The ability of Heat shock protein Complex (HspC) vaccines to boost the immune response to BCG vaccination in a murine challenge model of *Mycobacterium tuberculosis* infection

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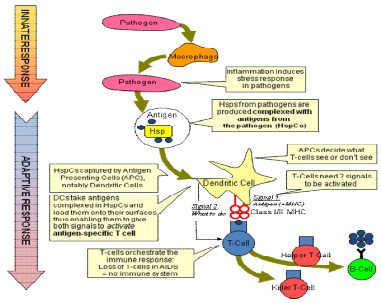
Introduction

Although a vaccine exists for the prevention of tuberculosis (TB) and antibiotics to treat *Mycobacterium tuberculosis* (*M.tb*) infections have been widely available for over half a century, TB remains endemic in developing countries and there is a continued threat of TB epidemics in developed countries. Whilst a new vaccine to replace BCG is essential, boosting the immunity of those already vaccinated with BCG is also a necessity. Here we show that a booster vaccine comprised of BCG-derived heat shock protein/peptide complexes enhances the immune response in BCG-vaccinated mice.

Microbial heat shock proteins (Hsps) have been implicated in the induction of both the innate and adaptive arms of the immune response. Hsps can bind to peptides, forming Hsp-peptide complexes (HspCs), and chaperone these peptides into the antigen processing/presentation pathways of professional antigen-presenting cells (APCs) such as dendritic cells (DCs). Extracellular peptides which are usually presented on MHC class II molecules, can be processed by APCs and displayed on MHC class I molecules and this cross-presentation activity is primarily found in DCs. This property of DCs could therefore allow mycobacterial-specific antigens carried on HspCs to stimulate both CD4 and CD8 T cells, potentially resulting in the production of protective responses against subsequent *M.tb* infection.

This study was performed to test the efficacy of using HspC vaccines as a boost to BCG vaccination and to identify *in vitro* correlates of *in vivo* protection. The immunogenicity of the HspC vaccines *in vivo* was measured *in vitro* by the detection of cytokines secreted by primed splenocytes in response to restimulation with *M.tb* or control antigens and the analysis of the antibody response in sera.

HspC ™ technology



Development Status

ImmunoBiology Ltd ("ImmBio") has a co-development agreement with Aeras Global TB Vaccine Foundation and is funded by the Bill & Melinda Gates Foundation.

The cell substrate for the T-BioVax™ vaccine is BCG, with cells produced by fermentation at the Aeras facility in Rockville MD, US. After fermentation of BCG and a heat induction step, the downstream process, developed by ImmBio, is centred on ion exchange chromatography. Assays and characterisation studies have been critical in establishing a stable, high-yield, reproducible process within a defined product specification. Two process patents were filed in 2008 and 2009.

Conclusions

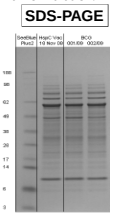
- Both the BCG and BCG/HspC boost vaccinations induced significant protection against challenge with *M.tb*, as measured by reduction in lung colony counts compared to the negative control.
- The data from this study show that when the HspC vaccines were used to boost immunity to BCG, increased levels of IFN-γ were detected, in splenocytes following restimulation *in vitro* with *M.tb* antigens, compared with the BCG only vaccinated group.
- Flow cytometry showed that in the BCG/HspC boost group the percentage of CD4+ multifunctional cells was higher than in the BCG only vaccinated group.
- Higher IgG1 and IgG2a antibody titres were detected in the sera after HspC booster vaccinations compared to titres seen in mice vaccinated with BCG alone.

In conclusion, these data suggest that T-BioVax™ vaccination can be an effective boost to BCG and may generate a more balanced Th1/Th2 immune response.

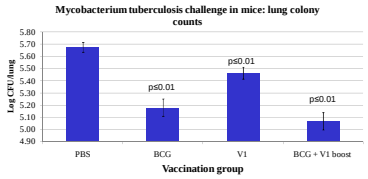
Methods & Results

Protection Against *M. tb* Challenge

Three batches of vaccine were produced using a standard downstream process (V1-3), which demonstrated good process robustness. This can be seen below in the stained SDS-PAGE profile and table of analytical results. Vaccines varied only in their final formulation.



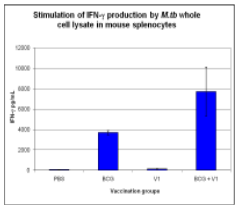
Test	Method	HspC 18Nov08 V1	BCG/ 002/09 V2	BCG/ 001/09 V3
Endotoxin (LAL) (EU/mg)	EndoSafe PTS	5-3	<0.2	<0.2
DNA (µg/mg)	Fluorometric dsDNA Quantitation	0.6	0.2	0.1
Protein Conc. (mg/mL)	BCA	2.5	3-7	3-7
Hsp 71 (µg/mg)	ELISA	2.3	1.6	1.4



HSP-5-2009

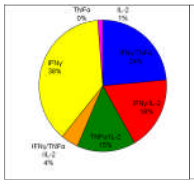
This *M. tb* aerosol challenge study was conducted at NIBSC. Groups (n=8) of Balb/c mice were vaccinated twice, 4 weeks apart, with or without a BCG prime, followed by challenge at week 12 and sacrifice at week 16. All vaccination groups showed a significant reduction in lung colony counts when compared to the PBS control.

Cytokine responses

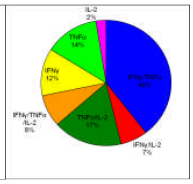


Vaccinated, unchallenged mice, in study HSP-5-2009 were analysed for cellular immunity.
Cytokines secreted from *in-vitro* primed splenocytes, in response to *M.tb* whole cell lysate, were measured by ELISA (R&D Systems).

BCG



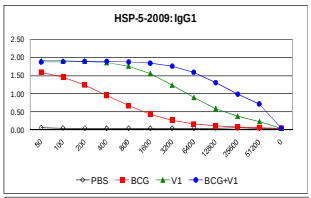
BCG + V1

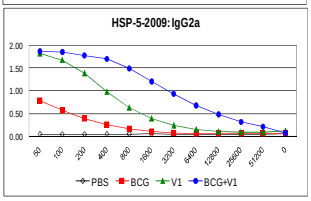


Further analysis was performed by intracellular cytokine staining of CD4+ cells. Multifunctional CD4+ cells have been shown to be of interest as a marker for vaccine efficacy. The data show that compared to the BGC vaccinated group, the group vaccinated with BCG and boosted with HspC vaccine showed an increase in the percentage of CD4+ cells expressing TNFα only, IL-2 only, IFN-γ/TNFα, TNFα/IL-2 and IFN-γ/TNFα/IL-2 with a decrease in the percentage of CD4+ cells expressing IFN-γ only and IFN-γ/IL-2.

Antibody responses

Sera from mice in study HSP-5-2009 were also analysed for antibody levels by titration of samples in an ELISA.
The isotypes were detected using anti-mouse IgG1 HRP or anti-mouse IgG2a HRP (both Jackson ImmunoResearch) followed by TMB substrate (Sigma).





Antibody responses to the vaccines were detected in the sera. Higher IgG1 and IgG2a antibody titres were detected in the sera of mice vaccinated with BCG followed by HspC (V1) booster vaccination compared to titres seen in mice vaccinated with BCG alone.