View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by University of Queensland eSpace

Indian J Med Res 119 (Suppl) May 2004, pp 104-107

Preclinical evaluation of a vaccine based on conserved region of M protein that prevents group A streptococcal infection

Michael Batzloff, Huaru Yan, Mark Davies, Jon Hartas & Michael Good

Cooperative Research Centre for Vaccine Technology, The Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, QLD 4029, Australia

Received August 7, 2003

***Background & objectives*: Infection with group A *Streptococcus* (GAS) may result in a number of human diseases ranging from the relatively benign pharyngitis to the potentially life-threatening invasive diseases and post-infectious sequelae. We have previously defined a minimal B-cell epitope from the conserved region of the M-protein. Here we report on the immunogenicity, opsonic potential of the resulting sera and the level of protection induced by this peptide in comparison to a pepsin extract of the M protein.**

***Methods*: Inbred mice were immunized with peptides derived from the M protein. Sera were collected from the immunized mice and its opsonic potential determined for M1 and M6 GAS strains. Mice were then intranasally challenged with a virulent M1 GAS strain to determine the protective efficacy of the peptides.**

***Results*: The peptides induced significant antibody responses when delivered subcutaneously and immunized mice demonstrated significantly enhanced survival compared to control groups following challenge.**

***Interpretation & conclusion*: The data obtained in the present study indicated that the chimeric peptide J8 from the conserved region of the M protein could form the basis for an anti-streptococcal vaccine in future.**

Key words Chimeric peptides - group A streptococcus - immunogenicity - M protein

Infection with group A *Streptococcus* (GAS) may result in a number of human diseases ranging from the relatively benign pharyngitis to the potentially life-threatening invasive diseases and post-infectious sequelae, such as rheumatic fever (RF) and rheumatic heart disease (RHD). The resurgence in severe invasive disease and increased incidence of GAS infection, in combination with the dynamic epidemiology of GAS serotypes highlight the need to develop a safe, broad-based, efficacious vaccine against this bacterium[1].

We have previously described a 20-mer peptide (p145) from the conserved region of the M protein which induced antibodies in vaccinated mice that opsonised multiple serotypes of GAS[2,3]. We also demonstrated that the prevalence of p145-specific antibodies amongst humans living in GAS-endemic areas increased with age being found in approximately 40 per cent of children and over 90 per cent of adults[4] - possibly increasing in parallel with the acquisition of natural immunity to GAS. We also demonstrated that affinity-purified human antibodies to p145 could directly opsonise multiple strains of GAS *in vitro*[4,5].

Peptide p145 would be a vaccine candidate except for the fact that humans may develop cross-reactive

T cells to this region of the M protein[6]. We therefore determined the minimal antibody epitope from the p145 peptide and separated this from any potential deleterious T cell epitope. We previously determined that the protective epitope in p145 was conformational with alpha helical propensity[7] a technique was developed to map the epitope by embedding small peptides from p145 within other longer and unrelated peptides which were also known to form a helical coil.

These peptides, referred to as chimeric peptides, maintained the appropriate tertiary structure, and enabled us to map the minimal antibody epitope. The antibody epitopes recognised by endemic human sera were chimeric peptides J2, J7 and J8[4], whereas the chimeric peptides recognised by p145 murine antisera were J7 and J8[7,8]. Human T cells respond to J2 and murine T cells from B10.BR mice immunized with p145 respond vigorously to J3[8]. The aim of this study was to compare the immunogenicity and protective potential of our chimeric peptide (J8) with p145 and a pepM extract.

Material & Methods

Peptide synthesis: Synthetic peptides were produced as described[9] and were purified by HPLC. The peptide sequences for the p145 and J8 peptides are LRRD LDASREAKKQVEKALEC and QAEDKVKQ SREAKKQVEKALKQLEDKVQ respectively.

Immunization of mice: Peptides were administered subcutaneously in a volume of 50µl at the tail base to B10.BR (inbred, H-2^k) mice (Animal Resources Centre, Western Australia). Each mouse received a total of 30µg of immunogen emulsified 1:1 in complete Freund's adjuvant (CFA) (Difco, USA). Mice were also given subsequent booster injections at days 21, 31 and 41 post-primary immunization.

