Antibody levels to the class I and II epitopes of the M protein and myosin are related to group A streptococcal exposure in endemic populations

Evelyn R. Brandt1, Penny J. Yarwood1, David J. McMillan1, Harpreet Vohra2, Bart Currie3, Layla Mammo4, Sumalee Pruksakorn5, J. Saour4 and Michael F. Good1

1CRC for Vaccine Technology, The Queensland Institute of Medical Research, PO Royal Brisbane Hospital, and the Australian Centre for International and Tropical Health and Nutrition, University of Queensland, Brisbane 4029, Australia
2Department of Experimental Medicine, Post-graduate Institute of Medical Education and Research, Chandigarh, India 160012
3Menzies School of Health Research, Casuarina, NT 0810, Australia
4King Faisal Specialist Hospital and Research Centre, Riyadh 11211, Saudi Arabia
5Department of Microbiology, Chiang Mai University, Chiang Mai 50002, Thailand

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Abstract

Rheumatic fever (RF)/rheumatic heart disease (RHD) and post-streptococcal glomerulonephritis are thought to be autoimmune diseases, and follow group A streptococcal (GAS) infection. Different GAS M types have been associated with rheumatogenicity or nephritogenicity and categorized into either of two distinct classes (I or II) based on amino acid sequences present within the repeat region (‘C’ repeats) of the M protein. Sera from ARF patients have previously been shown to contain elevated levels of antibodies to the class I-specific epitope and myosin with the class I-specific antibodies also being cross-reactive to myosin, suggesting a disease association. This study shows that immunoreactivity of the class I-specific peptide and myosin does not differ between controls and acute RF (ARF)/RHD in populations that are highly endemic for GAS, raising the possibility that the association is related to GAS exposure, not the presence of ARF/RHD. Peptide inhibition studies suggest that the class I epitope may be conformational and residue 10 of the peptide is critical for antibody binding. We demonstrate that correlation of antibody levels between the class I and II epitope is due to class II-specific antibodies recognizing a common epitope with class I which is contained within the sequence RDL-ASRE. Our results suggest that antibody prevalence to class I and II epitopes and myosin is associated with GAS exposure, and that antibodies to these epitopes are not an indicator of disease nor a pathogenic factor in endemic populations.

Introduction

Acute rheumatic fever (ARF) is one of the sequelae of nasopharyngeal group A streptococcal (GAS) infection. An increasing body of evidence implicates auto-immune processes in the development of both ARF and rheumatic heart disease (RHD). Antibody and T cell responses, initially generated against different GAS surface molecules, including the M-protein, and which also recognize host tissues, are important factors in the development of these diseases (1). The M protein is an α-helical coiled-coil protein on the surface of streptococci. The highly antigenically variable sequences located at the N-terminus are responsible for the serotype, whilst the C-terminus is highly conserved between GAS serotypes. Epidemiological studies suggest that certain serotypes of GAS are more strongly associated with ARF (M types
1, 3, 5, 6, 14, 18, 19, 24 and 29) and others with acute glomerulonephritis (M types 1, 4, 12, 49, 55, 57 and 60) (1). However, not all strains of a particular M type are equally nephritogenic or rheumatogenic.

Streptococci with M types associated with rheumatogenicity or nephritogenicity have previously been categorized into two distinct classes (I and II) based on epitopes present within the conserved C-repeat block region of the M protein (2,3). Class I GAS have been associated with ARF and do not exhibit opacity factor activity, unlike class II organisms (2,4). The antigenic epitopes of the class I and II M proteins were mapped to an exposed surface position of the C-repeat region of the M protein (3). A significant increase in recognition of the class I-specific epitope was shown in serum IgG from RF patients compared to control individuals and uncomplicated pharyngitis patients (2,5). Although these data suggested that RF is associated with an elevated titer to the class I epitope, we have recently shown that in highly endemic populations, there is no significant difference in the levels of class I antibodies between ARF, RHD and controls, suggesting that the level of class I antibodies reflects intense streptococcal exposure rather than disease (6).

