Comprehensive analysis of antibody responses to streptococcal and tissue antigens in patients with acute rheumatic fever

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Abstract

Acute rheumatic fever (ARF) is an autoimmune disease occurring in individuals following untreated group A streptococcal infection believed to be triggered by antibodies to bacterial components that cross-react with human tissues. We developed a multiplexed immunoassay for the simultaneous quantitation of antibodies to nine streptococcal-related antigens including streptolysin O (SLO), DNase B, collagen I and IV, fibronectin, myosin, group A carbohydrate, M6 protein and streptococcal C5a peptidase. Utilizing this method, we examined serum from 49 ARF, 58 pharyngitis patients and age- and sex-matched controls in samples collected at initial disease onset, and at 4 weeks, 6 months and 1 year after diagnosis. Antibody responses were significantly higher for SLO, DNase B, M6 protein, group A carbohydrate and the cross-reactive antigens collagen I and myosin in ARF compared with pharyngitis patients (P ≤ 0.05). Moreover, we found significantly elevated antibody responses in the ARF patients with rheumatic heart disease to fibronectin and collagen I compared with ARF patients without heart disease. The major differences between the ARF patients with and without carditis appear to be in the immune response to the putative heart valve components, collagen I and fibronectin.

Introduction

Acute rheumatic fever (ARF) is a multifocal autoimmune disease occurring in 0.1–3% of individuals following untreated group A streptococcal infections. Arthritis, carditis, Sydenham’s chorea, erythema marginatum and subcutaneous nodules make up the five major manifestations in the updated Jones criteria for the diagnosis of ARF (1). A resurgence of rheumatic fever and rheumatic heart disease (RHD), its major clinically significant sequela, was reported in several areas of the United States in the mid 1980s and has persisted in the intermountain area surrounding Salt Lake City, UT (2–6). Throughout the developing world, however, RHD remains the leading cause of acquired heart disease in individuals <50 years of age (7).

After infection with group A streptococci (GAS), antibody responses to both cellular and extracellular antigenic components of the organism are mounted. Extracellular antigens released by streptococci include streptolysin O (SLO) and S, which act as hemolysins, the DNase isoenzymes A, B, C and D as well as streptokinase and hyaluronidase. Cellular components of GAS that have been found to elicit antibody responses, which may be opsonic and/or protective, include M protein, an anti-phagocytic surface constituent (8, 9).
streptococcal C5a peptidase (SCPA), a surface endopeptidase (10–12), and group A carbohydrate, a cell wall component (13, 14).

While the exact pathogenesis of ARF and RHD remains unknown, it is believed to involve an autoimmune response triggered by antibodies specific for components of GAS that cross-react with human tissues (15–19). Cross-reactive antibodies between regions of the M protein and cardiac tissues, particularly myosin, as well as joint and neuronal tissues have been demonstrated in numerous studies (20–24). Collagen is also a likely autoimmune target as it is the most abundant protein in mammalian connective tissue. Collagen IV is a non-fibrillar form found primarily in connective tissue and cartilage (25, 26), while fibrillar collagen I is a major component of heart valves (27).

Employing a fluorescent microsphere immunoassay system, we have developed a multiplexed assay for the simultaneous quantitation of IgG antibody responses to nine different streptococcal or human tissue cross-reactive antigens, potentially involved in the pathogenesis of ARF and RHD (28). The multiplex assay includes two extracellular antigens, DNase B and SLO, and three virulence antigens, M protein, group A carbohydrate and SCPA. In addition, four tissue antigens, which could be involved in the mimicry process, collagens I and IV, myosin and fibronectin were included. We utilized this multiplexed assay to determine antibody profiles in 49 sequentially studied rheumatic fever patients, along with 58 pharyngitis patients and equal numbers of normal, age- and sex-matched controls (AMC).

