Vaccine-Mediated Protection in Genetically Diverse Collaborative Cross - A Model for Pre-Clinical Vaccine Testing

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Introduction

Vaccine development against TB has been hindered in part due to a lack of immune correlates that can be used to identify protective vaccine candidates. Although current mouse models have identified numerous immunological parameters that are associated with protective phenotypes, the lack of genetic diversity in traditional inbred lines makes it unclear if these correlates can be translated to diverse genetic backgrounds. We have elected to take advantage of a novel system that is able to capture this genetic diversity – known as the Collaborative Cross – in order to better understand the contribution of host genetics to both primary and vaccine-induced immunity against *Mtb*. Using this model, we have gained insights into the relative contribution of both adaptive and innate immunity in vaccine induced protection against TB across genetically diverse hosts.

BCG protective efficacy is dissociated with host susceptibility to TB

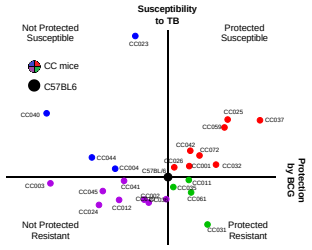

Figure 1 – BCG efficacy in CC mice is genetically dissociated from susceptibility to TB.

Experimental design

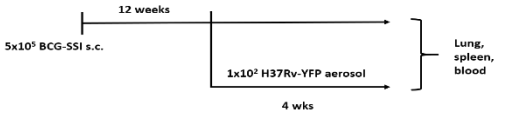

Figure 2 – Experimental schema for examining BCG-induced protection in CC mice.

Results

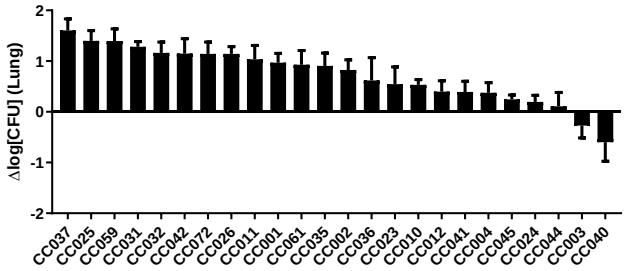

Figure 3 – Heterogeneity in the protective efficacy of BCG against pulmonary Mtb in CC mice. CC mice were immunized with BCG-SSI and rested for 12 weeks, after which they were infected with *Mtb* expressing YFP (*Mtb*-YFP). Bacterial burden was assessed in the lung at 4wks post infection, and the reduction of *Mtb* load in the lungs of BCG immunized mice compared to PBS control was examined.

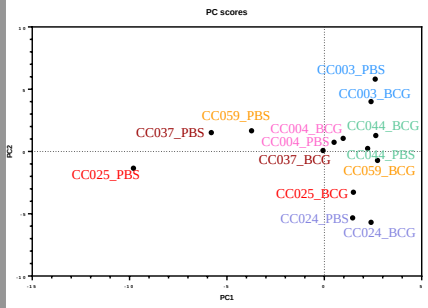

Figure 4 – BCG vaccination alters immunological profile in protected CC mice but not in non-protected CC mice. Principal Component Analysis (PCA) of the T cell responses from select protected and non-protected CC lines. Strains that are not protected by BCG are more similar immunologically.

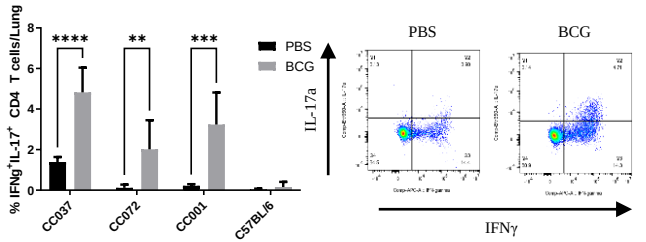

Figure 5 – Th1/17 cells emerge as an immune signature in protected CC mice. CC mice were immunized with BCG-SSI and rested for 12 weeks, after which they were infected with *Mtb*-YFP. Lung cells were isolated at 4wks pot infection and stimulated with p300 peptide pool for 5hrs. IFNγ and IL-17a responses were measured by intracellular cytokine staining.

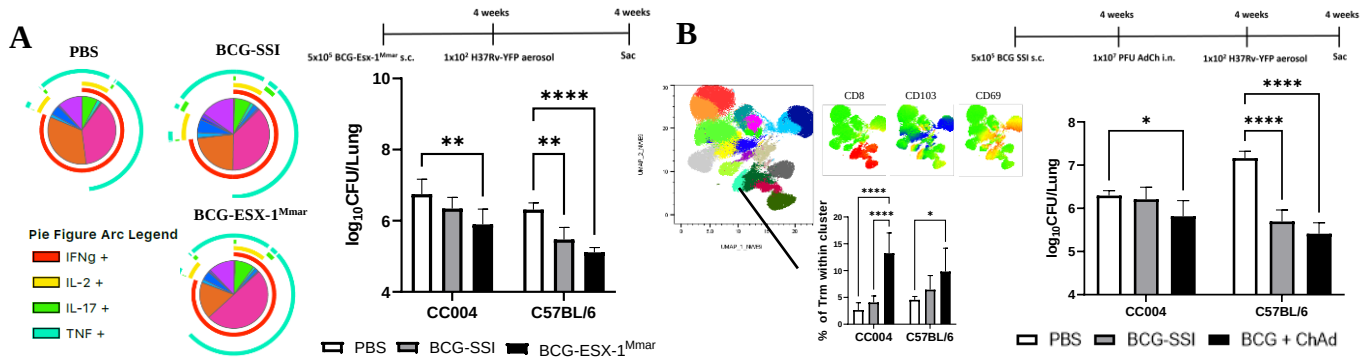

Figure 6 – Non-protected CC mice can be protected using alternate vaccine formulations. A) CC004 and C57BL/6 were vaccinated with either BCG or A) BCG-ESX-1^{Mmar} B) chimp Adenoviral vector vaccine (ChAd) as indicated. Mice were rested for minimum of 4wks, after which they were infected with *Mtb*-YFP. At 4wks post infection, bacterial burden and cellular responses were assessed at the lung via flow cytometry. Pie charts in **A** were created using SPICE software. UMAP projection and phenograph clustering in **B** were performed in FlowJo Software.

Future directions

- Investigate the role of Th1/17 responses as an immune correlate of protection following BCG vaccination
- Investigate the mechanism by which genetic backgrounds that are refractory to BCG vaccination are protected by alternate vaccines

Collaborative Cross as a model for preclinical vaccine testing?

Recombinant BCG	Viral vector	Subunit
1. **BCG ΔureC::hly** – Kaufmann Max Planck Institute for Infection Biology, Germany 2. **BCG ESX-1 *marinum*** – Brosch Institut Pasteur, France	1. **AdCh68Ag85A:rpfB:TB10.4** - Xing, McMaster University, Canada	1. **H74** – Andersen SSI, Denmark

Figure 7 – Current candidate vaccines that are being tested in CC mice.

Summary

- A diverse range of protection is observed in CC mice following BCG vaccination, which is dissociated with the susceptibility of the host to TB
- Th1/17 cells emerge as a immune signature in a subset of protected CC mice
- CC mice that are refractory to BCG are protected by alternate vaccination strategies

Reference

1. Smith et al, 2016. *mBio*. 7(5). pii: e01516-16
2. Smith, Proulx, Lai et al, 2019. *mBio*. 10(6). pii: e02791-19.

Authors have no conflicts of interest to disclose

This research is funded by a P01 grant (AI132130) from the National Institute of Health (NIH)