Recently, ARF sera were shown to contain antibodies to the class I epitope and to myosin (7). In addition, myosin and the class I epitope were shown to be immunologically cross-reactive, suggesting that the class I epitope may directly contribute to disease pathogenesis in the susceptible host (7). We have previously identified an opsonic peptide vaccine candidate, p145, from the conserved C-repeat region of the M protein which overlaps the class I epitope by 14 amino acids and which contains myosin sequences (8-11). Although no association between disease pathogenesis and p145-specific immune responses has been shown, it may still have significant bearing on the use of the conserved region as a broad-spectrum anti-GAS vaccine. Therefore, it was of particular interest for us to identify which residues within the class I sequence are involved in antibody binding.

In this study, we examine the prevalence of antibodies to class I, II and myosin peptides in human sera from populations that are highly endemic for GAS—Australian Aboriginal, Saudi, Thai and Indian patients with either RHD or ARF, and from controls. These populations have some of the highest prevalence rates of RF and RHD world-wide. The Australian Aboriginal population has an incidence of RF of >600/100,000/year (12). In Saudi school children, the prevalence of RHD is 240/100,000 (13), in Thais at least 12/100,000 (14,15) and Indians 210/100,000 (16). These populations, with their high prevalence of RF and RHD, are ideal to investigate whether reactivity to these peptides is associated with RF or intense streptococcal exposure.

Class II-specific antibodies have also been shown to be present in patients with high titer antibodies to class I (2). Antibody inhibition studies show that most of these antibodies may be directed to non-class-specific epitopes shared by class I and II (2). To further understand the immunological and molecular differences between the class I and II epitope, we identify the critical residues that define the class I epitope and the common epitope shared by class II antibodies.

### Table 1. Amino acid sequence of synthetic peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence*</th>
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<tbody>
<tr>
<td>Class I peptide</td>
<td>SRKGLRRLGASTREALKQVEK</td>
</tr>
<tr>
<td>Class II peptide</td>
<td>SRKGLRRLGASTREALKQVEK</td>
</tr>
<tr>
<td>CM1</td>
<td>SRKGLRRLGASTREALKQVEK</td>
</tr>
<tr>
<td>CM2</td>
<td>SRKGLRRLGASTREALKQVEK</td>
</tr>
<tr>
<td>144</td>
<td>NKISERKGLRRLGASTREALKQVEK</td>
</tr>
<tr>
<td>145</td>
<td>LRRDLGASTREALKQVEKALE</td>
</tr>
<tr>
<td>146</td>
<td>AKQVEKALEEANSKLALE</td>
</tr>
<tr>
<td>J1</td>
<td>QLEDKVQRLRRDLGASTREALKQVEK</td>
</tr>
<tr>
<td>J2</td>
<td>LEDKVQRLRRDLGASTREALKQVEKQ</td>
</tr>
<tr>
<td>J3</td>
<td>EDKVQAEQRRLGASTREALKQVEKQL</td>
</tr>
<tr>
<td>J4</td>
<td>DKVQAEQRRLGASTREALKQVEKQL</td>
</tr>
<tr>
<td>J5</td>
<td>VKQAEQRRLGASTREALKQVEKQL</td>
</tr>
<tr>
<td>J6</td>
<td>VKQAEQRRLGASTREALKQVEKQL</td>
</tr>
<tr>
<td>J7</td>
<td>QKAEDKVQASEALKQVEKALKQLEQDKV</td>
</tr>
<tr>
<td>J8</td>
<td>QALEDKVQASEALKQVEKALKQLEQDKV</td>
</tr>
<tr>
<td>Non-specific</td>
<td>EKLVSTLPLDQI1AAATMSK</td>
</tr>
</tbody>
</table>

*Bold residues indicate positions where the class I and class II sequences differ. Italicized sequences represent residues that are not included in the class I peptide sequence.

