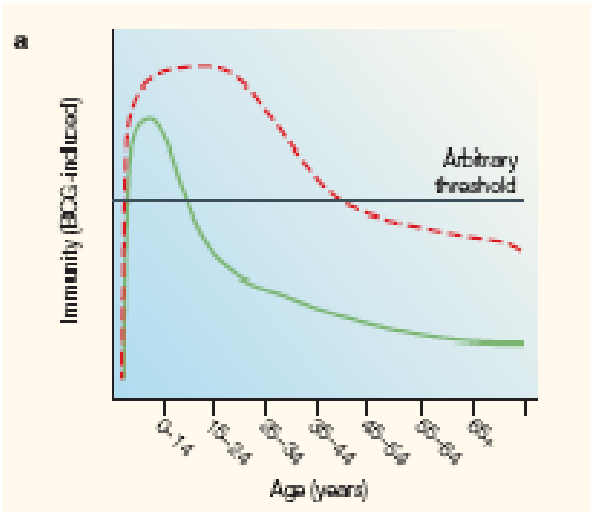
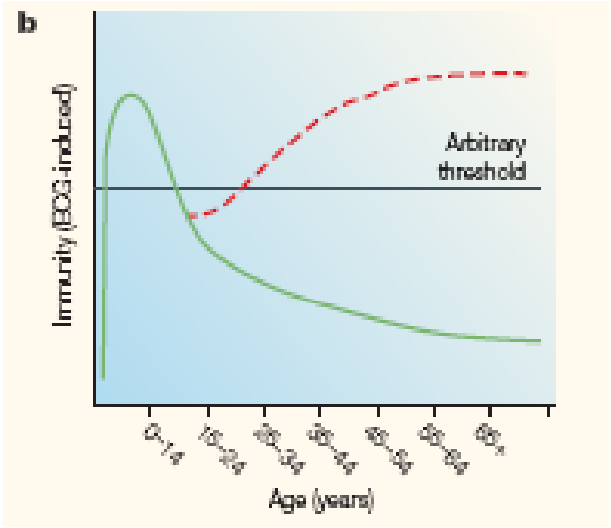
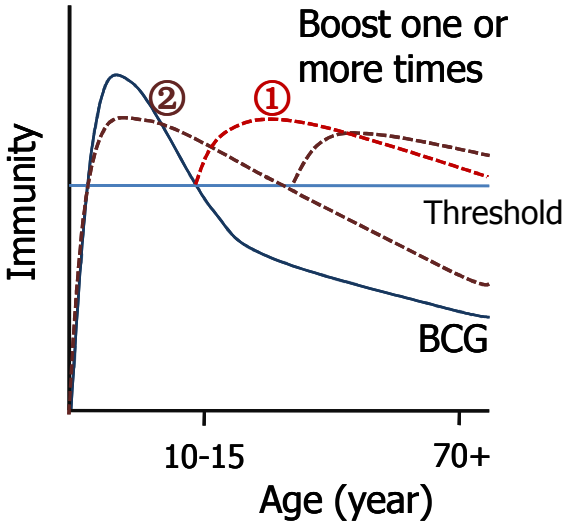
Tuberculosis Subunit Vaccine EAMMH and Vaccine-induced Immune Memory

Bingdong Zhu
Lanzhou University
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2014.09.26 Valencia

The strategies for tuberculosis vaccine development







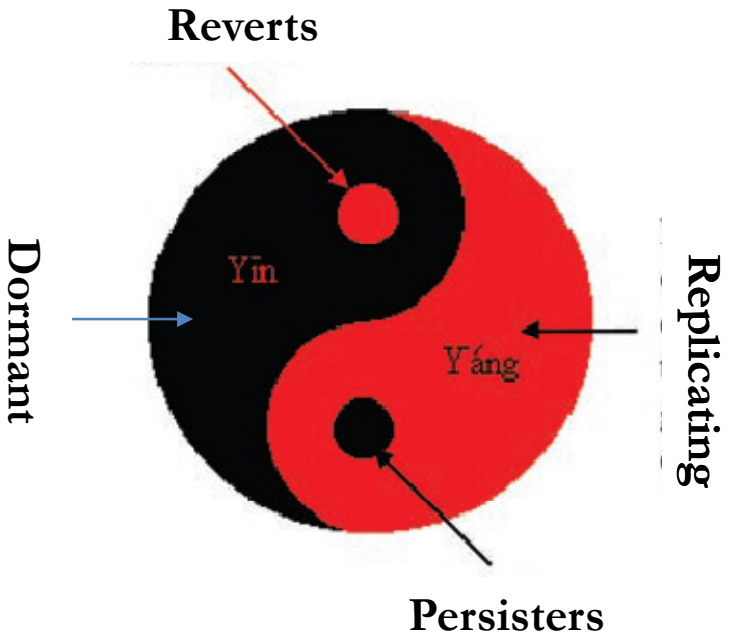
Replacement of BCG

Booster of BCG

Novel subunit vaccine

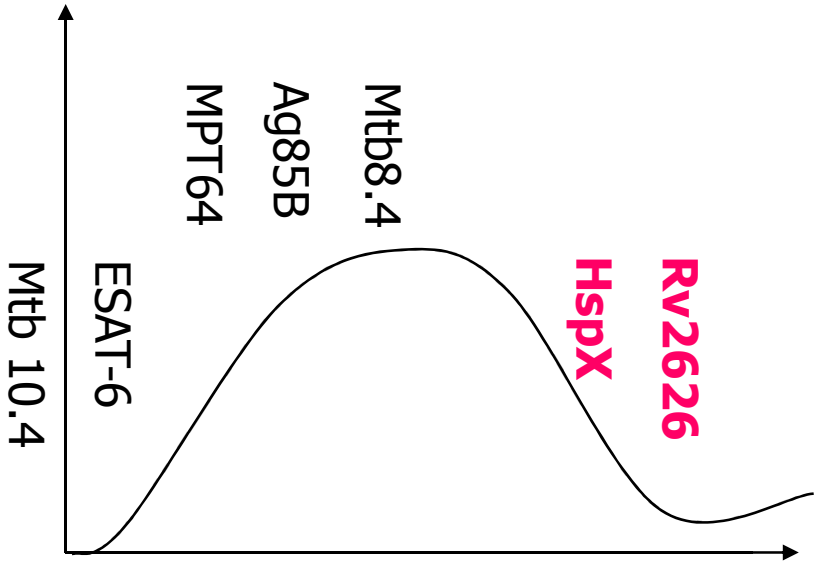
Andersen P, Nature reviews microbiology **2005**; 3:656-62.

