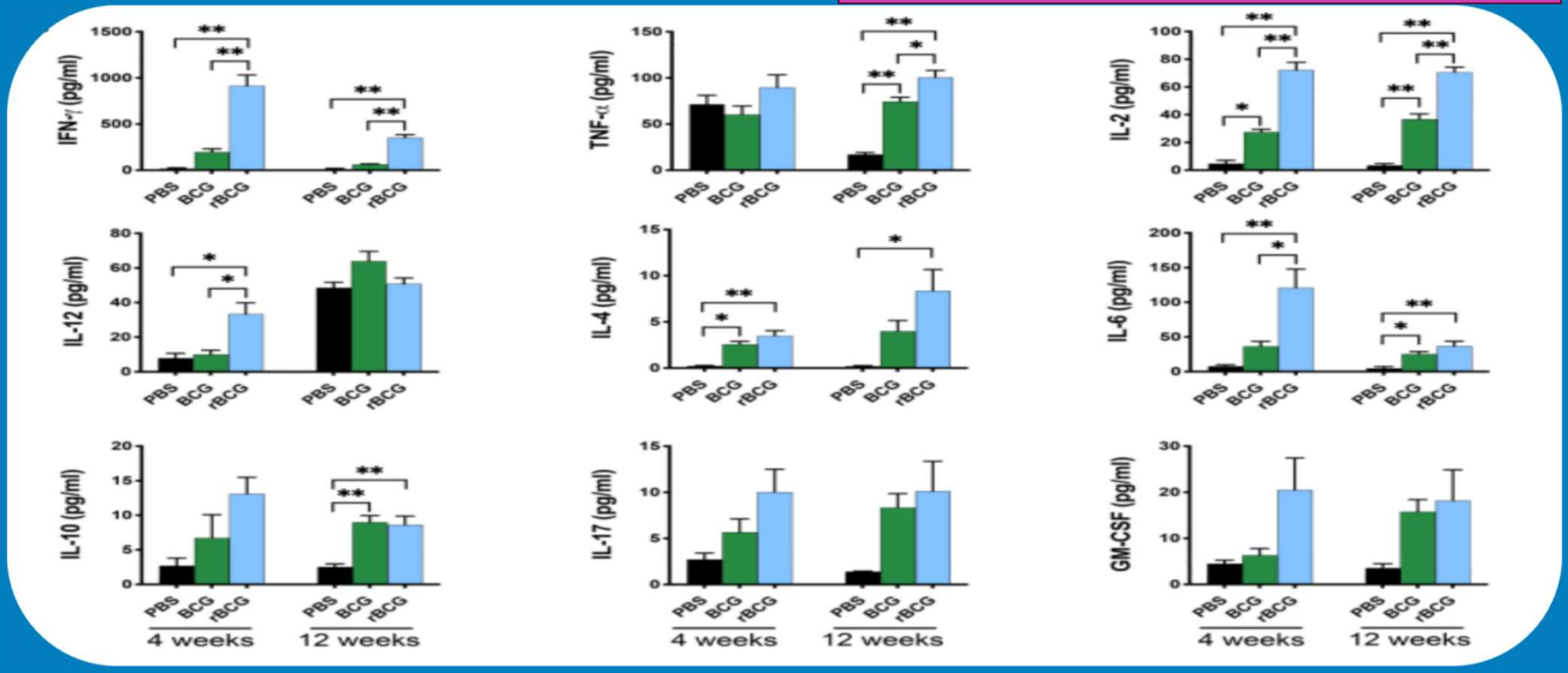
LUMINEX MULTIPLEX CYTOKINE ASSAY



spleen lymphocyte suspensions were seeded at 1 × 106 cells/well in 96-well U-bottomed plates and stimulated with	
10 μg/well of PPD	10 μg/well of ECD003
Medium alone as the negative control	
Incubation at 37°C for 24 h. after y centrifuge at 400 g for 5 min	
The cytokine productions including IFN-γ, TNF-α, IL-2, IL-12, IL-4, IL-10, IL-6, IL-17, and GM-CSF were detected by Luminex multiplex cytokine assay .	