### Patients and methods

#### Patients

Sera was obtained from a total of 109 Australian Aboriginals from The Northern Territory with established RHD (n=51), ARF (n=26) and control subjects (n=32); Saudis with established RHD (n=72) and control subjects (n=37); Thais with established RHD (n=39), ARF (n=8) and control subjects (n=51); and Indians with established RHD (n=49) and controls (n=49). All subjects were assessed by specialist physicians and patients with ARF met the revised Jones criteria for diagnosis (17). For patients with ARF or RHD, the date of onset of disease could not be determined. Hence sera from these patients is likely to represent random timepoints in the progression of these diseases. All endemic control subjects had no history of ARF and no clinical signs of RHD. Australian Caucasians (n=58) had no recent history of GAS infection.

#### Peptides and proteins

Synthetic peptides were made as described (18) and were purified by HPLC. All peptide sequences including class I and II peptides (3), class I substitution peptides CM1 and CM2, peptides 144, 145 and 146, the chimeric peptides J1–J8 (19), and the non-specific peptide (pNS) derived from a shistosoma tegumental antigen (8) are given in Table 1. Chimeric peptides contain 12mer amino acid sequences derived from the p145 sequence embedded within flanking sequences and were designed to maintain peptides in an α-helical conformation. Myosin used in this study was of porcine heart origin (Sigma-Aldrich, Castle Hill, NSW, Australia).

#### Detection of antibodies

Human sera was used in 2-fold dilutions from 1:100 to 1:12800 for ELISA which was carried out as previously described (8). Standard curves of optical density versus known concentrations of human IgG (0–100 ng/ml) were employed to quantitatively calculate antibody concentration. Comparison
of appropriately diluted sera samples that fell within the range of the standard curve was used to determine antibody concentration.

**ELISA inhibition studies**

Human sera used in the assay were pre-tested to determine antibody concentration to class I and II peptides, and then diluted to give an anti-peptide antibody concentration of 0.05 µg/ml. Sera were then pre-absorbed with various concentrations of competing peptide (0–5 µg/ml) and incubated at room temperature for 30 min. The mixture was then transferred to ELISA plates pre-coated with class I or II peptide and ELISA was performed as described (8,9). Results were calculated as the concentration of soluble peptide that inhibited 50% of antibody binding to peptide adsorbed to plastic (given as the IC50 value) (9).

For studies investigating peptide inhibition of class II antibodies, sera with class II antibody were diluted to 0.05 µg/ml and pre-absorbed with 10 µg competing peptide prior to incubation at room temperature for 30 min and then transferred to an ELISA plate coated with class II peptide. Class II peptides used at this concentration (10 µg) inhibited anti-class II antibodies from binding to immobilized class II peptide by >70%. ELISA was performed as described above. Percentage inhibition was calculated by comparing the mean OD at 450 nm of the sera in the presence and absence of competing peptide.

**Statistical analysis**

Geometric means were calculated and comparisons between groups of patients were made by Kruskal–Wallis one-way analysis of variance. Coefficient of correlation for linear regression was carried out by Pearson’s correlation test. A critical P < 0.05 was used for all analysis.

**Results**

**Prevalence of class-specific antibodies in endemic populations for GAS**

Sera were obtained from individuals living in areas of high GAS endemicity—Australian Aboriginals, Saudis, Thais and Indians—as well as non-endemic Australian Caucasians. For all endemic populations, clinical diagnosis was well defined, and included individuals with RHD, ARF and controls. The mean levels of serum IgG to class I and class II peptides (Table 1) were investigated for all population groups by ELISA, and antibodies were detected in 99% of Aboriginals and all Saudis, Thais and Indians tested. Aboriginal RHDs, ARFs and controls (Fig. 1a), Saudi RHDs and controls (Fig. 1b), and Thai RHD, ARF and controls (Fig. 1c) showed no significant difference in mean IgG levels to class I peptide between any of these patient groups (P > 0.05). Indian RHD patients had significantly higher levels of anti-class I IgG compared to controls (Fig. 1d; P < 0.0001). Indian controls used in this study were from affluent backgrounds, similar to Australian Caucasians, and therefore may have reduced streptococcal exposure.