The Yin-Yang model and antigens selection for TB vaccine



Yin-Yang Model

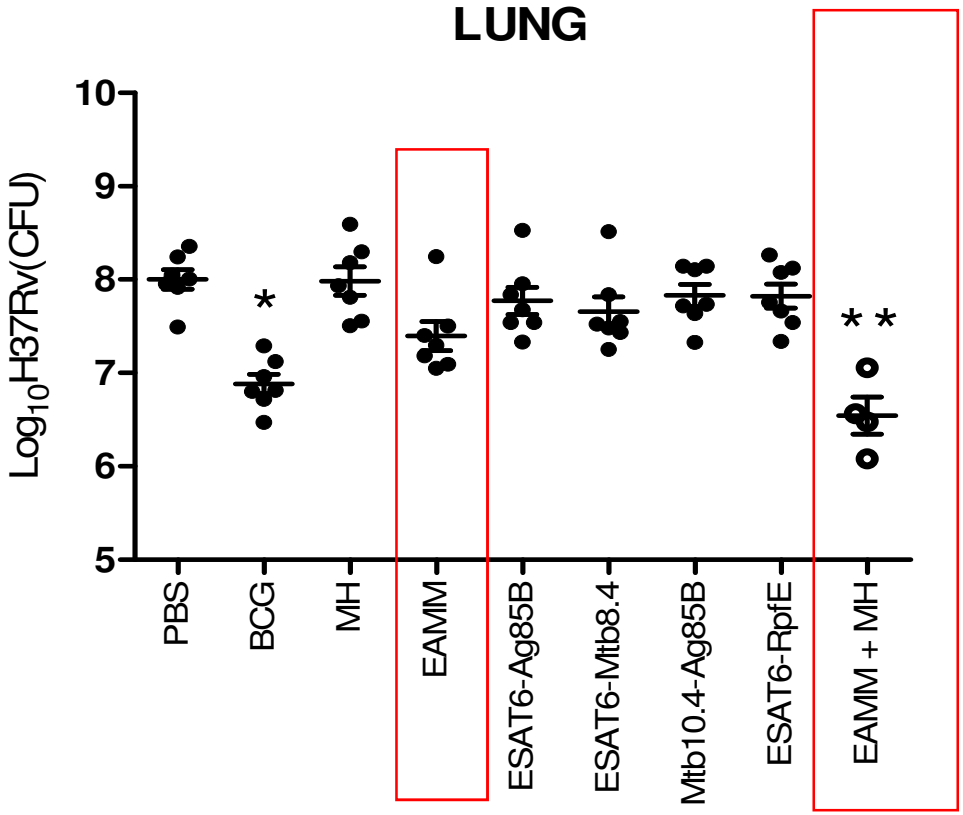
Ying Zhang, Clin Pharmacol Ther. 2007;82(5):595-600.



Resuscitation, Replication, Dormancy, Death of *M. tuberculosis*

The main fusion proteins constructed in our lab

- Ag85B-MPT64（190-198）-Mtb8.4 (AMM)
  (*Vaccine 2009*)
- ESAT6-AMM (EAMM)
(*PLoS One 2013*)
- Mtb10.4-HspX (MH)
(Vaccine 2011)



*$p < 0.05$ *vs.* PBS. ** $p < 0.05$ *vs.* PBS, EAMM and MH.

The novel multistage subunit vaccine candidate

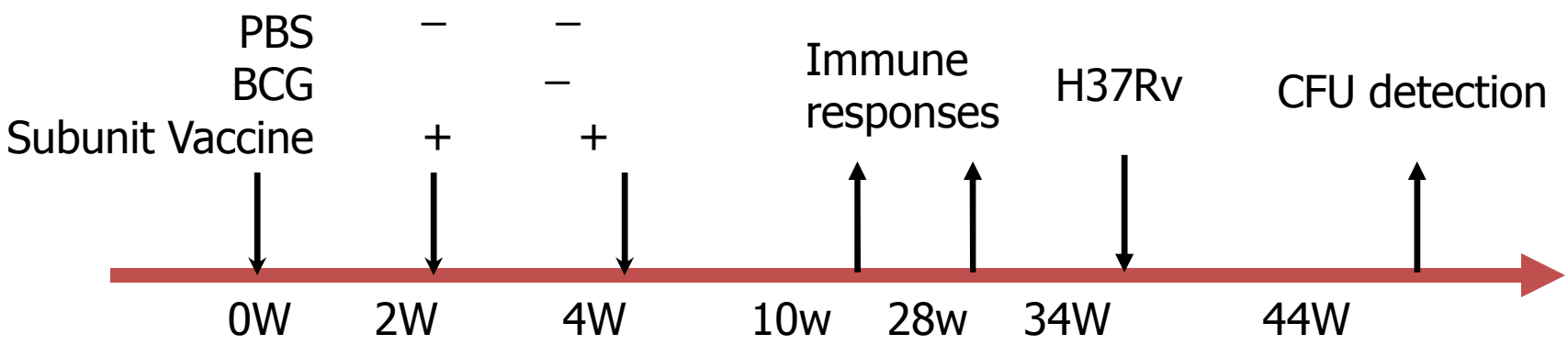
Fusion proteins:

–EAMM-HspX (EAMMH, LT69)