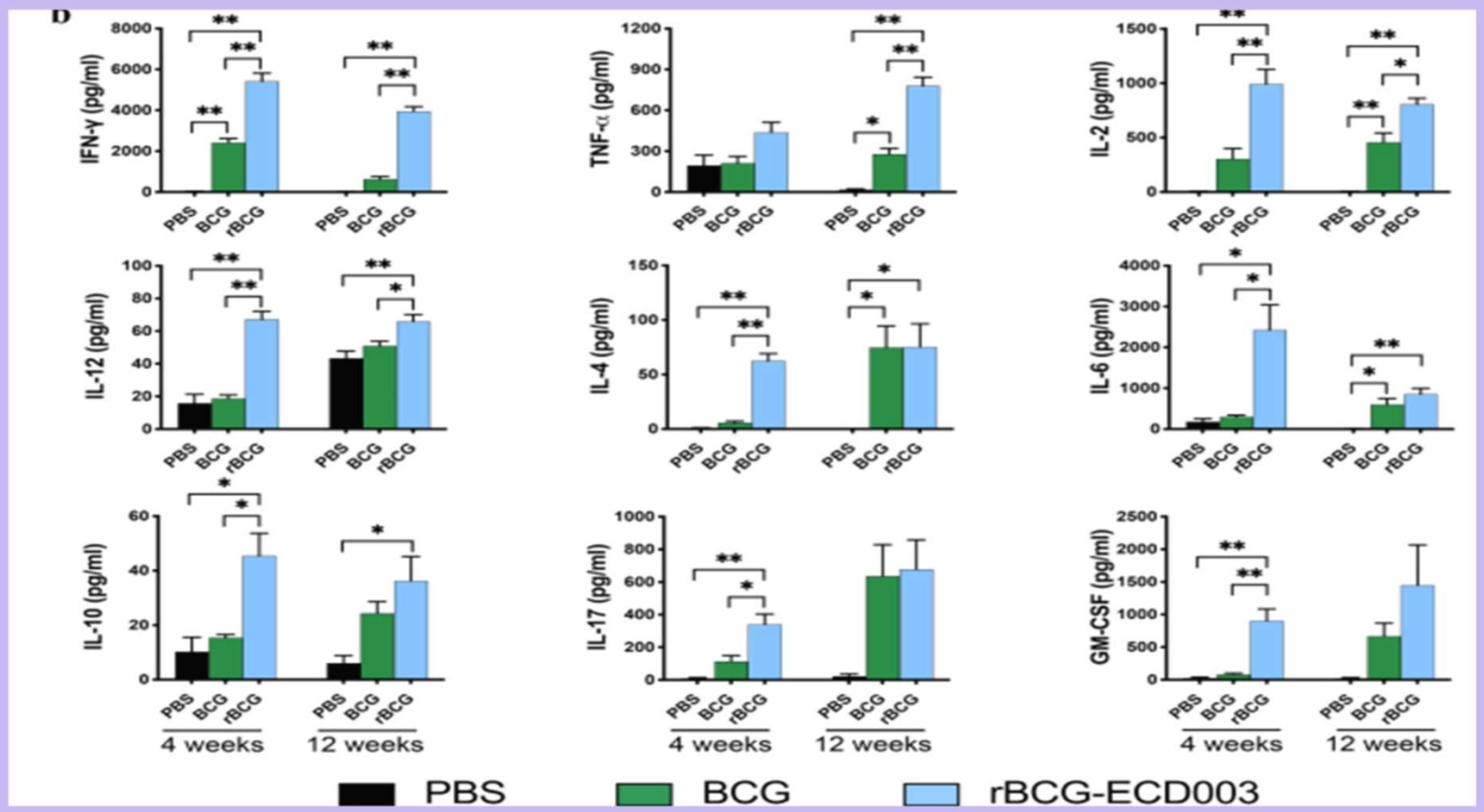
Cytokine production profile induced by rBCG-ECD003 in spleen