Methods

Patients

Patients were enrolled over a 3-year period at the University of Utah and Primary Children’s Medical Center, Salt Lake City, UT, USA, for the study of ARF. Three patient groups were analyzed: (i) 49 patients diagnosed with ARF based on the requirements of the updated Jones criteria (1) who had serum samples collected during the initial visit and at 4 weeks, 6 months and 1 year, (ii) 58 symptomatic, culture-positive group A streptococcal pharyngitis patients with initial, 4-week, 6-month and 1-year follow-up samples and (iii) equal numbers of normal healthy AMC obtained from the Associated Regional and University Pathologists Institute Child Diagnostics Normal Values Study. Patient demographics are presented in Tables 1 and 2. All pharyngitis patients were seen by local pediatricians who promptly administered appropriate antibiotic therapy on the first visit. The ARF patients were also routinely given an appropriate course of antibiotics when the diagnosis was established and of the 58 pharyngitis patients who were culture positive on the initial visit, only 15 (25.8%) were still positive on the 4-week follow-up visit. The mean duration between the onset of ARF and diagnosis was 3.37 weeks, with a standard deviation of 3.25 weeks. Fifty-three percent of ARF patients either had a positive culture or had remembered having a sore throat or respiratory illness in the preceding 8 weeks prior to the initial blood draw. All patient samples included in this study were approved for use by the University of Utah Institutional Review Board protocols #00008815 and #11475, respectively.

Multiplexed group A streptococcal antibody assay

We employed a flow cytometric microsphere-based profiling system (Luminex Corporation, Austin, TX, USA), which allows multiple analytes to be assayed simultaneously in a single sample (29). The development and validation of the multiplexed group A streptococcal assay has been previously described (28). Antigens were obtained from both commercial and private sources, Sigma (St Louis, MO, USA), SLO from Streptococcus pyogenes, myosin from calcium-activated porcine heart, collagen type I from calf skin, collagen type IV from human placenta and fibronectin from human plasma. DNase B was obtained from Wampole Laboratories, Cranbury, NJ, USA, SCPA was a full-length (150 kDa) recombinant protein derived from the M1 group A streptococcal strain 90226 (10–12). Highly purified M protein from the M6 strain contained the conserved and N-terminal regions of the molecule (8, 30). The group A-specific carbohydrate (ACHO) was purified from strain A374.

The nine different antigen-coupled microspheres were mixed together at a working concentration of 3000 of each

### Table 1. Demographics of ARF and pharyngitis study patients along with the AMC

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean age (years)</th>
<th>Age range (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cases</td>
<td>49</td>
<td>14.2 ± 6.1</td>
<td>4–40</td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>13.5 ± 6.7</td>
<td>4–40</td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>14.6 ± 5.8</td>
<td>7–33</td>
</tr>
<tr>
<td>Pharyngitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cases</td>
<td>58</td>
<td>15.8 ± 8.6</td>
<td>6–39</td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>13.9 ± 7.8</td>
<td>6–33</td>
</tr>
<tr>
<td>Female</td>
<td>36</td>
<td>16.9 ± 9.0</td>
<td>6–39</td>
</tr>
<tr>
<td>Pharyngitis AMC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cases</td>
<td>58</td>
<td>15.8 ± 8.5</td>
<td>7–39</td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>14.0 ± 7.8</td>
<td>7–33</td>
</tr>
<tr>
<td>Female</td>
<td>36</td>
<td>17 ± 8.9</td>
<td>7–39</td>
</tr>
</tbody>
</table>

### Table 2. Breakdown of ARF manifestations of the carditis and non-carditis patient groups

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carditis group</td>
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</tr>
<tr>
<td>Carditis only</td>
<td>7</td>
<td>26.9</td>
</tr>
<tr>
<td>Carditis and arthritis</td>
<td>14</td>
<td>53.8</td>
</tr>
<tr>
<td>Carditis and chorea</td>
<td>5</td>
<td>19.2</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Non-carditis group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthritis only</td>
<td>12</td>
<td>52</td>
</tr>
<tr>
<td>Chorea only</td>
<td>9</td>
<td>39</td>
</tr>
<tr>
<td>Other manifestation</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

Review Board protocols #00008815 and #11475, respectively.
Antibody responses in acute rheumatic fever

Initial antibody responses of 49 ARF with 58 pharyngitis patients and AMC groups for the initial, 4-week, 6-month and 1-year sample draws. Since the onset of acute rheumatic fever has a latency period of ~18–20 days after streptococcal pharyngitis (18, 31), the initial ARF samples were also compared with the 4-week pharyngitis samples. This is the time point at which antibody responses appeared to peak for these two groups.