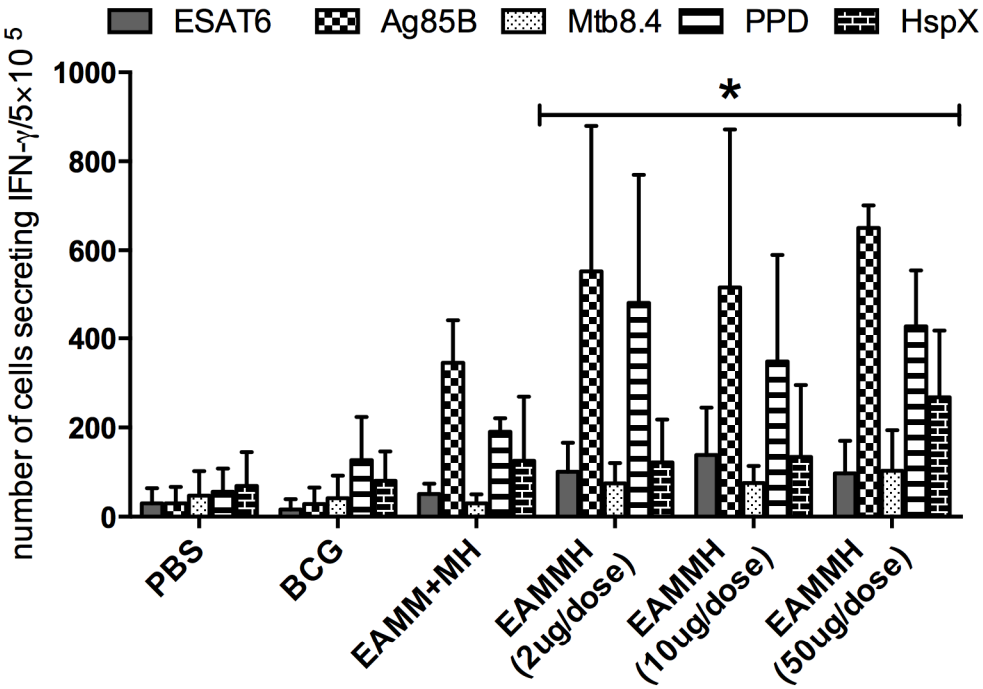
Adjuvant: DDA + Ploy I:C

The immunogenicity and protective efficiency of EAMMH

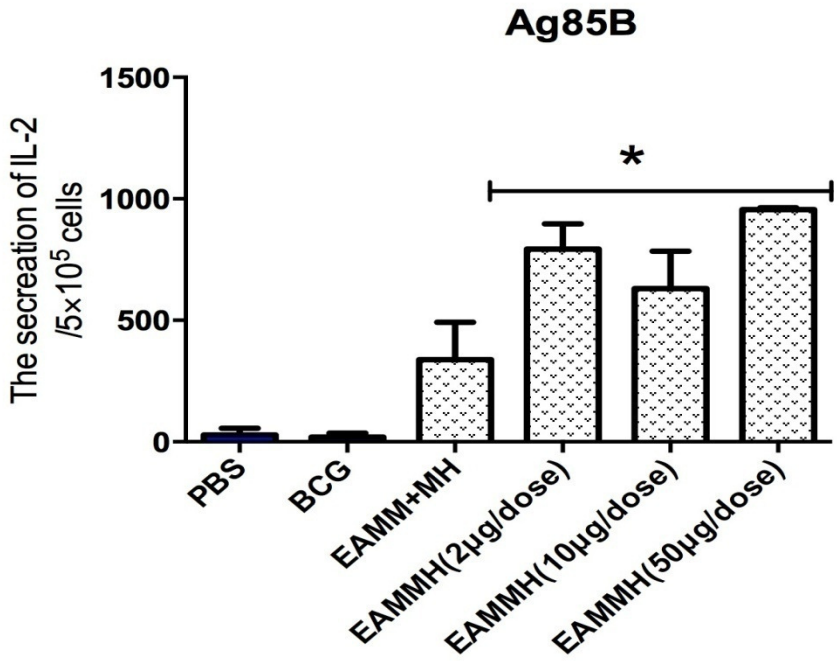
The schedule of immunization and challenge



The immunogenicity of EAMMH



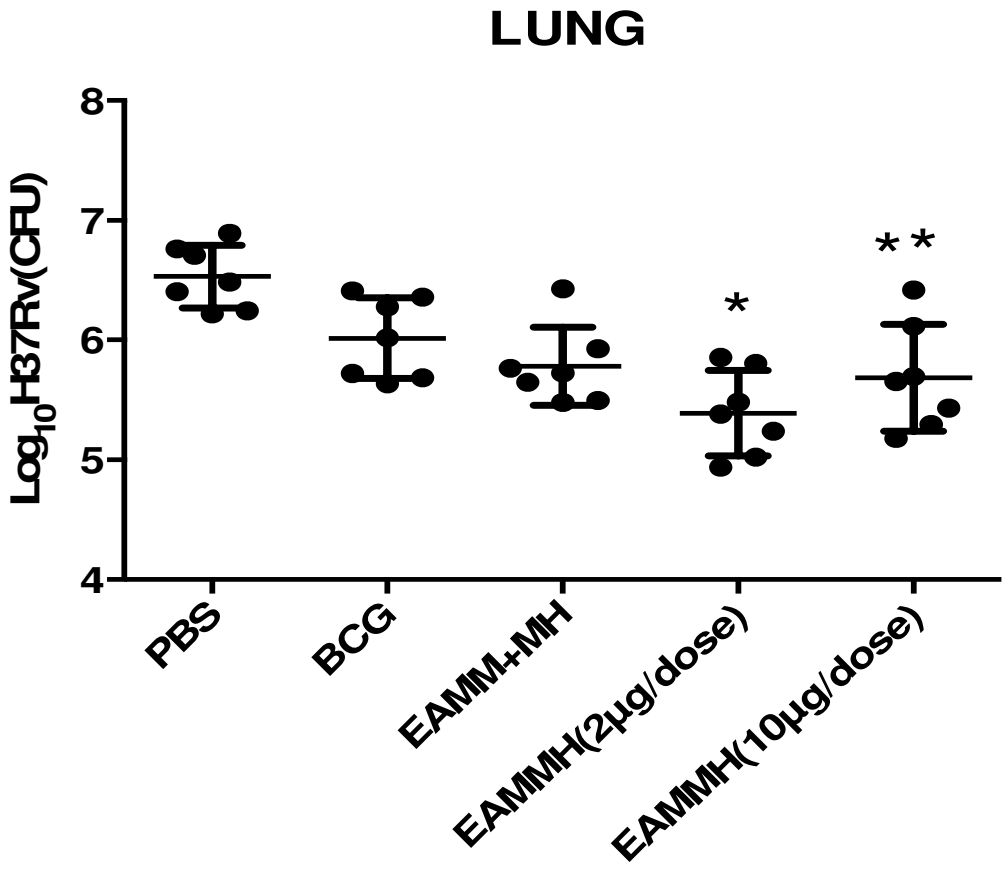
IFN-γ production with the stimulation of special antigens *in vivo*



