Infection with *Streptococcus pyogenes* (group A streptococcus, GAS) can lead to rheumatic fever (RF) and rheumatic heart disease (RHD), which are significant health concerns in the Indigenous populations of developed countries, including Australian Aboriginal people. The global burden of GAS diseases had been recently reviewed and multiple studies have demonstrated the high burden of these diseases in Australia. RF and RHD are autoimmune type diseases, in which T-cells and antibodies targeting the bacteria may also cross-react with human tissues, therefore rendering a whole cell vaccine impractical.

Research groups within Australia and overseas have focused on developing a subunit vaccine (proteins and peptides) to avoid inducing the potential cross-reactive T-cells and antibodies responsible for RF and RHD. Primarily, vaccine development has focused on the M-protein of GAS as it is the most abundant cell surface protein, of which the amino terminus is responsible for inducing type-specific opsonic antibodies. In contrast, the carboxyl terminus of the M-protein is highly conserved and is the main focus of this report. Other vaccine candidates focusing on different target antigens have been investigated and reviewed elsewhere.

A multivalent vaccine based on the variable amino-terminal regions of the M-protein has been evaluated in phase I and II human clinical trials in North America. This vaccine is designed to protect against 95% of currently circulating GAS strains in the USA; however, several studies have indicated that this vaccine candidate may have limited use outside of the USA. Similar to many developing countries, GAS burden in Indigenous communities in the Northern Territory of Australia is very dynamic, with rapid turnover of GAS strains in short periods of time. Moreover, GAS strains found in these communities are different to those found in the USA and, therefore, are unlikely to be affected by the multivalent amino terminal vaccine under development in the USA. Strain replacement, rather than streptococcal eradication, as observed with the multivalent pneumococcal vaccines, is another strong possibility of using a multivalent vaccine in regions with large strain diversity. Evidence for switching of *emm*-genes (the gene encoding the M-protein) and recombination between *emm*-genes, even in the absence of vaccine-induced selection pressure, has also been observed.

To overcome the limitations of an amino terminal vaccine, our research has focused on the carboxyl terminus, which is conserved between GAS strains. Our group has previously defined a minimal B-cell epitope that once embedded in a alpha helical framework (peptide J8) and conjugated to a carrier protein (diphtheria toxoid, DT) it can induce protection against different GAS strains including clinical isolates collected from the Northern Territory of Australia. In a number of preclinical studies, a positive correlation between antigen specific (J8) antibody titre and mice survival was observed, indicating that vaccine-induced protection is antibody mediated.

To expand on this work and to define the mechanism of protection induced by our vaccine candidate, a series of passive transfer and T-cell depletion experiments were conducted. To determine if CD8+ T-cells, responsible for cellular immunity, were involved in protection, cohorts of mice actively immunised
with the vaccine candidate (J8-DT/alum) were depleted of CD8+ T-cells using an α-CD8 monoclonal antibody (Figure 1a) and then subsequently intraperitoneally challenged with GAS to determine protection (Figure 1b). No significant difference in survival was observed between cohorts of J8-DT/alum-immunised mice who were depleted of their CD8+ T-cells and those administered the control antibody. As expected, the control mice administered DT/alum and PBS/alum (undepleted and depleted) succumb to infection. These data indicate that CD8+ T-cells (cellular immunity) are not involved in J8-DT mediated protection.

To further demonstrate that vaccine-induced protection was antibody mediated, IgG from rabbits (New Zealand White) immunised with J8-DT/alum was affinity purified and intraperitoneally administered to cohorts of naïve BALB/c mice

**Role of rabbit-J8-DT IgG and T-cells in protection mediated by J8-DT.**

**Figure 1a and b.** Depletion of CD8 T-cells and its effect on survival of BALB/c mice. (a) Post-parenteral immunisation with J8-DT/DT or PBS, BALB/c mice were depleted of their CD8+ T-cells and survival post-challenge was assessed. For *in vitro* depletion of CD8+ T-cells, post-immunisation, BALB/c mice were administered with 1mg of anti CD8 (α-CD8 beta clone 53.5.8) mAb over a set time-course before and after GAS challenge. The schedule resulted in 95-97% deletion of CD8+ T-cells as assessed by FACS. (b) Following intraperitoneal GAS challenge, the survival in J8-DT immunised and CD8+ T-cell depleted/undepleted BALB/c mice was monitored and is shown as percentage survival. The abbreviation D stands for immunised/depleted mice, whereas U/D represents immunised/undepleted.

**Figure 1c and d.** Protection in BALB/c and SCID mice following passive transfer of purified rabbit IgG. Rabbits were multiply vaccinated with J8-DT and DT preparations in alum. Purified IgG (0.5mg) was administered intraperitoneally into BALB/c mice on each of three days (days –1, 0 and +1). Controls received a similar amount of normal rabbit IgG (control R-IgG). The mice were challenged on day 0 with M1 GAS and their survival monitored. (c) Demonstrates the survival in BALB/c mice following a M1 GAS challenge (significance is represented as * where p<0.05) and (d) shows survival in SCID mice following routine (days –1, 0 and +1) and additional doses (on days 3, 5 and 8) of J8-DT R-IgG or control R-IgG post-GAS challenge. The abbreviation DS1 represents dose schedule 1 (days –1, 0 and +1) whereas DS2 represents dose schedule 2 (days –1, 0, +1, +3, +5 and +8). Significance is represented as * where p<0.05 and ** where p<0.01.
Under the Microscope

(Figure 1c) on days –1, 0 and 1 post-challenge. Mice administered purified IgG from rabbits immunised with J8-DT/alum had significantly (p<0.05) increased survival compared to control groups. This is consistent with previous observations \(^6\), where mice administered DT/alum or PBS/alum were not significantly protected following challenge.

Expanding on this study, affinity purified IgG from rabbits immunised with J8-DT/alum were administered to cohorts of naïve immunodeficient SCID mice (which lack both B and T-cells) on days –1, 0, 1, 3, 5 and 8 post-challenge. While immunocompetent BALB/c were able to generate an immune response to the bacteria by days 5-7 post-challenge, the immunocomprised SCID were unable to mount any T-cell or B-cell specific response to the GAS. SCID mice administered IgG from J8-DT/alum immunised rabbits were also significantly protected compared to control cohorts (Figure 1d). Taken together, these data indicate that J8-DT/alum-induced protection is antibody mediated and that CD8\(^+\) T-cells responsible for cellular-based immunity is not required for protection.

The epidemiology of GAS in Australian Indigenous communities, like many developing countries, poses several significant hurdles when compared with the USA and Europe. The ever-changing strain diversity, combined with a subset of unique strains, demonstrates the need for the development of a vaccine that can protect against a range of GAS strains. The J8-DT vaccine has previously been shown to protect against a range of isolates and its mechanism of protection has now been elucidated, highlighting its potential in both Australia and overseas.

References


MALDI-TOF MS

Bruker Biosciences Pty Ltd Australia Phone +61 3 9474 7000 sales@bruker-daltonics.com.au