restimulated with the fusion protein ECD003



PBS BCG rBCG-ECD003

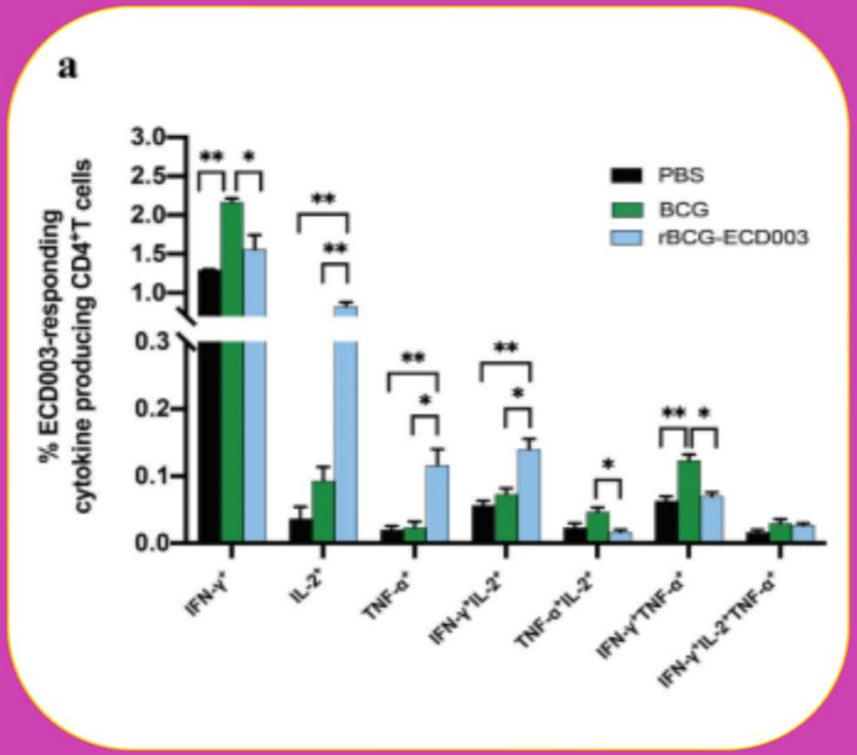
FOR STIMULATION WITH PPD

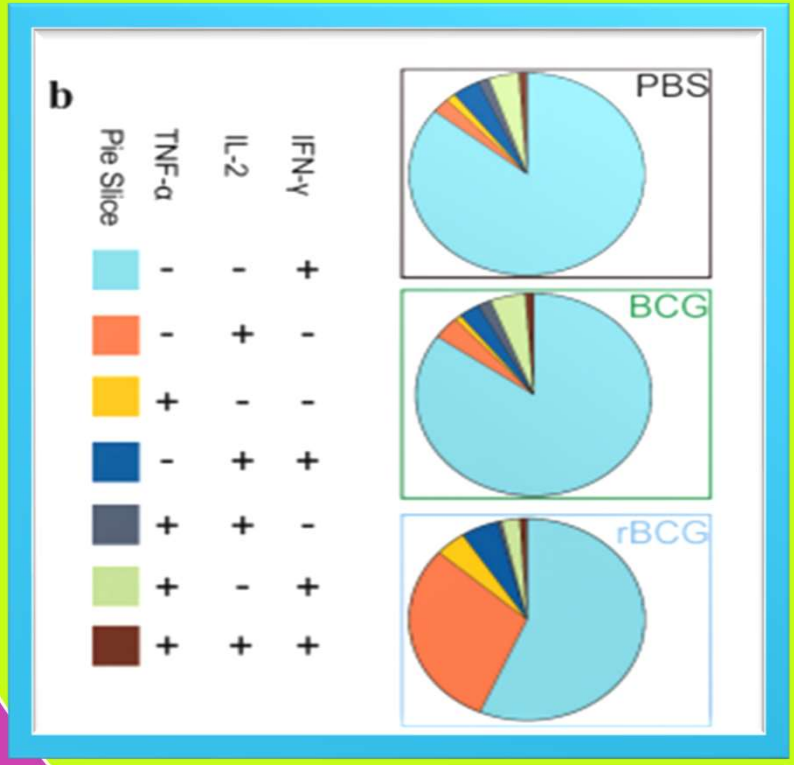


Polyfunctionality CD4+ T cells induced by rBCG-ECD003

restimulated with the fusion protein ECD003

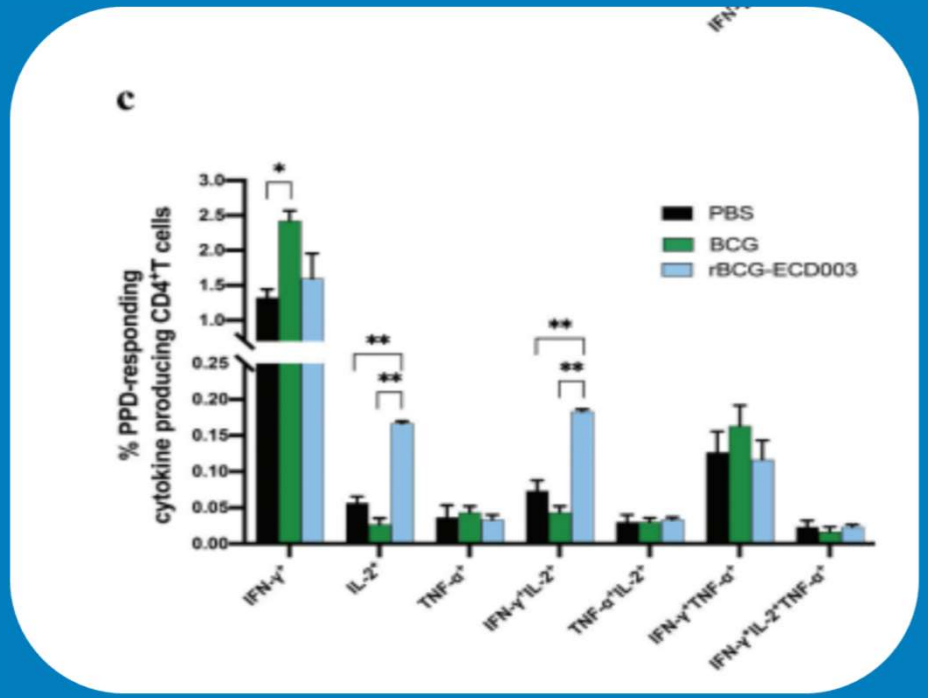
Increase in single-positive IL-2+ CD4+ T cell proportion in positive CD4+ T