* $p < 0.05$ *vs.* PBS，BCG and EAMM+MH groups

IL-2 production with the Ag85B stimulation *in vivo*

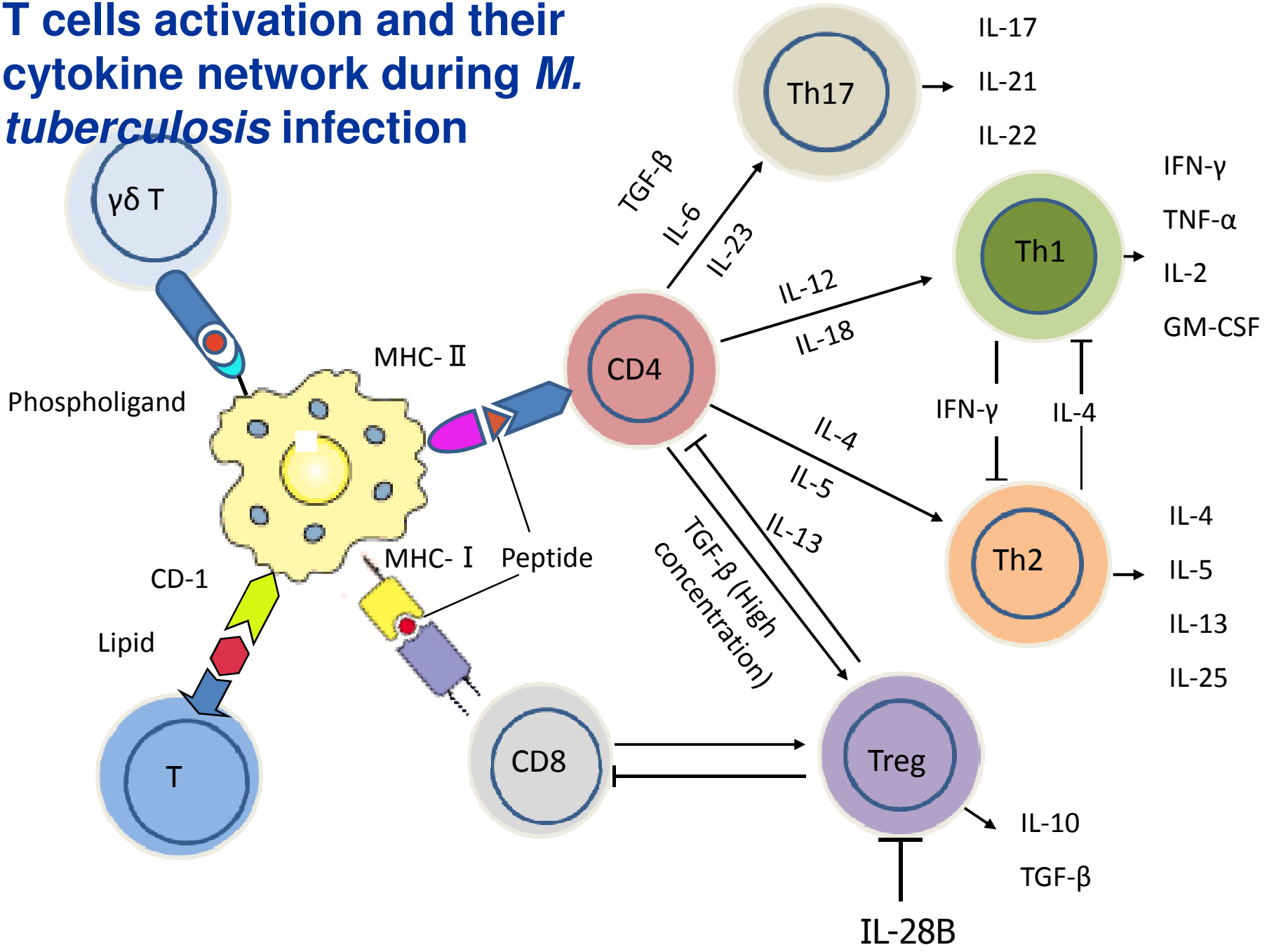
The protective efficacy of EAMMH



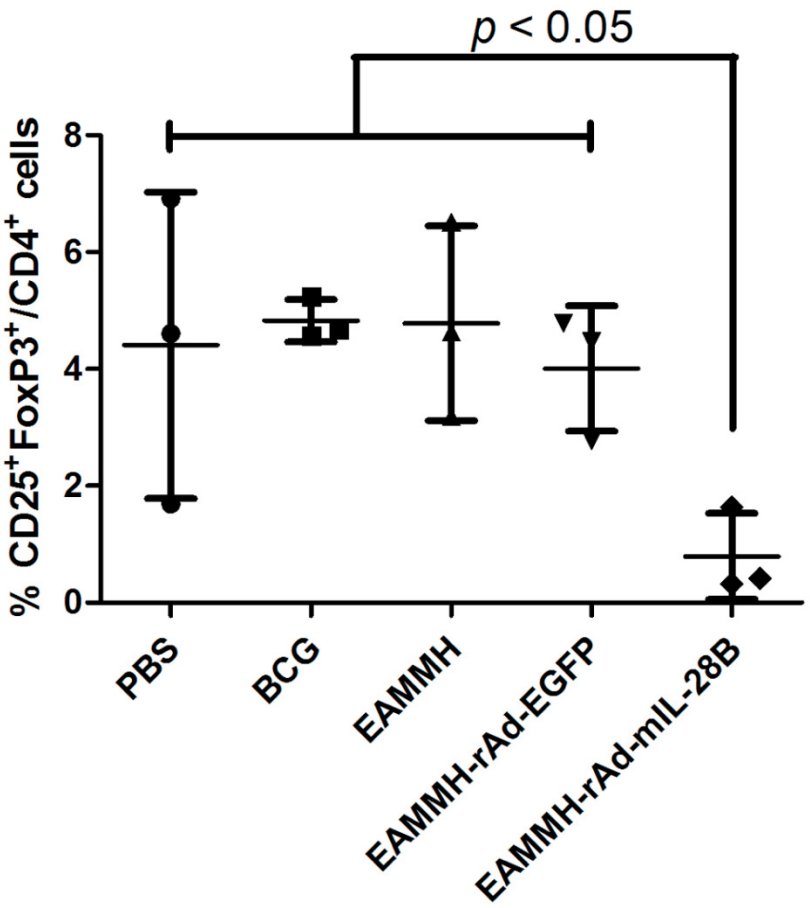
* $p < 0.05$ *vs.* PBS，BCG and EAMM+MH groups;
** $p < 0.05$ *vs.* PBS and BCG groups.