Mean anti-class I IgG levels were compared between the different endemic populations to Australian Caucasians, with no recent history of streptococcal infection and low exposure to GAS. Aboriginal controls, Saudi RHDs, Thai RHDs and controls, and Indian RHDs and controls had significantly higher levels of the class I peptide antibody compared to Caucasians (Fig. 1a–d; P < 0.0001). No significant difference in antibody levels to the class I peptide was observed between Australian Caucasians and the other groups (P > 0.05).

The concentration of anti-class II peptide antibodies in Aboriginal, Saudi, Thai and Indian patients was also investigated. No significant difference in mean anti-class II peptide antibody level was seen between patient groups for all Aboriginal, Saudis, Thai and Indians (data not shown) (P > 0.05). There was no significant difference between levels of class II peptide IgG in Caucasian sera compared to any of the other patient population groups (P > 0.05).

**Relationship of class I and II antibodies in patient groups**

The relationship of class I peptide and class II peptide antibody levels in all population groups was investigated. The level of class I peptide-specific antibodies correlated directly with levels of class II antibodies for Aboriginal RHD and controls (Fig. 2a and c), Thai ARF and controls (Fig. 2e and 2f), Saudi RHD and controls (Fig. 2g and h), Indian RHD and controls (Fig. 2i and 2j), and Caucasians (Fig. 2k) (P < 0.05). No significant associations of class I and II antibody levels were observed in Aboriginal ARF and Thai RHD patients (P > 0.05) (Fig. 2b and d respectively).

**Fine epitope mapping within the class I peptide**

To determine the specificity of antibodies recognizing the class I peptide and the residues important for binding to class I, a peptide inhibition ELISA was performed using sera from Aboriginal patients with RHD (n = 8), ARF (n = 5) and controls (n = 6). Sera were pre-absorbed with class I and II peptides, derived from the conserved region of the M protein, the overlapping peptides 144 and 145, and peptide J1 (a chimeric conformational peptide) (19) (which contain a window of 17, 14 and 12 amino acids of class I sequence respectively) (Table 1). Also, two peptides, CM1 and CM2, were included which have amino acid substitutions at position 4 (Gly → Ser) and 10 (Glu → Asp) respectively of the class I peptide sequence—the two residues that differ in sequence between class I and II peptides (Table 1).

For all patients, pre-incubation of sera with low concentrations of class I peptide resulted in 50% inhibition of binding of class I-specific antibodies to class I peptide coated on the plastic (Fig. 3). The mean IC50 for the RHD, ARF, and controls in these groups were <0.1 µM. In comparison to class I peptide, IC50 ranging from 0.45 to 1.24 µM for class II, 144, 145 and J1 peptides were required to inhibit serum class I antibodies from binding to class I peptide coated on the plate (Fig. 3). The mean concentration of peptides 144, 145 and J1 to inhibit class I-specific sera from binding to class I coated on an ELISA plate by 50% (IC50) was significantly higher compared to the class I peptide for all RHD and control patients, and all patient groups when class II was the inhibiting peptide (P < 0.05). In only three of 19 cases did the non-
specific control peptide result in 50% inhibition of serum class I antibodies binding to immobilized class I peptide (Fig. 3).

In ARF and control patient sera, peptide CM1 (amino acid substitution at position four of the class I peptide) could inhibit 50% of the binding of class I-specific antibody to immobilized class I peptide to the same level as class I peptide ($P > 0.05$). For all patient sera, significantly higher levels of peptide CM2 (amino acid substitution at position 10), compared to class I peptide, were required to inhibit 50% of class I-specific antibodies from binding to immobilized class I peptide ($P < 0.05$).