ASO and anti-DNase B responses

The patients with ARF had significantly elevated anti-DNase B antibody concentrations compared with the pharyngitis patients (median 174.1 versus 42.3 ng ml$^{-1}$, $P = 0.000002$) and the AMC (median 174.1 versus 28.0 ng ml$^{-1}$, $P = 0.00004$) for the initial sample draw (Fig. 1). By week 4, ARF patient median values had dropped by 45%, but were still significantly higher than pharyngitis patients (96.6 versus 55.3 ng ml$^{-1}$, $P = 0.02$) and AMC ($P = 0.001$). The antibody responses in ARF patients were not significantly different for the 6-month and 1-year follow-up samples compared with either the pharyngitis or age-matched controls.

ASO responses were also significantly elevated in the ARF patients at the time of the initial draw compared with pharyngitis patients (ARF 162.2 ng ml$^{-1}$ versus pharyngitis 30.9 ng ml$^{-1}$, $P = 3.0 \times 10^{-11}$), and the AMC (33.9 ng ml$^{-1}$, $P = 0.00005$) (Fig. 2). At week 4, the ARF patients’ ASO responses were still elevated compared with pharyngitis patients (71.3 versus 46.3 ng ml$^{-1}$, $P = 0.008$) and AMC (33.9 ng ml$^{-1}$, $P = 0.002$). By 6 months, ARF patients still had significantly higher antibody responses to SLO compared with the AMC (46.6 versus 33.9 ng ml$^{-1}$, $P = 0.02$), but there were no significant differences at 1 year.

Antibody responses to group A streptococcal virulence antigens

Antibody concentrations to the M6 protein were significantly greater in the ARF patients compared with the pharyngitis patients for the initial visit (151.9 versus 47.1 ng ml$^{-1}$, $P = 1.94 \times 10^{-9}$) and at 6 months (76.5 versus 44.5 ng ml$^{-1}$, $P = 0.00003$) and 1 year (80.5 versus 50.1 ng ml$^{-1}$, $P = 0.03$) (Fig. 3). Peak antibody responses, ARF initial versus pharyngitis at week 4, were still significantly higher in the ARF group compared with pharyngitis patients. By week 4, antibody responses to M6 protein were significantly higher in both the ARF (73.2 ng ml$^{-1}$, $P = 0.00004$) and pharyngitis (75 ng ml$^{-1}$, $P = 0.0002$) patients compared with the AMC group (33 ng ml$^{-1}$). Antibody responses remained

Assay validation

Our assay for anti-streptolysin O (ASO) antibodies showed good correlation with a commercial nephelometric assay (Dade Behring, Deerfield, IL, USA) with an agreement of 100% between positive and negative samples and an $R^2$ value of 0.8594. There was also good correlation between our multiplexed assay and an enzyme inhibition assay (Wampole Laboratories) for anti-DNase B antibodies with 100% agreement between positive and negative samples. For the antigens that had no commercial or established assay, validation was accomplished by reacting mAbs or polyclonal antibodies to the antigen-coupled microspheres (28). Antibodies against collagen I, collagen IV, myosin, group A carbohydrate, fibronectin and M6 protein reacted strongly to their respective specific antigen-coupled microspheres, with only slight or no cross-reactivity to any of the other antigen-coupled microspheres.

Statistical analysis

Statistical analysis was performed to compare the antibody concentrations between patients with ARF and pharyngitis and AMC. Non-normally distributed analyte levels were transformed using the natural logarithm prior to the analysis.

For the comparison of ARF to pharyngitis patients, we compared each analyte concentration independently. We used a two-sided $t$-test for log-transformed analyte values for an equivalent time point comparison of analyte levels in patients with pharyngitis and patients with ARF. We also compared log-transformed analyte levels of patients with ARF with patients with pharyngitis as well as age-matched controls using Dunnett’s test for multiple pair-wise comparison at various time points. ARF patients were further divided into two groups, carditis and non-carditis, and analyzed with Dunnett’s test for multiple pair-wise comparisons.

Results

Initial analysis compared antibody responses of 49 ARF with 58 pharyngitis patients and AMC groups for the initial, 4-week, 6-month and 1-year sample draws. Since the onset of acute rheumatic fever has a latency period of ~18–20 days after streptococcal pharyngitis (18, 31), the initial ARF samples were also compared with the 4-week pharyngitis samples. This is the time point at which antibody responses appeared to peak for these two groups.
significantly elevated in both patient groups at 6 months (ARF 76.5 ng ml⁻¹, \( P = 0.00003 \) and pharyngitis 44.5 ng ml⁻¹, \( P = 0.03 \)) and 1 year (ARF 80.5 ng ml⁻¹, \( P = 0.027 \) and pharyngitis 50.1 ng ml⁻¹, \( P = 0.034 \)) compared with the AMC group (33 ng ml⁻¹), but were always higher in the ARF patients.