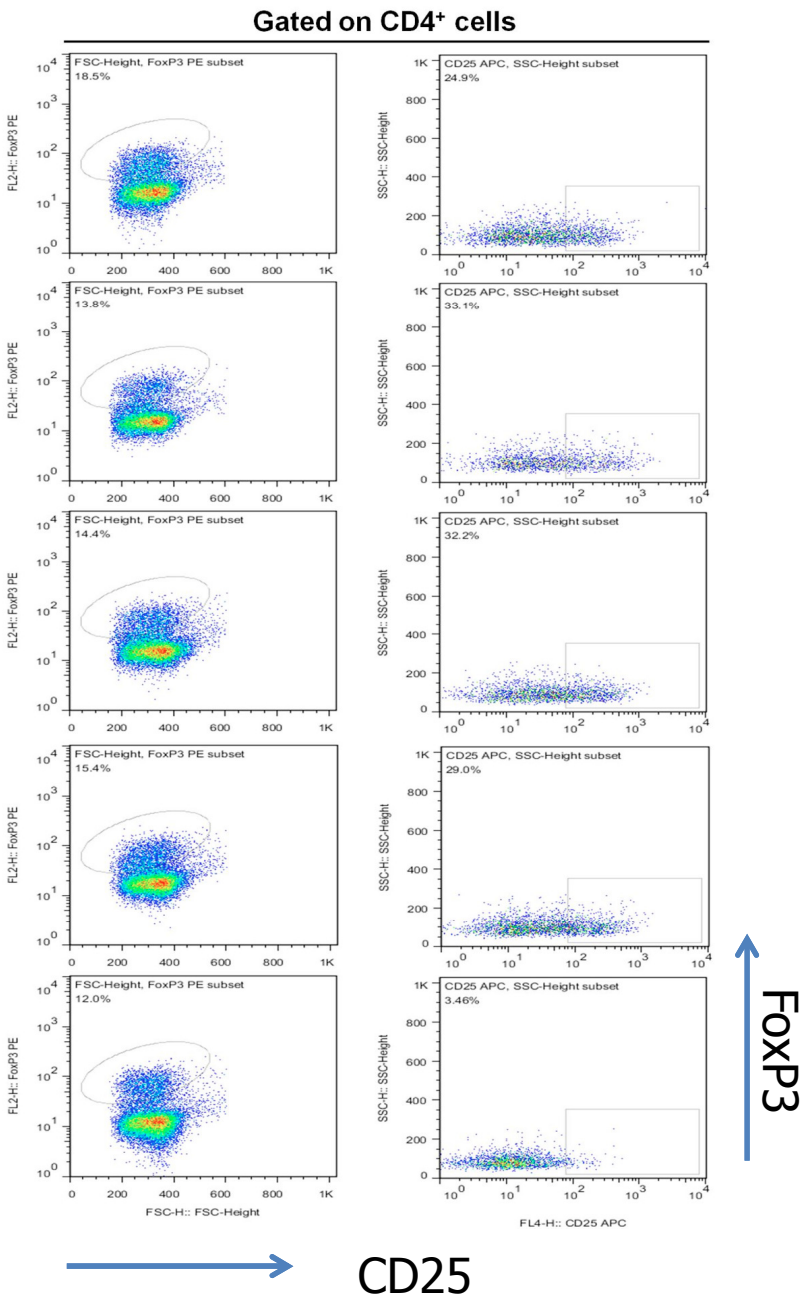
Regulation the immune responses induced by EAMMH

