THE DETECTION OF ANTIBODIES TO GROUP A STREPTOCOCCAL M PROTEIN IN RHEUMATIC FEVER

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Summary

Rheumatic fever as a sequela of group A streptococcal pharyngitis continues to be an important cause of cardiovascular morbidity not only in the developing world but also in aboriginal communities within Australia. The clinical diagnosis of rheumatic fever or rheumatic heart disease can be difficult, particularly, in remote aboriginal communities. Current serological methods to support the diagnosis are not specific for rheumatic fever. The group A streptococcus (*Streptococcus pyogenes*) has on its outer surface an antiphagocytic protein, M-protein. Antibodies to M-protein have been shown to persist in rheumatic fever. There are however, few studies looking at the use of this as a diagnostic test.

In the first part of this study, pepsin extracts of *Streptococcus pyogenes* M24 and M18 were used in a Western blot against sera from 31 subjects, (both Aboriginal and non-Aboriginal). Eighteen had proven rheumatic heart disease or rheumatic fever. Of these, 16 had definitive bands at regions corresponding to specific M24 and/or M18 proteins. There was no difference between Aboriginal and non-Aboriginal subjects with a history of rheumatic fever or rheumatic heart disease. Of the remaining 13 subjects with no history of rheumatic fever, 12 were non-reactive. This would suggest that the detection of antibodies to M-protein, may have a place in the serodiagnosis of rheumatic fever.

In the second part of this study, linear epitopes of the M24 protein were mapped, using overlapping synthetic biotinylated 16-mer peptides in a streptavidin based enzyme immunoassay system, against sera from eighty two subjects, Aboriginal and non-Aboriginal with either acute rheumatic fever, previous rheumatic heart disease or with no history of disease. Of

the eighty two peptides tested, five commonly reactive peptides were identified. These five peptides (Peptides 89, 95, 102, 103 and 105), were all from the carboxy-terminal end of M24 protein and had significant amino-acid sequence homology with each other. Parameters for the enzyme immunoassay were determined and included using 0.01 μg/well streptavidin, 0.28 μg/well biotinylated peptide and sera at a 1 in 500 dilution.

Sera from Aboriginal subjects with acute rheumatic fever, previous rheumatic fever or rheumatic heart disease, were significantly more reactive with peptides 89 and 95, than sera from matched control subjects,

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There was no significant difference in reactivity between sera from non-Aboriginals with previous rheumatic fever and matched controls.

Using peptides in combination, or using related 20-mer peptides with the same panel of sera, did not reliably differentiate between subjects with rheumatic fever and those without.

It is concluded that related peptides at the C-terminal end of M24 protein represent linear epitopes recognised by sera from Aboriginal subjects with acute rheumatic fever and previous rheumatic fever.

It is proposed that these peptides could be used as antigens in the serodiagnosis of acute rheumatic fever.