**Antibody binding specificity of the class II peptide**

The specificity of class II peptide antibody binding to class II peptide was investigated. Sera from eight Aboriginals were pre-absorbed with class I, II, p145 and pNS (a non-specific peptide from Schistosoma) peptides (Table 1), prior to addition to class II peptide-coated ELISA plates. Table 2 shows that addition of class II peptide to the sera completely inhibited binding of the antibodies to class II peptide coated on the plate. Addition of p145 and class I peptide to the sera also inhibited binding of the antibodies to class II peptide coated on the plate and to the same extent as class II peptide (Table 2). Pre-absorption with pNS had no effect on antibody binding (Table 2).

To determine which residues form part of a common epitope between the class II, I and p145 peptides, a peptide inhibition ELISA was used with the overlapping peptides 144, 145 and 146 (Table 1) as inhibitors of serum antibodies from Aboriginal donors when class II peptide was the immobilized antigen. Addition of class I peptide, 144 or 145 inhibited antibody binding to class II peptide to similar levels as class II peptide (Fig. 4; $P > 0.05$). In contrast, addition of p146 to the sera did not inhibit sera binding to immobilized class II peptide (Fig. 4; $P < 0.001$).

We next used overlapping conformationally intact peptides of the p145 sequence, i.e. the chimeric peptides J1–J8 (Table I). For most sera, the addition of pJ1, pJ2 or pJ3 significantly inhibited the binding of antibodies to the class II peptide coated on the plate (Fig. 4). Addition of peptides J4–J8 did not significantly affect antibody binding to class II peptide coated on the plate. Consequently levels of peptide inhibition were significantly lower when compared to class II peptide (Fig. 4; $P < 0.001$).

**Prevalence of myosin antibodies in endemic Aboriginal sera and correlation to class I**

Recent studies have identified class I and myosin cross-reactive epitopes in ARF and uncomplicated pharyngitis sera and question whether the class I epitope is involved in ARF pathogenesis (7). To determine whether anti-myosin antibodies are prevalent in sera from Australian Aboriginal RHDs, ARFs and controls living in a streptococcal endemic region, mean anti-myosin IgG levels were investigated. Individuals from all groups had detectable anti-myosin IgG. The amount of anti-myosin IgG in control Aboriginal sera was less than seen in the RHD and ARF patient groups, but this difference was not significant (Fig. 5a; $P > 0.05$). All Aboriginal
Fig. 2. Relationship between class I and class II peptide antibody levels in sera grouped by population and disease categories. Aboriginal sera (a) RHD, (b) ARF and (c) control; Thai sera (d) RHD, (e) ARF and (f) control; Saudi sera (g) RHD and (h) control; Indian sera (i) RHD and (j) controls; and (k) non-endemic Caucasian sera.
Class I and II responses in streptococcal infection

Fig. 3. Competitive inhibition of binding by class I-specific human antibodies from Aboriginal RHD, ARF and control patients to class I by synthetic peptides. Sera were diluted to give an anti-class I antibody concentration of 0.05 µg/ml. All sera were pre-incubated with 0–5 µg/ml of inhibiting peptide prior to being reacted with class I peptide by ELISA. The concentration of the soluble peptide that inhibited 50% of antibody binding to class I peptide adsorbed to plastic (IC50, µM) was measured for each patient. Crosses represent mean peptide IC50 for each patient group.

Table 2. Competitive Inhibition of binding of class II peptide-antibodies from Aboriginal RHD, ARF and control patients by synthetic peptides

<table>
<thead>
<tr>
<th>Patient condition</th>
<th>Competing peptide (IC50) (µM)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Class I</td>
</tr>
<tr>
<td>RHD</td>
<td>0.04</td>
</tr>
<tr>
<td>RHD</td>
<td>0.12</td>
</tr>
<tr>
<td>RHD</td>
<td>0.08</td>
</tr>
<tr>
<td>ARF</td>
<td>0.08</td>
</tr>
<tr>
<td>ARF</td>
<td>0.12</td>
</tr>
<tr>
<td>Control</td>
<td>0.1</td>
</tr>
<tr>
<td>Control</td>
<td>0.16</td>
</tr>
<tr>
<td>Control</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Sera were diluted to give an anti-class II peptide antibody concentration of 0.05 µg/ml.

