

A vaccine to provide strain-transcending immunity to group A streptococci



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Organisms such as *Streptococcus pyogenes* (group A streptococcus), *Plasmodium spp* parasites and HIV present significant obstacles to vaccine development. They can subvert the immune system and present dominant antigens that can display a vast array of allelic types of variants. In spite of these obvious decoys, the main strategy to develop vaccines for these organisms has been to focus on dominant antigens or epitopes. The obvious reason for this approach is that such antigens and epitopes are easy to define. Early successes are often posted largely because homologous organisms are typically used to challenge animals post-vaccination. However, success in efficacy trials with heterologous challenge is yet to be seen.

Our approach to vaccine development for both group A streptococcus (GAS) and malaria has been to avoid dominant antigens and attempt to define protective immune responses and antigens/epitopes that do not constitute the normal immune responses and repertoire that follow natural infection. Our data suggest this strategy can unveil an 'Achilles heel' for the organism as it is then presented with an immune attack for which it has not evolved an evasion strategy.

For GAS, an organism responsible for the loss of up to 500,000 lives per year as a result of post-streptococcal rheumatic heart disease or sepsis¹, we searched for and defined a cryptic epitope in the C3-repeat of the M protein. Our approach was quite simple: we used a series of overlapping peptides from the highly conserved C3 repeat, generated antibodies to each and asked whether any could induce antibodies capable of killing GAS organisms in an opsonophagocytosis assay.

We identified p145, a 20-mer that induced functional antibodies in mice. We further observed that although children living in streptococcal-endemic areas rarely displayed antibodies to p145 (demonstrating that it was 'cryptic'), adults mostly did have

antibodies and that affinity purified human antibodies could also kill multiple strains of GAS *in vitro*. Thus, p145, buried within the sugars on the surface of GAS, was poorly immunogenic in its natural state and was thus not under any immune pressure, resulting in it being highly conserved. However, the isolated p145 peptide epitope was highly immunogenic and antibodies induced by immunisation were able to recognise the epitope on the bacterial surface. Thus, in its native configuration, p145 was antigenic, but not immunogenic.

To develop this as a vaccine candidate, we next defined the minimal protective epitope within p145. We undertook this task to minimise the possibility that a vaccine might induce autoimmunity. This was a major concern for us because GAS infection can lead to rheumatic fever, an autoimmune condition involving the heart (rheumatic heart disease), the brain (chorea), skin (subcutaneous nodules) and joints (arthritis). To define the minimal epitope it was necessary to maintain the natural alpha helical structure of the p145 epitope and to achieve this we developed (and patented) a folding technology².

The vaccine epitope that we thus defined and folded correctly, J8 (or J14), was then presented to the immune system in various ways and was able to induce IgG antibodies (Figure 1) when conjugated to diphtheria toxoid and administered intramuscularly with alum³, and was able to induce IgG and IgA antibodies when administered intranasally in either a proteosome vesicle⁴ or when conjugated to certain lipids⁵⁻⁹.