T cells activation and their cytokine network during *M. tuberculosis* infection

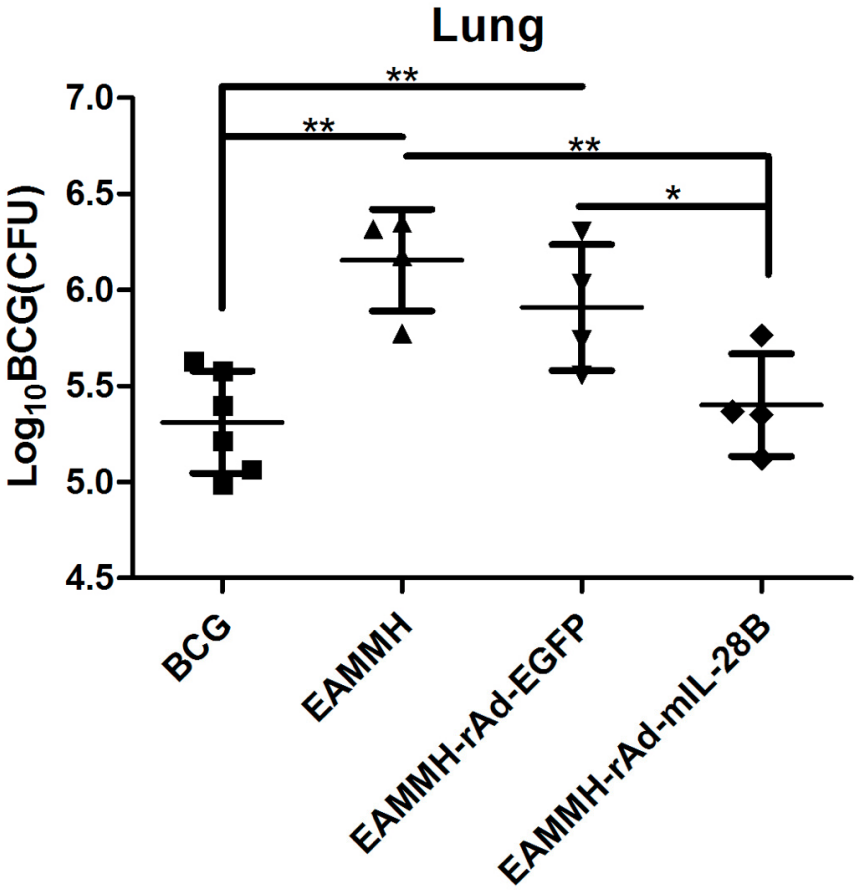


IL-28B down-regulated Tregs following vaccination with EAMMH





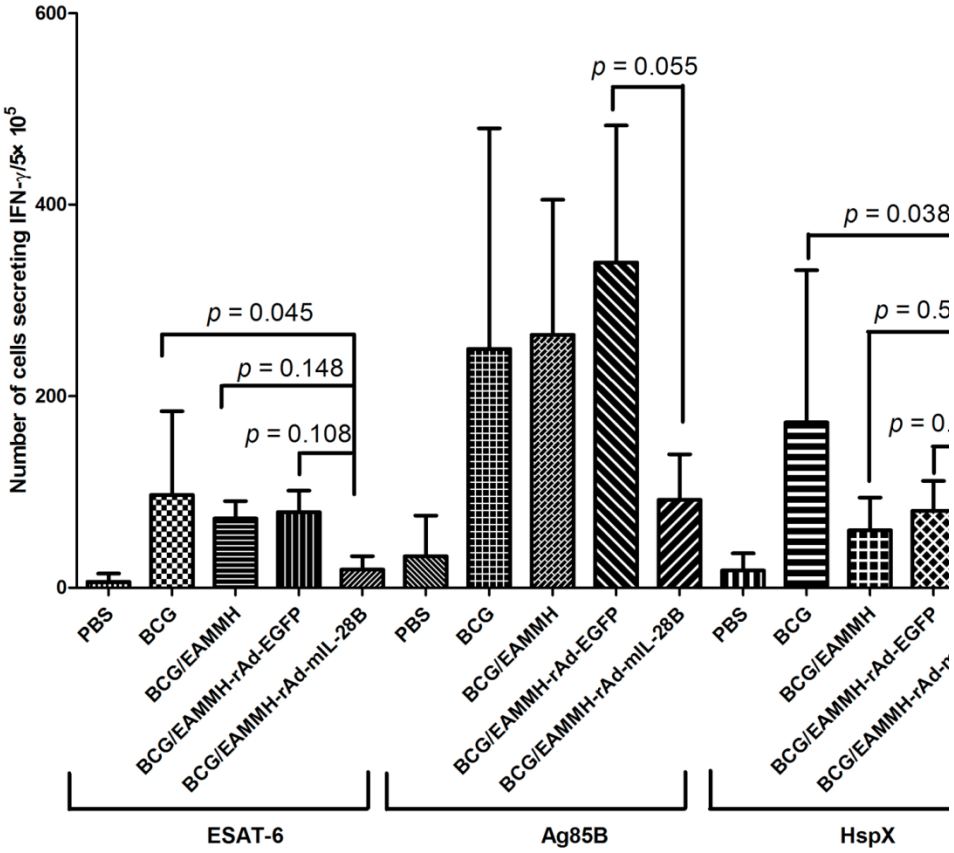
IL-28B enhanced short-term protective efficacy induced by the EAMMH vaccination

