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

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Being a thesis submitted to the University of Adelaide for the degree of  
Doctor of Medicine.

Date: 17th March 1998

# Contents

<b>Table of abbreviations</b> .....	5
<b>Declaration</b> .....	6
<b>Acknowledgements</b> .....	7
<b>Summary</b> .....	8
<b>Chapter 1 Rheumatic fever and streptococcal M protein - a review of the literature</b> .....	10
1.1 Introduction .....	10
1.2 Historical background.....	11
1.3 Epidemiology.....	11
1.4 The pathogenesis of rheumatic fever.....	13
1.5 The clinical diagnosis of rheumatic fever.....	16
1.6 Serological tests used to demonstrate previous streptococcal infection (antibodies to extracellular antigens).....	18
1.7 Streptococcal M protein - background .....	19
1.8 The nature and structure of M protein .....	20
Figure 1.1 A diagrammatic representation of streptococcal M protein .....	21
Figure 1.2 A comparison of amino acid sequences of nine M proteins. ....	23
1.9 M associated proteins and class I and II M proteins.....	25
1.10 Antiphagocytic activity of M protein .....	26
1.11 The role of M protein in adherence .....	26
1.12 Other virulence factors of the group A streptococcus .....	27
1.13 Structural similarity and immunological cross-reactivity between components of group A streptococci and mammalian protein .....	27
1.14 The extraction of M protein.....	31
1.15 The <i>emm</i> gene coding for M protein.....	32
1.16 B and T cell epitope mapping of M protein. ....	34
1.17 The serological detection of antibodies to cellular streptococcal antigens (including M protein).....	34
1.18 Streptococcal M protein and rheumatic fever - Conclusions.....	39
1.19 Aims of this study.....	40
<b>Chapter 2 Study population</b> .....	42
2.1 Subjects with acute rheumatic fever .....	42
2.2 Aboriginal subjects with previous rheumatic fever .....	44
2.3 Aboriginal control sera .....	48
2.4 Non aboriginal subjects with rheumatic heart disease.....	48
2.5 Non aboriginal control sera .....	51
2.6 Study population - conclusions.....	52

<b>Chapter 3 Materials and methods</b> .....	53
3.1 Bacterial strains .....	53
3.2 Culture .....	53
3.3 Extraction of M Protein by mild pepsin digestion.....	53
3.4 Polyacrylamide gel electrophoresis of extracted M protein .....	54
3.5 Western blotting .....	55
3.6 Sera used in the Western blot using pepM24 and pepM18 as antigens.....	55
3.7 ASOT determination.....	56
3.8 ADB determination.....	56
3.9 Enzyme immunoassay using biotinylated peptides .....	57
Table 3.1 Amino acid sequences of the eighty two biotinylated peptides.....	59
3.10 Titration of streptavidin concentration for coating wells .....	61
3.11 Determination of optimal peptide and sera concentrations .....	63
3.12 Enzyme immunoassay of test sera.....	64
3.13 Peptide epitope mapping of M24 protein .....	65
3.14 Screening of test sera with commonly reactive peptides.....	65
3.15 The use of the five reactive peptides identified, in combination at varying concentrations.....	66
3.16 A comparison of two 20 mer related peptides with peptides used in this study, using an alternative EIA method .....	66
3.17 Statistical analysis.....	68
<b>Chapter 4 Use of the M protein extract in a Western blot serological survey of the study group</b> .....	69
4.1 Polyacrylamide gel electrophoresis (PAGE).....	69
4.2 Western blots .....	70
Figure 4.1 PAGE of pepM18 and pepM24.....	71
Figure 4.2 Representative reactive Western blots .....	72
Table 4.1 Western blots on Aboriginal subjects with rheumatic fever .....	73
Table 4.2 Western blots on non-Aboriginal subjects with rheumatic fever .....	74
Table 4.3 Western blots on non-Aboriginal controls.....	75
Table 4.4 Western blots on Aboriginal controls .....	76
4.3 Western blot serological survey of the study group - conclusions .....	78
<b>Chapter 5 Determination of optimal parameters for the assay</b> .....	79
5.1 Determination of optimal peptide and sera concentrations .....	79
Figure 5.1 Titration of peptide and sera concentrations .....	80
Figure 5.2 Titration of peptide and sera concentrations .....	81
5.2 The use of five reactive peptides in combination at varying concentrations.....	82
Table 5.1 Mean absorbance values using reactive peptides in combination .....	82
5.3 Titration of streptavidin for coating wells .....	83
Figure 5.3 Titration of streptavidin concentration for coating wells.....	85
Figure 5.4 Preabsorption of sera with streptavidin.....	86
Figure 5.5 Peptide reactivity with different concentrations of streptavidin .....	87
5.4 Determination of optimal parameters - Conclusions .....	88

<b>Chapter 6 Peptide epitope mapping</b> .....	89
6.1 Preliminary screening of the peptide bank .....	89
Figure 6.1 Peptide epitope mapping - Aboriginals with rheumatic fever.....	91
Figure 6.2 Peptide epitope mapping - Aboriginal controls .....	92
Figure 6.3 Peptide epitope mapping - Non Aboriginals with previous rheumatic fever .....	93
6.2 Screening of test sera with universally reactive peptides .....	94
Table 6.1 Aboriginal sera (Acute rheumatic fever) .....	95
Table 6.2 Aboriginal sera (Previous rheumatic fever) .....	96
Table 6.3 Aboriginal control sera .....	97
Table 6.4 Non-Aboriginal sera (Previous rheumatic fever) .....	99
Table 6.5 Non-Aboriginal control sera.....	100
Figure 6.4 Screening of eighty two sera against the five reactive sera.....	101
6.3 ASOT and ADB results .....	102
6.4 A comparison of two 20-mer related peptides with peptides used in this study .....	103
6.5 Peptide epitope mapping - Conclusions .....	104
Table 6.6 Mean absorbance values obtained with the related peptide.....	104
<b>Chapter 7 Discussion and Conclusions</b> .....	105
7.1 Pepsin extraction of M protein and PAGE .....	105
7.2 Screening of sera by Western blot.....	106
7.3 Preliminary screening of the peptide bank by EIA.....	108
7.4 Screening of test sera with commonly reactive peptides.....	108
7.5 A comparison of the two 20-mer related peptides.....	111
7.6 The use of the five reactive peptides in combination .....	112
7.7 Titration of streptavidin concentration for coating wells .....	113
7.8 A comparison of the Western blot and EIA results .....	113
7.9 A comparison between the EIA and current serological tests when used in rheumatic fever.....	115
7.10 Possible role of the peptide epitopes identified in this study in the pathogenesis of rheumatic fever .....	116
7.11 Conclusions .....	118
<b>References</b> .....	120



## 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,

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.