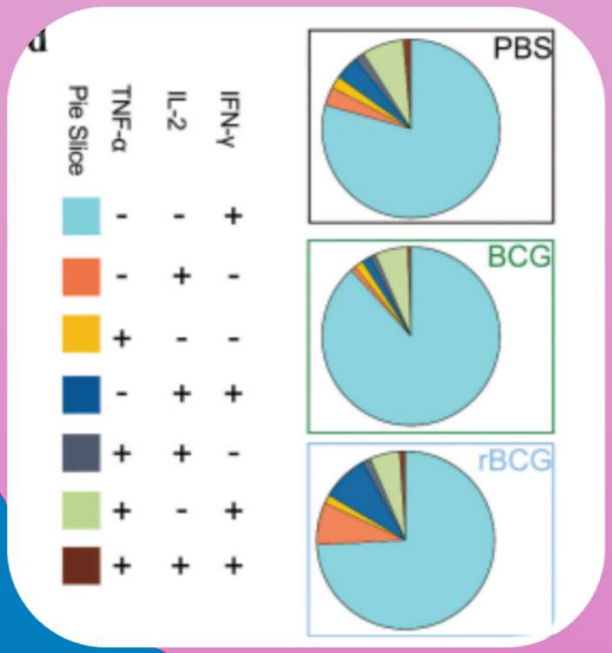


Polyfunctionality CD4+ T cells induced by rBCG-ECD003

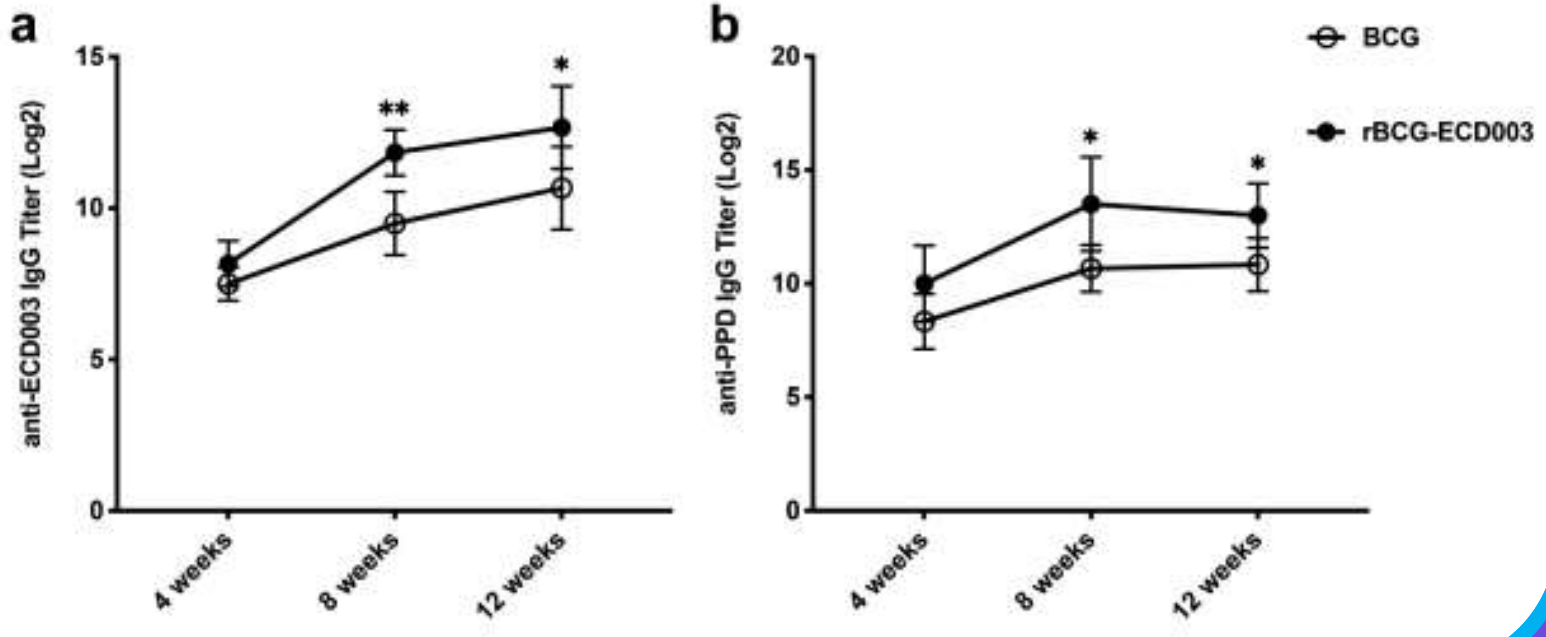
Stimulation with PPD



The pie charts reflect there is a wider variety of combinations of cytokine production in CD4+ T cells in rBCG-ECD003 groups.