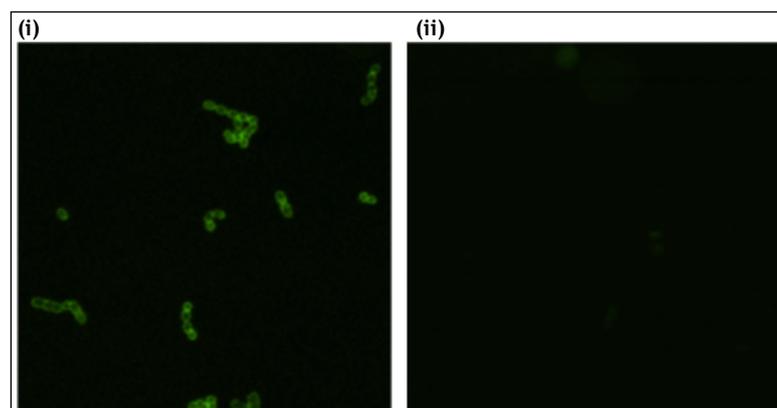
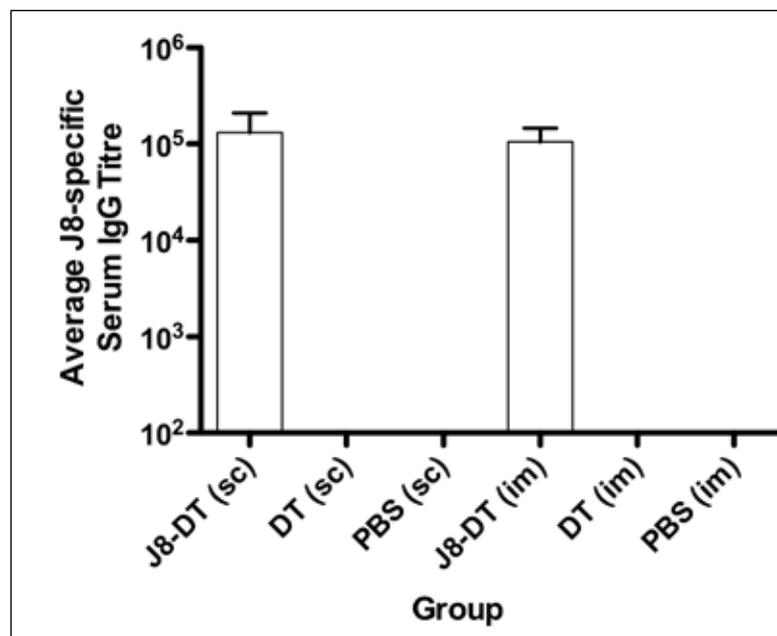
The alum formulation has now been further developed for a phase I clinical trial. GMP-grade peptide and diphtheria toxoid have been conjugated and vialled with alum. The vaccine is undergoing formal toxicology testing in the USA and, pending the outcomes of those studies, will then be administered to volunteers in a phase I study. The measured outcomes will be safety and the development of antibodies.

Although we have known for many years that the vaccine can

protect mice from GAS administered intraperitoneally, our latest data also show that the vaccine can completely protect mice from skin infection (M. Pandey, M. Batzloff and M.F. Good, unpublished). This is significant because there is now accumulating evidence that rheumatic heart disease can follow skin infection (at least in Indigenous Australians who suffer the highest rates of streptococcal diseases in the world¹⁰).

If the vaccine is ultimately successful, the crux will be that the p145 peptide is antigenic in its native configuration but not immunogenic. For this reason alone, the organism has not had to evolve multiple allelic epitopes; however, vaccine-induced antibodies can target the epitope and kill the organism. If this vaccine is successful, then this strategy of defining non-dominant immune responses and epitopes will be more seriously considered for other organisms that readily evade natural immunity.

Figure 1. Immunogenicity of the vaccine formulations when administered intramuscularly (im) or subcutaneously (sc) to outbred ARC-SWISS mice (n=10). Cohorts of mice were administered antigen (30ug) on days 0, 21 and 28 post-primary immunisation. Sera from individual mice were collected on day 35 post-primary immunisation for ELISA which was used to determine J8-specific serum IgG titres. Mean and SEM shown for all groups. Immunofluorescent micrograph of GAS (M6) exposed to (i) J8-DT/alum and (ii) PBS/alum antisera. All images captured using the same settings and are representative of the complete slide as previously reported⁹.



References

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Biography

Professor Michael Good is a NHMRC Australia Fellow at Griffith University, the past Director of the Queensland Institute of Medical Research, a past President of the Association of Australian Medical Research Institutes, and a past Director of the Cooperative Research Centre for Vaccine Technology. In 2006 he was appointed as Chair of the National Health and Medical Research Council of Australia. In 2008 he was a Steering Committee member and Co-Chair of the “long-term national health strategy” of the 2020 Summit. Also in 2008 he was awarded an Officer of the Order of Australia (AO) for service to medical research and contributions to education. In 2009 he won the Australian Museum CSIRO Eureka Prize for Leadership in Science. In 2010 he was named a “Queensland Great” by the Queensland Premier. He graduated MD PhD DSc from the University of Queensland and the Walter and Eliza Hall Institute of Medical Research in Melbourne. He undertook postdoctoral training at the National Institutes of Health in Bethesda, Maryland before returning to Australia in 1988. His interests are in the field of immunity and immunopathogenesis to malaria and group A streptococcus/rheumatic fever, with particular relevance to the development of vaccines.