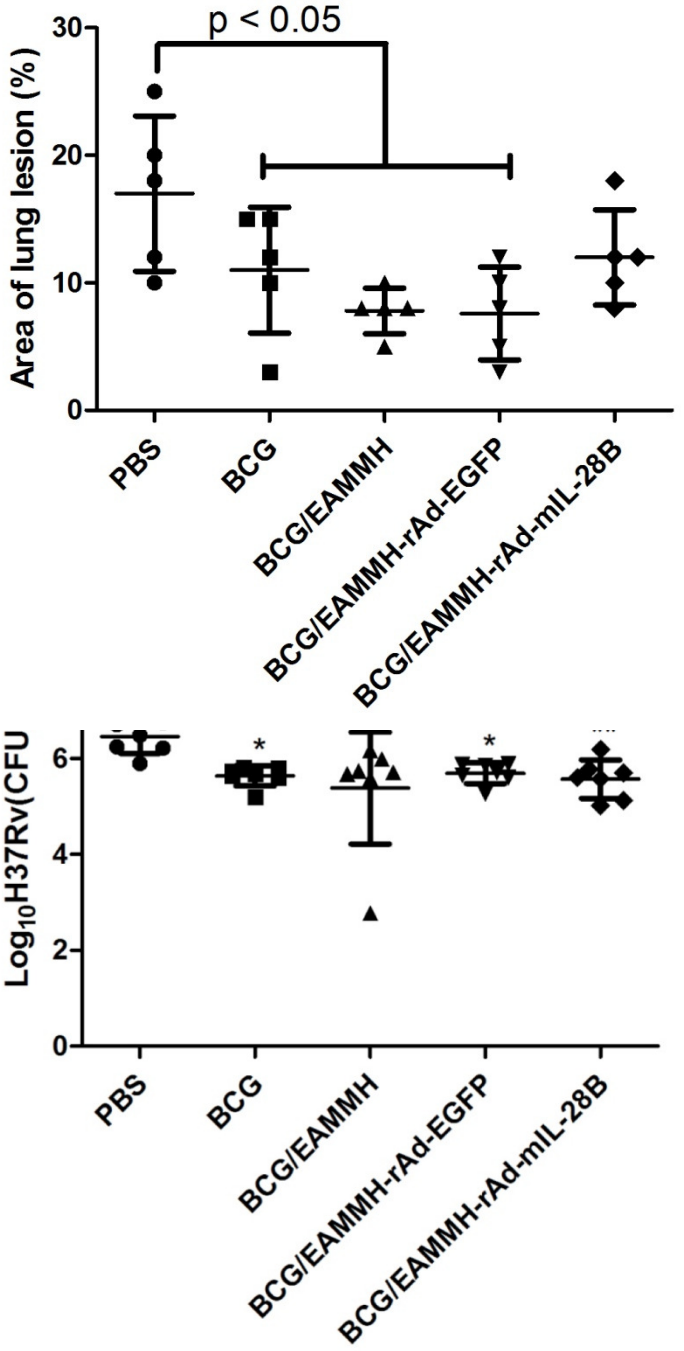




IL-28B impaired long-term antigen-specific immune memory





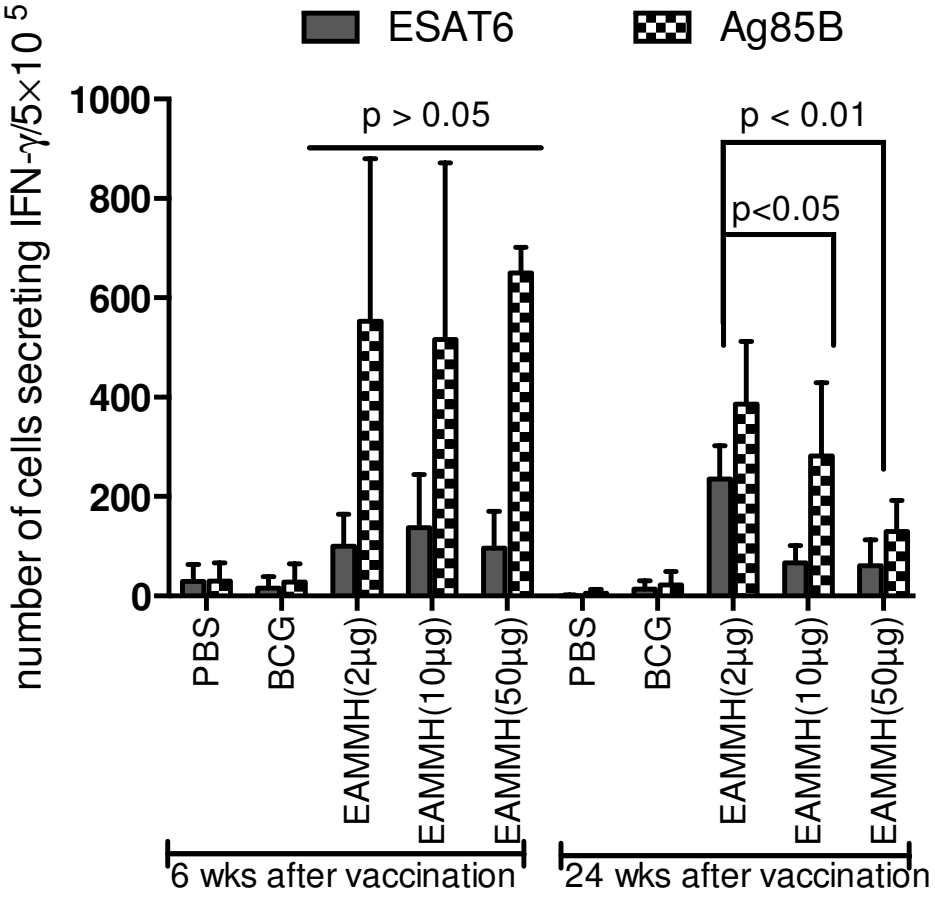


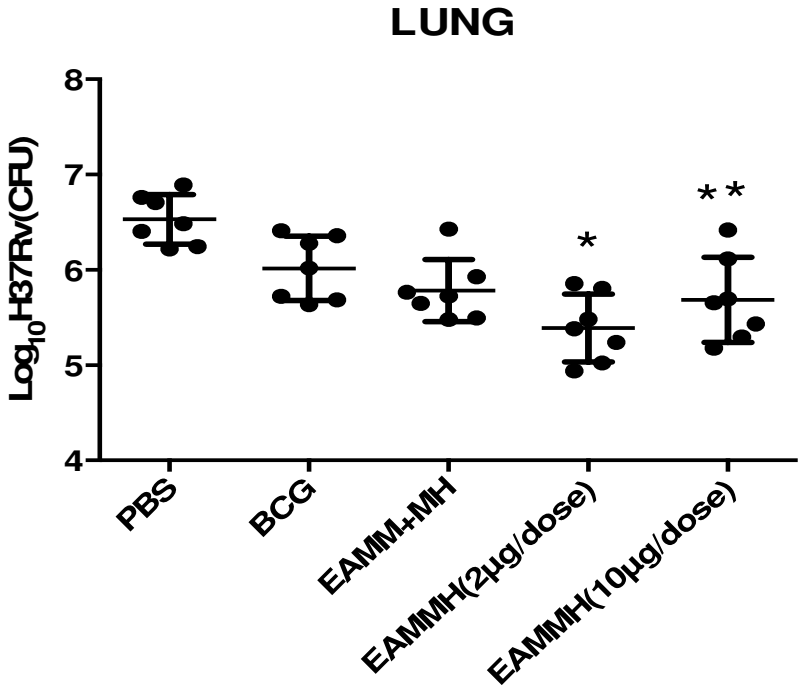
Factors that affect the immune memory induced by EAMMH

– Antigen dose

– Rapamycin

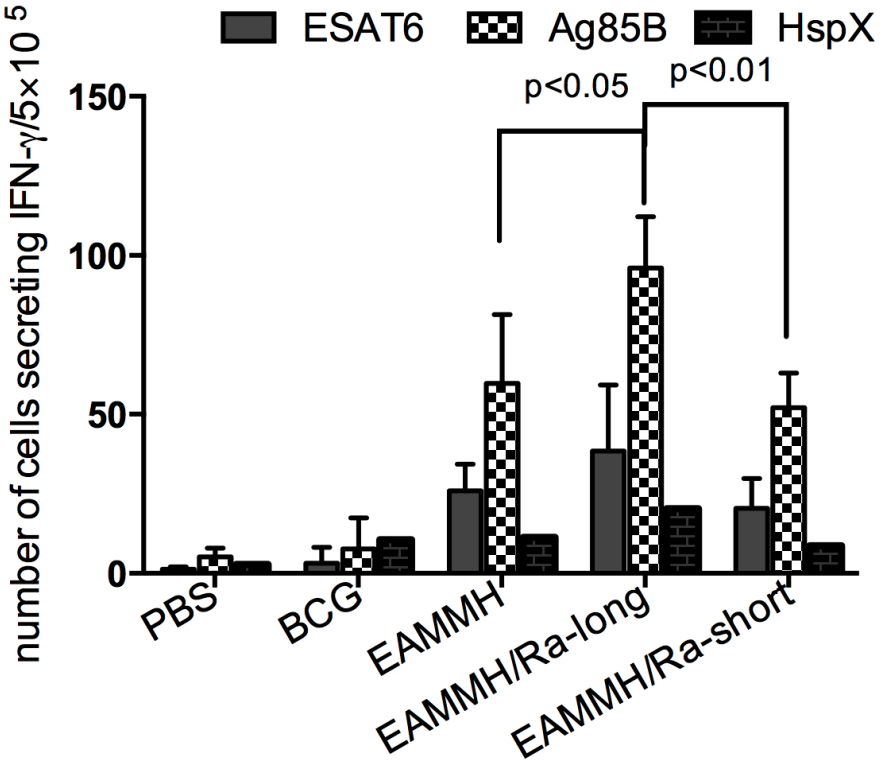
Low dose of antigen generated long-lived cellular immune responses



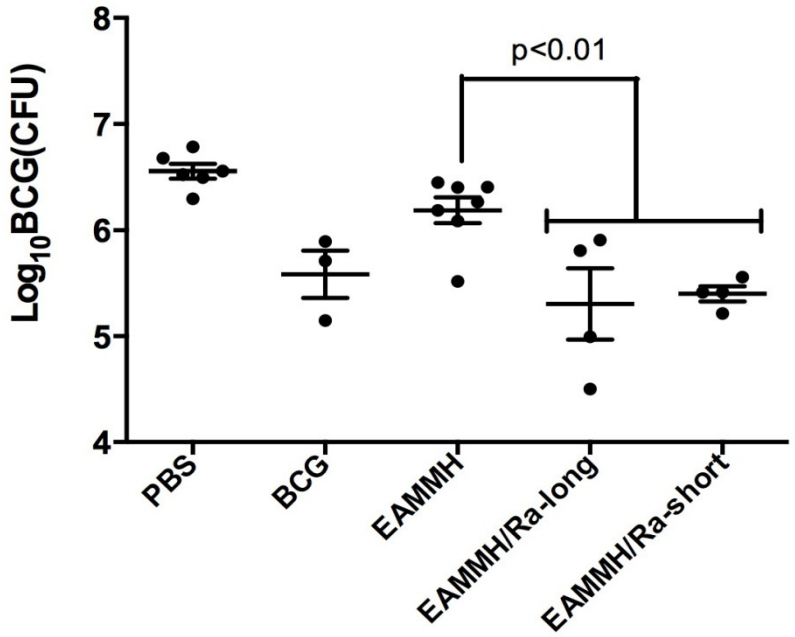


* $p < 0.05$ *vs.* PBS，BCG and EAMM+MH groups;
** $p < 0.05$ *vs.* PBS and BCG groups.