ELIZA assay for IGg antibody



rBCG-ECD003 conferred superior humoral immunity in BALB/c mice

Mycobacterial growth inhibition assay in vitro

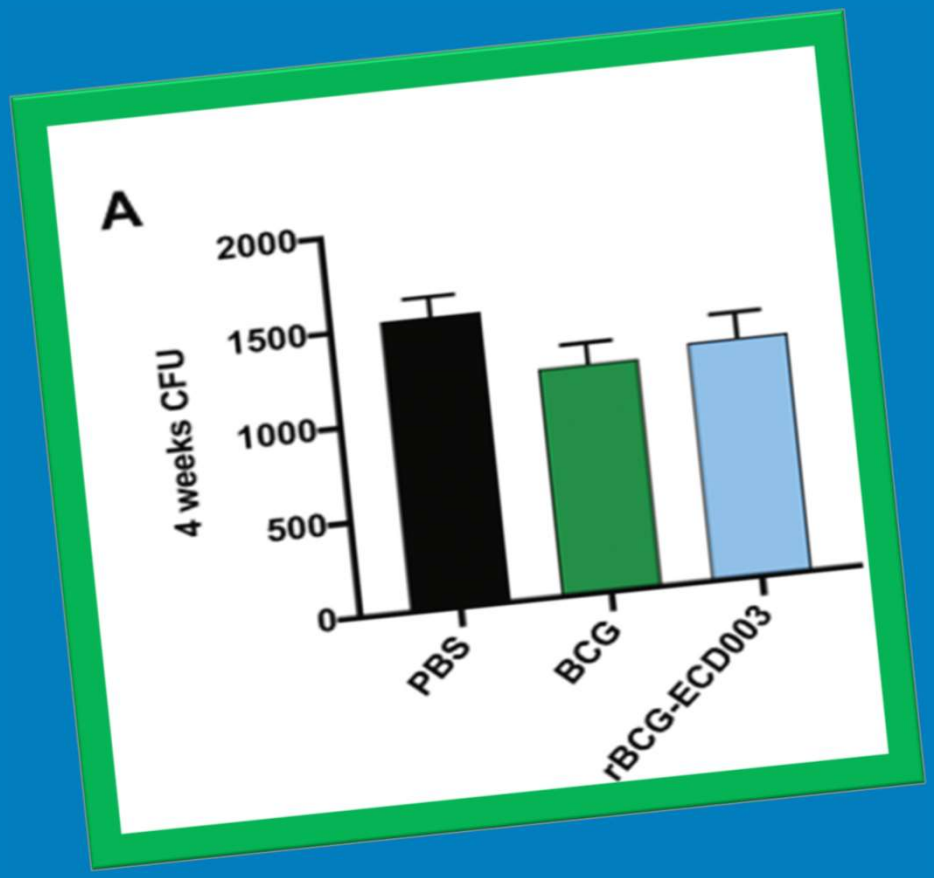
1. Spleen lymphocytes (1 × 105 /well) were cocultured with ultrasonic dispersed Mtb H37Rv (500 CFU/well) for 72 h in 24-well plates