ARF patients had 2- to 3-fold higher concentrations of antibodies to group A carbohydrate at the initial time point compared with pharyngitis patients (2144.9 versus 913.6 ng ml⁻¹, \( P = 0.00007 \)) and the AMC (2144.9 versus 798.7 ng ml⁻¹, \( P = 0.008 \)) (Fig. 4). When comparing peak antibody responses, ARF initial versus pharyngitis at week 4, there were no significant differences in antibody responses to group A carbohydrate. For the remaining time points, there were no significant differences between either of the patient groups or the AMC.

Antibodies to SCPA were initially elevated in both the ARF (273.9 ng ml⁻¹, \( P = 0.00004 \)) and pharyngitis patients (211.1 ng ml⁻¹, \( P = 0.01 \)) when compared with the AMC (126.9 ng ml⁻¹) group, but no significant differences were observed between ARF and pharyngitis patients at this time point or subsequently.

Antibody responses to cross-reactive tissue antigens

Antibodies to collagen I were found to be significantly elevated in the ARF patients in the initial draw compared with pharyngitis patients (0.6 versus 0.4 ng ml⁻¹, \( P = 0.002 \)) and AMC (0.2 ng ml⁻¹, \( P = 0.00002 \)). The ARF patients still had significantly elevated antibody concentrations compared with the AMC at 4 weeks (0.5 versus 0.2 ng ml⁻¹, \( P = 0.03 \)).

Both ARF and pharyngitis patients had significantly elevated concentrations of antibody to collagen IV compared with the AMC. For the ARF patient group versus AMC, significant differences were observed at the initial (1.1 versus 0.3 ng ml⁻¹, \( P = 0.000007 \)), 4-week (0.7 versus 0.3 ng ml⁻¹, \( P = 0.003 \)) and 6-month (0.6 versus 0.3 ng ml⁻¹, \( P = 0.007 \)) time points. There were no significant differences between the ARF and pharyngitis patients for any of the time points.

Antibody responses to myosin were found to be significantly elevated on the initial draw in the ARF patients (1.8 ng ml⁻¹) compared with the pharyngitis patients (1.0 ng ml⁻¹, \( P = 0.00003 \)) and the AMC (0.9 ng ml⁻¹, \( P = 0.008 \)) (Fig. 5). Antibody responses to myosin in the ARF group were no longer significantly different when comparing the week 4 visit to the pharyngitis group at week 4, but the initial ARF group values (1.8 ng ml⁻¹) were still significantly greater than the week 4 pharyngitis group (1.1 ng ml⁻¹, \( P = 0.0005 \)). No significant differences were observed at 6 months or 1 year, nor were any differences observed between pharyngitis patients and AMC for myosin antibodies at any of the time points.

Antibody responses in carditis versus non-carditis ARF patients

We next examined serum from ARF patients with carditis (\( n = 26 \)) and compared these results with those from ARF patients without carditis (\( n = 23 \)) (Table 2). Since the onset
of ARF has a latency period of ~18–20 days after streptococcal pharyngitis (18, 31), the initial ARF samples were compared with the 4-week pharyngitis samples, the equivalent time points at which antibody responses appear to peak.

Antibody responses to M6 protein were very similar in both the carditis and non-carditis group at all three time points and showed no significant differences. When these two ARF groups were compared with the pharyngitis patients, however, antibody responses were significantly greater for all three time points: initial carditis (143.8 ng ml\(^{-1}\), \(P = 0.00003\)) and non-carditis (158.6 ng ml\(^{-1}\), \(P = 0.0007\)) versus pharyngitis (76.5 ng ml\(^{-1}\), \(P = 0.0001\)) and non-carditis (79.7 ng ml\(^{-1}\), \(P = 0.005\)) versus pharyngitis (44.5 ng ml\(^{-1}\), \(P = 0.02\)).

Antibody responses to group A carbohydrate were significantly elevated in the carditis compared with pharyngitis patients (2974 versus 1341 ng ml\(^{-1}\), \(P = 0.01\)) for the initial visit, but no significant differences were observed between the carditis and non-carditis patients. Likewise, no significant differences in antibody response to SLO were observed between the carditis and non-carditis groups.