The concentration of the soluble peptide that inhibited 50% of antibody binding to class II peptide adsorbed to plastic (IC50, µM) was measured for each patient.

groups had significantly higher levels of myosin antibodies compared to non-endemic Caucasian controls (P < 0.01). No correlation was found between levels of class I and myosin antibodies in Aboriginal RHD, ARF and control sera and Caucasians (Fig. 5b–e respectively; P > 0.05).

Discussion

Human antibodies to class I peptides have recently been shown to cross-react with myosin (7). Several reports have also identified epitopes along the M-protein that can induce host cross-reactive antibodies (21–25), but there is limited

Fig. 4. Definition of the common epitope shared by class II, I and p145 peptides. Data is given as percentage inhibition of peptide inhibiting the binding of human sera (n = 10) to class II peptide immobilized on an ELISA plate. Crosses represent average percentage inhibition. Asterisked peptides represent a significant difference in mean percentage inhibition by peptide compared to addition of class II peptide (P < 0.001).
Class I and II responses in streptococcal infection

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Fig. 5. (a) Prevalence of myosin antibodies in endemic Aboriginal sera and correlation to class I. Serum Ig response to myosin grouped by disease categories: Aboriginal RHD, ARF and controls, and Caucasians. Crosses represent geometric mean anti-class I peptide IgG concentration (µg/ml sera). Arrows represent significant difference in mean anti-class I peptide IgG concentration (P < 0.05) between the groups. Each data point may represent more than one set of measurements. (b) Relationship between class I and myosin peptide antibody levels in Aboriginal sera grouped by disease categories, RHD, (c) ARF, (d) control and (e) non-endemic Caucasian sera.

Evidence linking these antibodies to disease (21,22,26). While previous studies suggest that ARF and/or RHD sera have significantly higher levels of anti-myosin and anti-class I antibodies than controls (4,22), this study found that individuals living in areas of high GAS endemicity, including ARFs, RHDs and controls, have high levels of antibodies to both class I and myosin. As we do not know the time when sera samples were collected post onset of RF/RHD, we cannot rule out the possibility that anti-myosin and anti-class I antibodies were further elevated at the time of onset of these diseases, however. In contrast, Australian Caucasians, with low streptococcal exposure, have significantly lower levels of both class I peptide-specific and myosin-specific antibodies compared to endemic populations. Rapid, effective penicillin treatment of individuals with streptococcal infection within the Caucasian population is likely to be another factor in the reduced levels of antibodies seen in this population.

The incidence of RF and RHD is much greater among the Australian aboriginal population than the Caucasian population. The repeated exposure to GAS and the concomitant increase in anti-streptococcal antibodies are therefore important factors in the development of RF and RHD. However, as control patients from endemic regions also have high levels of anti-class I and anti-myosin antibodies, high levels of these antibodies are not the sole cause of RF/RHD. Other groups have shown cross-reactive anti-streptococcal/anti-myosin antibodies are capable of recognizing heart tissue, including heart valves, and have also been shown to be cytotoxic for some human cells (28,29). Our data would seem to indicate that other factors (e.g. genetic predisposition) are required for elaboration of these diseases.

Although no direct association with disease pathogenesis has been shown, immunological cross-reactivity for class I epitope antibodies and myosin (7) might nevertheless have a bearing on the use of the conserved region as a broad-spectrum anti-streptococcal vaccine. The vaccine candidate, p145, overlaps the class I epitope by 14 amino acids (8,9,10,11); therefore it was important to identify which residues within the class I sequence are specifically involved in antibody binding. We showed that overlapping peptides containing the class I sequences SRKGLRRDLASRE (from p144), LRRDLASREAKKQVEK (from p145) and LRRDLASREAK (from J1) did not inhibit antibody binding to class I peptide. Of significance for vaccine development, we have shown that the conserved region candidate, p145, in almost all cases, is not recognized by class I antibodies. We have also shown that within the class I epitope, the residue at position 10 rather than position 4 is important for binding to class I antibodies as addition of a peptide with an amino acid substitution of Gly → Ser (position 4) inhibited antibody binding to class I peptide on an ELISA plate but substitution at position 10 from Asp → Glu did not.