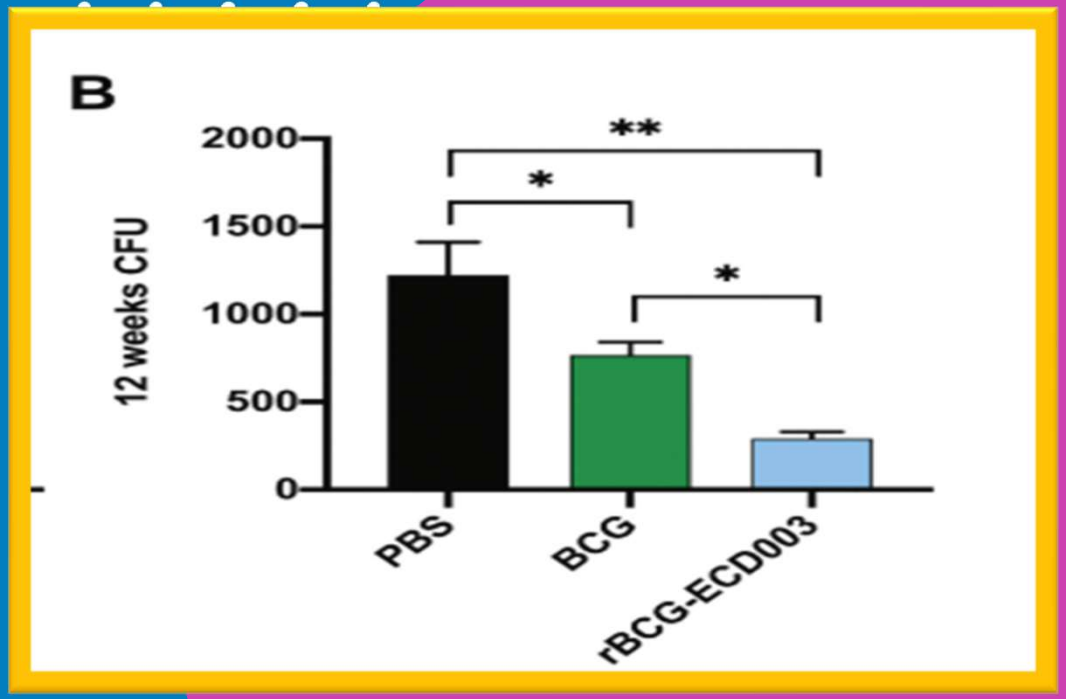
2. The co-cultures were harvested at 12,000 rpm for 10 minutes, then the supernatants were carefully removed by pipetting

3. After mixing with repetitive pipetting, the lysates were transferred to the corresponding tubes containing the co-cultures.

4. The tubes were pulse vortexed and 50 μl bacterial suspensions were spread-plate on Middlebrook 7H10 agar plate supplemented with 5 μg/ml TCH was used specifically to inhibit BCG growth

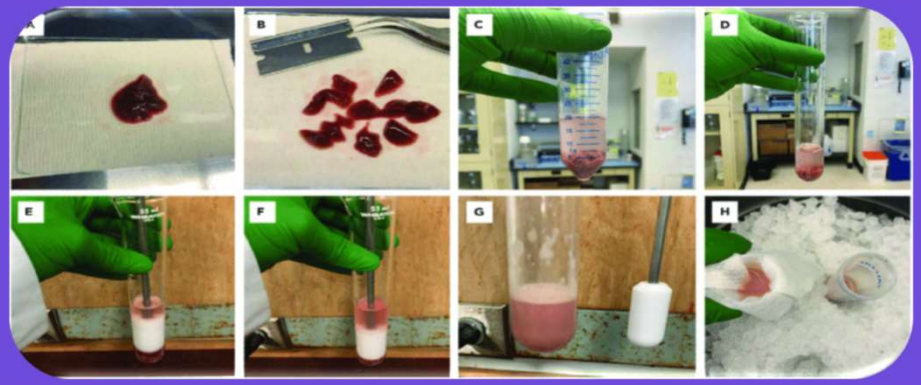
The bacterial load results of the 4th week