Antibodies to collagen IV, a component of heart valves, were significantly elevated in the carditis group compared with the non-carditis group (0.8 versus 0.5 ng ml\(^{-1}\), \(P = 0.02\)) for the initial visit. Significant differences were also observed between the carditis group and pharyngitis group (\(P = 0.04\)), but not between the non-carditis and pharyngitis patients (\(P = 0.8\)). In contrast, for collagen IV, we did not find any significant differences between the carditis and non-carditis patient groups.

Antibodies to myosin were significantly elevated in both the carditis (1.8 ng ml\(^{-1}\), \(P = 0.006\)) and non-carditis (1.5 ng ml\(^{-1}\), \(P = 0.005\)) patient groups when compared with the pharyngitis patients at the first time point (1.1 ng ml\(^{-1}\)). There were no differences, however, in myosin antibody concentrations when comparing the carditis and non-carditis groups for any of the time points.

Significantly greater antibody concentrations to fibronectin were observed in the carditis compared with the non-carditis patient groups (0.6 versus 0.4 ng ml\(^{-1}\), \(P = 0.02\)) and the carditis versus pharyngitis patients (0.6 versus 0.4 ng ml\(^{-1}\), \(P = 0.002\)), at the first time point. After this initial spike, however, no further differences were observed.

**Discussion**

We have assessed antibody responses to nine different antigens associated with streptococcal infections in 49 well-defined ARF patients, 58 group A streptococcal pharyngitis patients and AMC for each patient group. Results were generated using a quantitative, objective multiplexed immunoassay as opposed to subjective assays, such as indirect immunofluorescence, reported in other studies. This is the only study, to our knowledge, which has measured all these group A streptococcal-specific and potentially cross-reactive antibodies in a well-defined population of ARF patients and compared them over time with appropriate controls.

Antibody responses to SLO and DNase B were characterized >40 years ago and are still the standard tests for the serodiagnosis of a preceding GAS infection. In patients with group A streptococcal pharyngitis, the ASO response rises ~1 week to 10 days after initial GAS infection, reaching a peak response 3 to 6 weeks later, after which they begin to decline (18, 32, 33). This was consistent with the results in our pharyngitis study group, as we observed peak antibody responses at 4 weeks, which then declined (Fig. 2). Anti-DNase B responses peak later than ASO titers and demonstrate a longer persistence of 2–3 months (18, 32, 33). This was again consistent with our findings in which the highest concentrations of anti-DNase B were observed at 6 months in our pharyngitis patients (Fig. 1). We also found...
significantly elevated ASO and anti-DNase B responses in our ARF patients compared with the pharyngitis patients, which has also been reported in numerous publications (34–36). In addition, our ASO and anti-DNase B multiplexed assays compared well with Food and Drug Administration-approved commercial assays, which are the standard in use in many diagnostic laboratories (28).

Both ARF and pharyngitis patient groups showed persistently elevated antibody responses to M6 protein for up to 1 year compared with the AMC group, which is consistent with the long-lived protective antibody response to these virulence proteins. The M6 protein utilized in our assay was the complete recombinant molecule and contained the conserved region that is found in all M protein molecules. M or emm typing was performed on the nine positive group A streptococcal cultures we obtained from the ARF patients and 23 of the pharyngitis patients. The ARF patients had predominately emm types 1, 5, 7 and 118, while pharyngitis patients were predominately 1, 3, 1, 4, 6, 12, 22 and 58. Only emm 1 was found in common between the ARF and pharyngitis patients. Since none of the ARF patients included in our study was positive for emm type 6 streptococci, the antibody response measured in our assay is most certainly directed against the conserved region of the molecule. Antibody to this conserved region of M6 protein has been associated with opsonic activity (37) and protection in a murine model of group A streptococcal infection (38, 39). M-associated proteins have also been speculated to have a role in the pathogenesis of RHD (40). Previous studies by Bessen et al. (40) using a surface-exposed conserved region of the M protein molecule showed elevated serum IgG levels to this synthetic peptide in ARF patients compared with pharyngitis and normal control groups. In agreement with Bessen, we found significantly elevated antibody concentrations to M6 protein in ARF patients compared with pharyngitis patients as well as in AMC, with antibodies in our patients persisting for at least 1 year.