Rapamycin treatments increased the immune responses and protective efficacy

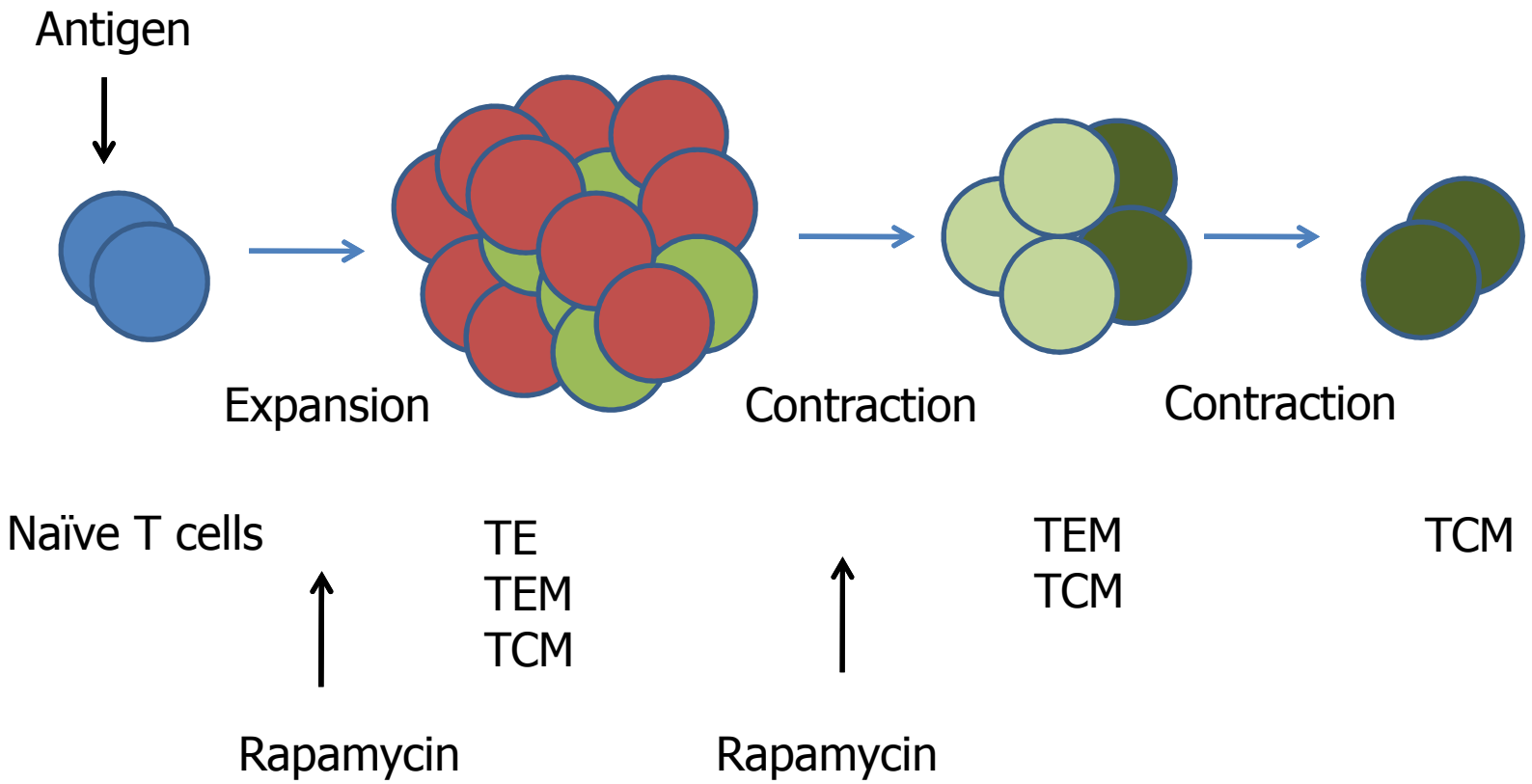


The antigen specific IFN-γ expression 24 wk after the last immunization



Mice were challenged with BCG by tail vein 30 wk after last immunization

Vaccine induction of memory T cells



- **More antigens stimulate more effector T cells and less memory T cells**
- **Down-regulating Tregs induces more effector T cells, less memory T cells**

Conclusion

- Subunit vaccine EAMMH induced long-term T cell immune responses in mice, and its protective efficacy against *M. tuberculosis* was even higher than traditional vaccine BCG.
- Down-regulation of regulatory T cells does not benefit to long-term immunity.
- Low-dose EAMMH had the benefit to generate long-term immune memory.
- The EAMMH-induced immune memory could be enhanced by additional treatment with rapamycin during vaccination.

Acknowledgement

Lanzhou University

Prof. JING Tao Dr. MA Xingming
YU Hongjuan Dr. TANG Jiying
Dr. LI Qin Dr. LUO Yu
NIU Hongxia LUO Yanping
LIU Xun SONG Nannan DA Zejiao
XIN Qi JIANG Wenwen PENG Jinxiu
LI Zhi ZHANG Guoping LIU Wanbo
WANG Yue BAI Chunxiang CHEN Huiyu
SUN Hongjia TANG Kefeng BAI Chunxiang

Lanzhou Institute of Biological Products

Dr. WANG Bingxiang
HU Lina

Gansu Province CDC

Lanzhou Lung Hospital

Fudan University

Prof. WANG Honghai

Johns Hopkins University

Prof. Ying Zhang

MIT

Prof Jianzhu Chen

ABSL-3 of Wuhan University

AERAS

Funding

863 Project of China
National Major S&T Projects of China
NSFC of China