The bacterial load results of the 12th week

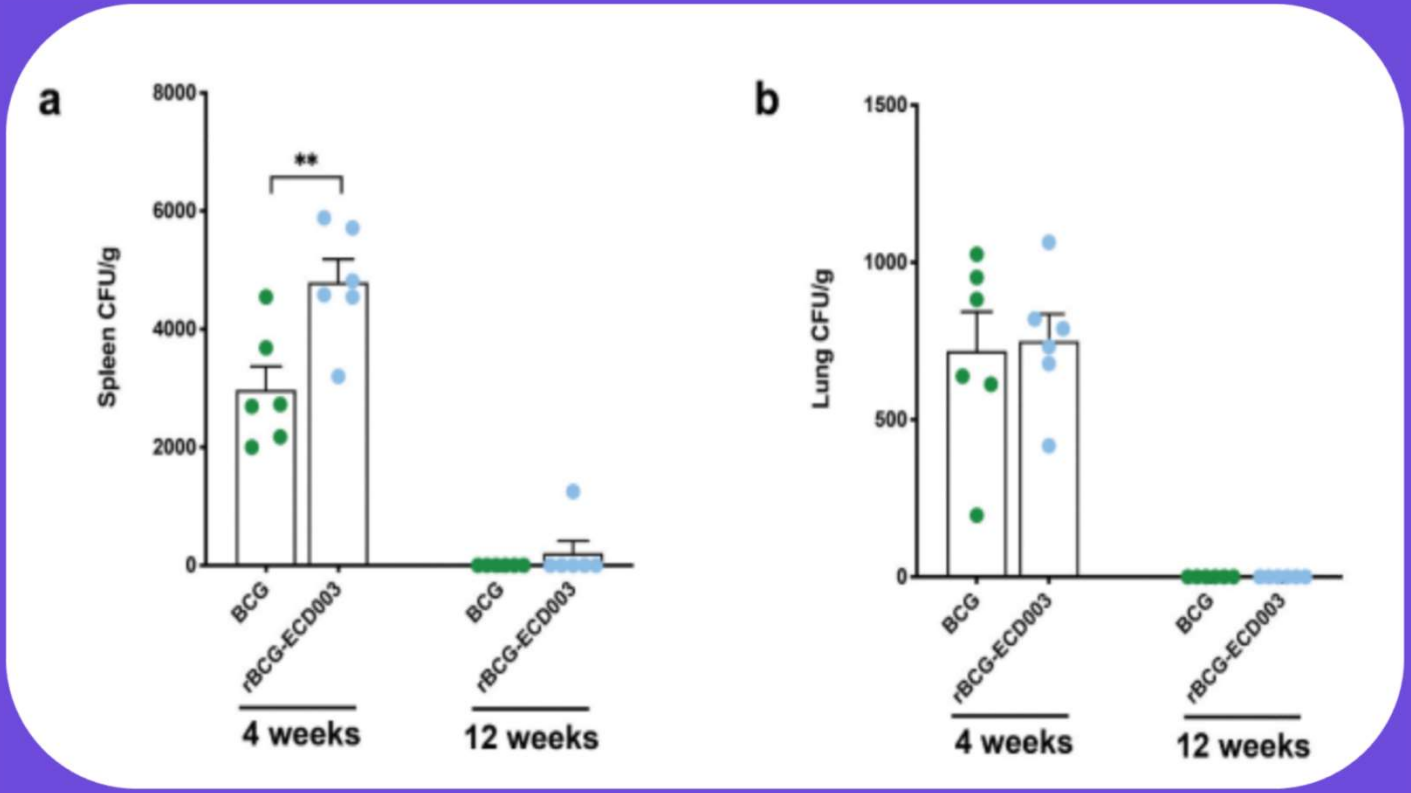
RECOMBINANT BCG SAFETY ASSAY



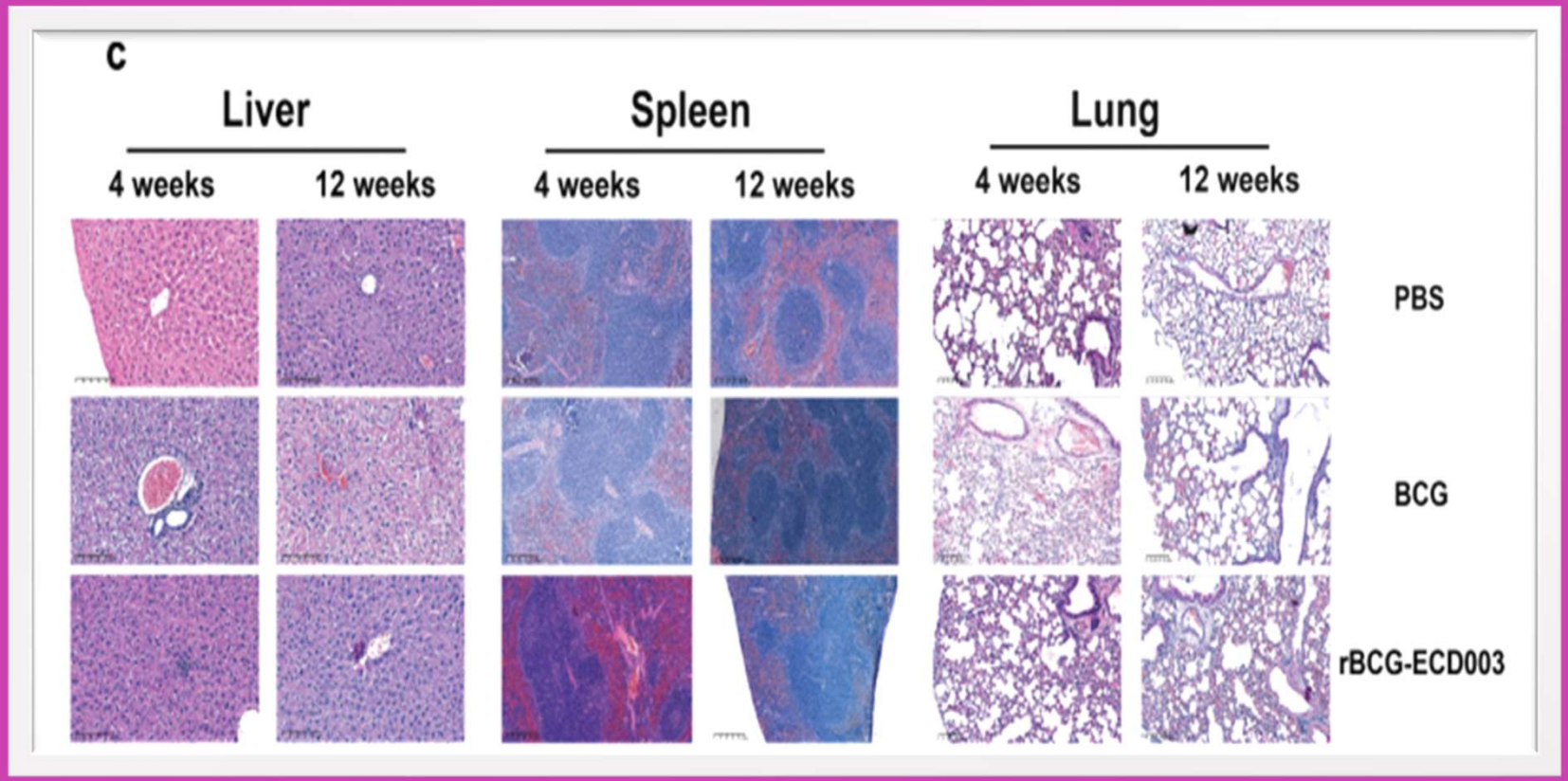
H&E stained

100 µl each sample onto Middlebrook 7H10 agar plate

The safety analysis of the rBCG-ECD003 vaccine after immunization



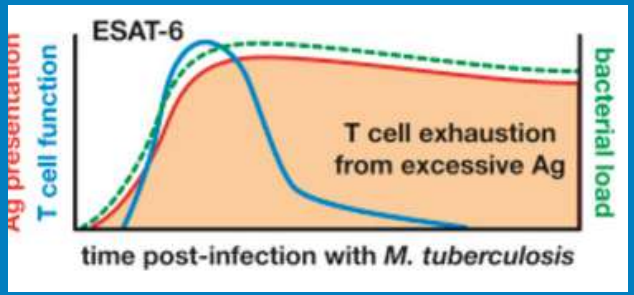
The typical pathological changes in each group



A subunit vaccine H56, which contains early antigens Ag85B, ESAT-6, and a latent antigen Rv2660c, has shown greater protective efficacy in the late stage of Mtb infection than Ag85B-ESAT-6 vaccine proven the superiority ofmultistage antigens strategy.(Aagaard C.2020)

It was observed that DnaK had increased abundance in a dormancy model that mimics Mtb latent infection state in vivo. (Trutneva KA.2020)

- The ESAT-6-specificCD4+ T appeared more differentiated and likely to undergo functional exhaustion, which may lead to loss of protective capacity in long term. (Barber DL-2017)



The rBCGECD003 vaccine also exhibits a greater capacity to control Mtb growth in vitro in the long term compared to the parental BCG, without obviously elevating virulence in mice. However, further testing is required to determine the exact level of protection provided by rBCG-ECD003 against Mtb infection in animal models.

HUMAN VACCINES & IMMUNOTHERAPEUTICS
2024, VOL. 20, NO. 1, 2299607
https://doi.org/10.1080/21645515.2023.2299607

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RESEARCH ARTICLE

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Recombinant BCG vaccine expressing multistage antigens of *Mycobacterium tuberculosis* provides long-term immunity against tuberculosis in BALB/c mice