Serum collection: Blood was collected from mice via the tail artery and allowed to clot at 37°C for at least 30 min. Serum was collected after centrifugation at 1000 *g* for 10 min, heat inactivated at 56°C for 10 min and stored at -20°C. Serum was collected from all of the mice 1-day prior to boosts and 15 days after the final boost.

Detection of murine antibodies: An ELISA was used to measure J8-specific murine serum IgG antibodies and the antibody isotypes were determined as described[2,8]. Titre was defined as the highest dilution that gave an optical density (OD) reading of more than three standard deviations (SD) above the mean OD of control wells containing normal mouse sera.

Indirect bactericidal assay: Murine anti-peptide sera were assayed for their ability to opsonise GAS *in vitro* as previously described[2,4,5,10]. Briefly, bacteria were grown overnight at 37°C in 5ml Todd-Hewitt broth (THB). GAS was then serially diluted to 10^{-5} in phosphate-buffered saline (PBS). For each individual mouse, 50µl of fresh heat-inactivated serum was mixed with 50µl of the bacterial dilution and incubated for 20 min at room temperature. After the incubation, 400µl of non-opsonic heparinised human donor blood was added. All donor blood was pre-screened prior to performing the assay to ensure that it could support the growth of the GAS strain by at least 32 times the inoculum in a 3 h incubation at 37°C.

The mixtures were incubated end-over-end at 37°C for 3 h and 50µl from each tube was plated out in duplicate on 2 per cent blood THB agar pour plates. The plates were incubated overnight and the number of colonies on each plate was determined. Opsonic activity of the anti-peptide sera [% reduction in mean colony forming units (cfu)] was calculated as [1-(cfu in the presence of anti-peptide sera) / (mean cfu in the presence of normal mouse sera)] X 100.

Intranasal GAS challenge procedure: GAS was cultured overnight in THB with 1 per cent neopeptone and 200 mg/ml streptomycin, spun at 1500 *g* for 10 min and washed twice with THB containing 1 per cent neopeptone and resuspended in 25 per cent of the original volume. The inoculum dose (cfu/ml) was determined by optical density at 600nm and plating out 10-fold dilutions of bacterial suspensions on 2 per cent horse blood THB agar plates. Following overnight incubation of the plates at 37°C, colony counts were determined. Immunized and control mice were challenged intranasally with a pre-determined dose of GAS in 30 µl (15 µl/nare).

Statistical analysis: The T-tests, mean and the standard error of the mean were calculated using standard

formulae. The log rank or Mantel-Haenszel test was used to compare survival curves of challenged mice[11]. $P < 0.05$ was considered significant.

Results

Inbred B10.BR (H-2^k) mice were immunized with the conserved region peptide p145 and its chimeric derivative J8. Control mice received PepM extract or PBS in CFA. The mice developed strong antigen-specific serum IgG titres (Fig.1). IgG isotypes were predominantly the IgG2a > IgG1 > IgG2b for both the J8 and p145 peptide. However for the pepM extract IgG1was the predominant isotype followed by IgG2a and IgG2b (data not shown). While IgA was not detectable in the serum, antigen-specific serum IgM was shown to be present albeit 5-fold lower then IgG1.

Serum collected from the immunized mice was capable of the *in vitro* opsonisation of the M1 GAS strain (Fig.2). Mice immunized with p145 ($P < 0.001$), J8 ($P < 0.01$) and pepM ($P < 0.001$) had significantly higher levels of opsonisation when compared to the PBS control group. Interestingly, serum from both peptide (p145 and J8) immunized mice was capable of the *in vitro* opsonisation of M1 GAS strain at similar levels to that of the pepM mice ($P>0.05$). The antiserum was also shown to opsonise the M6 GAS strain at similar levels to that observed for the M1 strain (data not shown).

Mice were then challenged intranasally with the M1 GAS strain (Fig.3). Mice immunized with p145 ($P < 0.05$), J8 ($P < 0.05$) and pepM ($P < 0.05$) had significantly higher levels of survival compared to the PBS/CFA immunized mice. There was no significant difference in survival in the p145, J8 and pepM immunized groups, indicating the conserved region peptide was able to induce protection equal to that of the pepM immunized mice.