Since the M protein is a coiled-coil protein, the conformational
presentation of the class I peptide might be important. In the
typical seven residue pattern a–b–c–d–e–f–g of a helical coil,
a + d are usually apolar residues and form part of the a-helical
core, residues e and g lie next to the core, and b, c and f are
in the outer positions (29). The Gln in position four of the class I
epitope is less likely to be exposed to the immune system than
the Ser, a hydrophilic residue, at this position in the class II
epitope. At position 10 of the class I and II epitope, both res-

ides and likely to be surface exposed on the helix. There-
fore, if Asp is a key residue, the substitution to Glu may knock
out epitope recognition. It is also interesting to note that myosin
contains the class I epitope homologous sequence LKRDID
(Table 3), maintaining the Asp as a key residue in the helix.

Antibodies to class II peptides have previously been shown
to cross-react with class I peptide (4). Antibody levels to class
II peptide in the Aboriginal, Thai, Saudi, Indian and Caucasian
individuals investigated correlated significantly with antibody
levels to class I peptide. We were able to show that class II
antibody binding could not only be inhibited by the class I
epitope but by peptides p144, p145 and three chimeric pep-
tides (J1–J3) that in total include the sequence SRKGLRDL
ASREAKKQVEK. The other chimeric peptides which include the sequences DLASREAKKQVEKAL did not
inhibit antibody binding to the class II epitope. The data suggest
that residues RDL-ASRE, shared by class I, p144, p145 and
J1–J3, form part of a common epitope recognized by class II
antibodies. Parts of this sequence are also found within the
heavy chain of myosin (Table 3), and this supports the observa-
tion that class I and II antibodies may recognize myosin
sequences.

This study has shown that both myosin- and class I-specific
antibodies are highly prevalent in all individuals, including ARF,
RHD and controls of endemic populations, and would not be
suitable as a disease marker. The class I epitope may be con-
formational and share potential structural homology with
myosin (i.e., both molecules have a similar quaternary structures
in the overlapping region of homology). However, if the M-
protein sequences are involved in the pathogenesis of ARF, it
is more likely to be through a cell-mediated mechanism. Myosin
and many other cardiac proteins are cytoplasmic, and as such
would not be available to bind to antibodies. However, T cells
recognize internal proteins of cells following processing to short
peptides, binding to MHC molecules and presentation to the
cell surface.

CD4+ and CD8+ T cells have been reported in cardiac
lesions in high numbers (30). Although normal heart myocytes
are not thought to express MHC class II molecules (31), expres-
sion could be up-regulated by some coincidental infection or
damage to the heart (32) providing a rational basis to implicat-
cell-associated pathology in the pathogenesis of ARF. Clinical
evidence has emerged supporting the role of T cells that recog-
nize selected M-protein epitopes in disease pathogenesis
(33,34). T cell epitopes within the A-, B- and C-repeat regions
of the M-protein have also been identified and some of these
epitopes include the class I sequence (23,35,36,37,10,38,39).
Further studies are required to determine whether the C-repeat
region, including class epitopes of the M-protein play a role in
RF disease pathogenesis.

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ARF</td>
<td>acute rheumatic fever</td>
</tr>
<tr>
<td>GAS</td>
<td>group A streptococcal</td>
</tr>
<tr>
<td>RF</td>
<td>rheumatic fever</td>
</tr>
<tr>
<td>RHD</td>
<td>rheumatic heart disease</td>
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References
   biologically distinct classes of group A streptococcal M protein by
   limited substitutions of amino acids within the shared region of M