Characterisation of a novel heat shock protein-enriched multivalent *Streptococcus pneumoniae* protein antigen vaccine


UCL Respiratory, Rayne Building, 5 University Street, London, UK
ImmunoBiology Ltd., Babraham Research Campus, Cambridge, UK

**INTRODUCTION**

*Streptococcus pneumoniae* is the leading cause of community-acquired pneumonia which causes up to a million deaths a year in children worldwide. There are over 90 different serotypes of the vaccine which vary in virulence and geographical prevalence.

**Current vaccines**

There are two pneumococcal vaccines available in the UK:

1. Pneumococcal polysaccharide vaccine (PPV) – a mixture of capsular polysaccharides from 23 serotypes
2. Pneumococcal conjugate vaccine (PCV) – 7 or 13 capsular polysaccharides conjugated to a carrier protein

**Need for a new vaccine**

- Serotype replacement – the emergence of non-vaccine serotypes in carriage and disease
- Lack of geographic coverage
- Cost of production

**Heat shock proteins and PnuBioVax**

ImmuBiology (ImmBio) are developing a new vaccine, based on the over-expression of heat shock proteins (Hsps) and multiple protein antigens (Figure 1.).

**Functions of Hsps**:

- Conserved *S. pneumoniae* proteins will protect against all serotypes
- As chaperones they ensure correct folding and degradation of misfolded proteins, forming complexes with these proteins
- Present these complexed proteins to antigen presenting cells (APCs) triggering the adaptive immune response
- Also triggers innate responses such as the release of cytokines and activate killer T cells and NK cells

**AIM**

The aim of this study is to produce a *S. pneumoniae* vaccine that is enriched with heat shock proteins, to show that it is protective and able to induce cross-reactive antibodies and finally, to identify key antigens in the vaccine.

**Vaccine production**

Western blotting of the vaccine showed that the preparation was enriched in Hsp60 and Hsp70. Other key antigens such as PspA and pneumolysin (Ply) were also present. Blotting with human immunoglobulin (VG) detected some bands indicating presence of immunogenic components in the vaccine (Figure 3.).

**RESULTS**

**In vivo protection and antibody production**

A vaccine (TIGR4.1) was made from heat shocked *S. pneumoniae* TIGR4 and administered to mice which were subsequently infected in the pneumonia model.

Degree of protection was assessed by counting colony-forming units in lung and blood 48 hours after infection

- Mice were significantly protected (p<0.004) against infection with log-fold lower CFU than control (Figure 4.)
- Western blotting and whole cell ELISA showed that sera from vaccinated mice contained high concentrations of antibody (Figure 5.)
- Flow cytometric IgG surface-binding assay shows that TIGR4.1 vaccinated mouse sera contained specific IgG surface-binding antibody (Figure 6)

**Cross-protection**

Rabbits were vaccinated with a heat shock vaccine and a double (heat and acid) shock (DS) Rx1apol strain vaccine and sera was collected from terminal bleed.

- Cross-reactive antibodies were present to the 5 other serotypes that were tested (Figure 7.)
- Conserved protein antigens are therefore present across different serotypes

**CONCLUSIONS**

- The manufacturing process of PnuBioVax enriches the vaccine with heat shock proteins, and contains immunogenic targets
- A TIGR4 strain vaccine protects mice from infection in the lung, reducing bacteraemia in a pneumonia infection model
- The vaccines induce the production of antibodies, which demonstrate cross-reactivity with other strains and ability to specifically bind the bacterial surface

**References**