Discussion

This study was carried out to determine the immunogenicity of a conserved M protein epitope, J8, with CFA and compare the levels of opsonisation and protection induced by immunization with the parent peptide p145 and the M protein extract pepM. It was demonstrated that J8 induced significant antibody responses when delivered

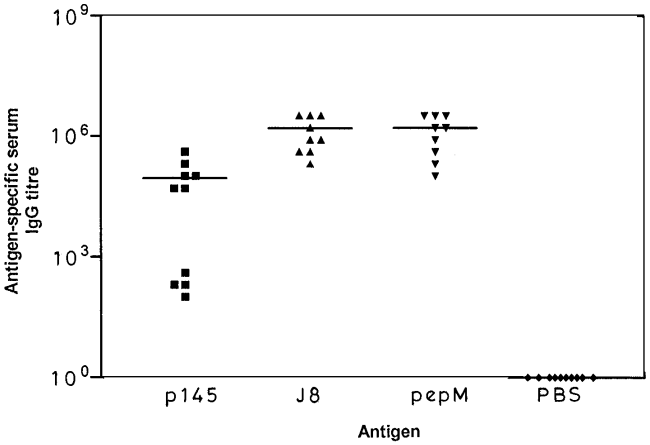

Fig. 1. Antigen-specific serum IgG titres of immunized mice taken at day 56 post-primary immunization. Horizontal bar indicates average. PBS, phosphate buffer saline.

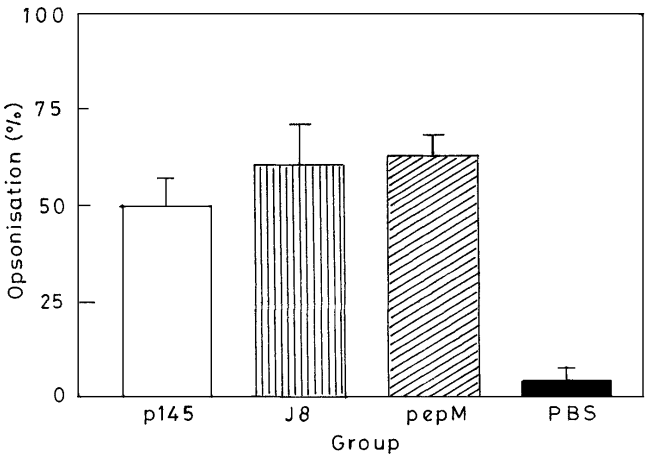

Fig.2. Average per cent opsonisation of an M1 GAS strain by serum taken at day 56 post-primary immunization. PBS, phosphate buffer saline.

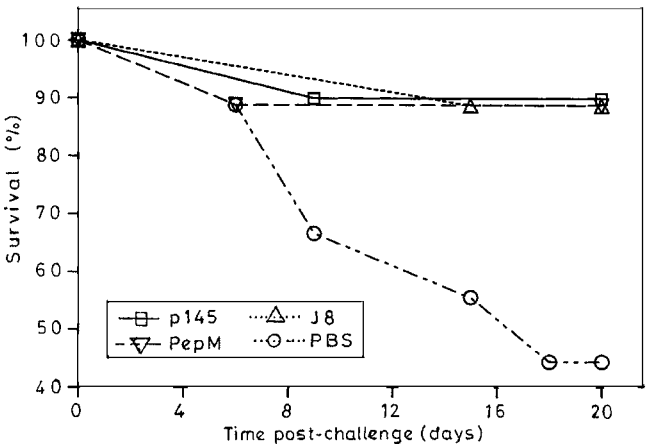

Fig.3. Per cent survival of immunized mice following intranasal challenge with an M1 GAS strain.

subcutaneously and that immunized mice demonstrated significantly enhanced survival compared to control groups.

J8 when administered with CFA can protect up to 80 per cent of H-2^k responder strain mice (B10.BR) from virulent challenge[12]. However, minimal epitopes such as J8 are unlikely to be immunogenic in an outbred population. To render such epitopes immunogenic they must be conjugated to carrier proteins capable of inducing T cell help. Diphtheria toxoid (DT) has been used as a carrier protein in many systems and preliminary data from our own laboratory suggested that anti-DT antibodies may in fact be capable of opsonising GAS making DT an obvious choice for a carrier protein for J8 in future studies.