Antibody responses to group A carbohydrate usually peak 1–3 weeks after initial streptococcal pharyngitis, while circulating antibodies are present in most of the adult population (41). The opsonic properties of group A carbohydrate-specific antibodies have been demonstrated in vitro (14) and have been suggested to have a protective role in mice immunized with the antigen conjugated to tetanus toxoid (42). Earlier studies by Dudding and Ayoub (34) reported group A carbohydrate antibody titers in patients with rheumatic valvulitis remained elevated for at least 1 year and even up to 20 years compared with patients with glomerulonephritis or rheumatic fever patients with no valvular involvement. As in our study, patients had received penicillin prophylaxis after the last acute episode. We found significantly elevated antibodies to group A carbohydrate in our carditis group when compared with pharyngitis patients and AMC at the initial time point. For these differences, were no longer significant at subsequent sampling times.

SCPA is a virulence factor of GAS and appears to be highly conserved in many different serotypes (12). Due to the conserved nature of the protein, immunogenicity studies have been conducted to determine the vaccine potential of SCPA (43, 44). It has also been suggested that 67% of the children studied had at least a 15% increase in antibody response to SCPA in the 4-week period after infection and that antibody response to SCPA remains elevated through adulthood (10, 43). In our studies, we found a significant initial increase in antibody concentrations in patients with either ARF or pharyngitis compared with the AMC. We did not find any significant differences between ARF and pharyngitis patients. By 6 months antibody responses in both these patient groups were comparable to that of the AMC. A recent study by Karmarker et al. (45) also found increased antibody concentrations to SCPA in Indian children and adults diagnosed with rheumatic fever or RHD when compared with normal subjects.

Structural similarities of the α-helical coiled-coil region of M protein and the rod region of cardiac myosin have been described (21, 23), and it has been proposed that immunologic mimicry between these two proteins may be involved in the pathogenesis of RHD. Antibodies to myosin were significantly elevated in our ARF patients when compared with both the pharyngitis and AMC groups at the initial time points, but these differences did not persist past 4 weeks. While we did find elevated anti-myosin antibodies in our carditis group compared with pharyngitis patients, they were also significantly elevated in the non-carditis ARF group. Collagen, a major component of connective tissue, heart valves and joint cartilage, has been theorized to be an autoimmune target in the pathogenesis of ARF and RHD. Components of connective tissue, such as hyaluronic acid and N-acetylglucosamine, are also found, or have structural similarities to components found, in the capsule and cell wall of GAS (31, 46, 47). In our study, there were elevated antibody responses in the ARF and pharyngitis patients when compared with the AMC to both collagen I, a component of heart valves, and collagen IV, which is abundant in basement membrane. When examining ARF patients with or without carditis, the carditis group had significantly elevated collagen I antibody concentrations compared with both the non-carditis and pharyngitis patients. As far as we know, this is the first published report documenting elevated antibody responses to collagen I in well-defined rheumatic fever patients. Others, Dinkla et al. (25), have shown significantly greater antibody concentrations to collagen IV in ARF compared with pharyngitis patients. Their study, however, was limited to five ARF patients with carditis and arthritis and did not include any non-carditis ARF patients. Since collagen I is a major structural component of heart valves, we suggest that these antibodies may have a significant role in the development of valve damage that represents the only long-term sequelae of ARF. We speculate that the mitral and aortic valves are affected more often because of pressure differences across these valves, resulting in greater mitral damage.

Fibronectin is a glycoprotein found on cells present on mucosal surfaces of the oral cavity, as well as a component of the extracellular matrix of heart valves and other tissues. Several fibronectin-binding proteins expressed on the cell surface of GAS have been described which aid in adherence, and later invasion of mucosal surfaces of the host (16, 48–50). To our knowledge, this is the first report on the analysis of antibodies to fibronectin in ARF and pharyngitis
patients. We found significantly elevated fibronectin antibody concentrations in the carditis patients compared with both the non-carditis ARF and pharyngitis patients.

Even though we did not find persistently elevated tissue-specific antibodies, the development of rheumatic fever is still likely due to cross-reacting antibodies causing initial damage and inflammation of the host tissue, which in turn stimulates T-cell infiltration and the initiation of a cellular immune response leading to mitral and aortic valve damage and RHD.

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Abbreviations
AMC  age- and sex-matched controls
ARF  Acute rheumatic fever
ASO  anti-streptolysin O
GAS  group A streptococci
RHD  rheumatic heart disease
SCPA  streptococcal C5a peptidase
SLO  streptolysin O

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