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ABSTRACT

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) persistently kills nearly 1.5 million lives per year in the world, whereas the only licensed TB vaccine BCG exhibits unsatisfactory efficacy in adults. Taking BCG as a vehicle to express Mtb antigens is a promising way to enhance its efficacy against Mtb infection. In this study, the immune efficacy of recombination BCG (rBCG-ECD003) expressing specific antigens ESAT-6, CFP-10, and nDnaK was evaluated at different time points after immunizing BALB/c mice. The results revealed that rBCG-ECD003 induced multiple Th1 cytokine secretion including IFN-γ, TNF-α, IL-2, and IL-12 when compared to the parental BCG. Under the action of PPD or ECD003, rBCG-ECD003 immunization resulted in a significant increase in the proportion of IL-2$^+$ and IFN-γ$^+$IL-2$^+$ CD4$^+$T cells. Importantly, rBCG-ECD003 induced a stronger long-term humoral immune response without compromising the safety of the parental BCG vaccine. By means of the protective efficacy assay *in vitro*, rBCG-ECD003 showed a greater capacity to inhibit Mtb growth in the long term. Collectively, these features of rBCG-ECD003 indicate long-term protection and the promising effect of controlling Mtb infection.

ARTICLE HISTORY
Received 14 September 2023
Revised 7 December 2023
Accepted 22 December 2023

KEYWORDS
Tuberculosis; *Mycobacterium tuberculosis*; recombinant BCG vaccine; multicomponent fusion antigen; cytokine