The mechanism for J8 mediated protection of mice from GAS challenge has not been completely defined. However it has been demonstrated that the N-terminal epitopes of the M protein induce high levels of serum antibodies, which are highly opsonic to the corresponding GAS strain[13-15]. These epitopes have also been shown to induce high levels of protection from GAS challenge in mice[12], indicating that protection is antibody mediated.

In conclusion, the findings of the present study showed that our peptide from the conserved region of the M protein could form the basis of an anti-streptococcal vaccine.

Acknowledgment

The authors acknowledge the financial support by the NHMRC (Australia), The Prince Charles Hospital Foundation, National Heart Foundation of Australia and the Cooperative Research Centre for Vaccine Technology.

References

1. Kaplan EL, Wotton JT, Johnson DR. Dynamic epidemiology of group A streptococcal serotypes. *Lancet* 2002; *359* : 2115-6.
2. Pruksakorn S, Galbraith A, Houghten RA, Good MF. Conserved T and B cell epitopes on the M protein of group A streptococci. Induction of bactericidal antibodies. *J Immunol* 1992; *149* : 2729-35.
3. Pruksakorn S, Currie B, Brandt E, Martin D, Galbraith A, Phornphutkul C, *et al*. Towards a vaccine for rheumatic fever: identification of a conserved target epitope on M protein of group A streptococci. *Lancet* 1994; *344* : 639-42.
4. Brandt ER, Hayman WA, Currie B, Carapetis J, Wood Y, Jackson DC, *et al*. Opsonic human antibodies from an endemic population specific for a conserved epitope on the M protein of group A streptococci. *Immunology* 1996; *89* : 331-7.
5. Brandt ER, Hayman WA, Currie B, Pruksakorn S, Good MF. Human antibodies to the conserved region of the M protein: opsonization of heterologous strains of group A streptococci. *Vaccine* 1997; *15* : 1805-12.
6. Pruksakorn S, Currie B, Brandt E, Phornphutkul C, Hunsakunachai S, Manmontri A, *et al*. Identification of T cell autoepitopes that cross-react with the C-terminal segment of the M protein of group A streptococci. *Int Immunol* 1994; *6* : 1235-44.
7. Relf WA, Cooper J, Brandt ER, Hayman WA, Anders RF, Pruksakorn S, *et al.* Mapping a conserved conformational epitope from the M protein of group A streptococci. *Pept Res* 1996; *9* : 12-20.
8. Hayman WA, Brandt ER, Relf WA, Cooper J, Saul A, Good MF. Mapping the minimal murine T cell and B cell epitopes within a peptide vaccine candidate from the conserved region of the M protein of group A streptococcus. *Int Immunol* 1997; *9* : 1723-33.
9. Houghten RA. General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc Natl Acad Sci USA* 1985; *82* : 5131-5.
10. De Malmanche SA, Martin DR. Protective immunity to the group A streptococcus may only be strain specific. *Med Microbiol Immunol* 1996; *183* : 299-306.
11. Altman D. *Practical statistics for medical research*. New York: Chapman and Hall; 1991.
12. Brandt ER, Sriprakash KS, Hobb RI, Hayman WA, Zeng W, Batzloff MR, *et al*. New multi-determinant strategy for a group A streptococcal vaccine designed for the Australian aboriginal population. *Nat Med* 2000; *6* : 455-9.
13. Dale JB, Simmons M, Chiang EC, Chiang EY. Recombinant, octavalent group A streptococcal M protein vaccine. *Vaccine* 1996; *14* : 944-8.
14. Lancefield RC. Current knowledge of the type specific M antigens of group A streptococci. *J Immunol* 1962; *89* : 307.
15. Brandt ER, Teh T, Relf WA, Hobb RI, Good MF. Protective and nonprotective epitopes from amino termini of M proteins from Australian aboriginal isolates and reference strains of group A streptococci. *Infect Immun* 2000; *68* : 6587-94.

Reprint requests: Dr Michael R. Batzloff, The Queensland Institute of Medical Research, PO Royal Brisbane Hospital
Brisbane QLD 4029, Australia
e-mail: michaeBa@qimr.edu.au