

## CONCISE REPORTS

## Rheumatic fever: autoantibodies against a variety of cardiac, nuclear, and streptococcal antigens

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### Abstract

**Objective**—To measure antibody titres to cardiac, nuclear, and streptococcal antigens in different groups of rheumatic fever (n = 60) and control subjects (n = 80) with the aim of identifying cross reactive antigens of potential laboratory diagnostic value.

**Methods**—Enzyme linked immunosorbent assays (ELISA), immunocytochemical, and electrophoretic techniques were used to measure titres of antibodies to a variety of cardiac, nuclear, and streptococcal antigens in seven groups comprising patients with rheumatic fever and control subjects.

**Results**—Increased concentrations of antibodies to several streptococcal and cardiac antigens, in addition to increased IgA and IgG levels, were noted in sera from patients with acute rheumatic fever and chronic rheumatic heart disease. Autoantibodies to nuclear antigens were evident in three rheumatic fever sera.

**Conclusion**—Although we were unable to identify any unique cross reactivity between cardiac and streptococcal antigens, these results demonstrate that there is an exaggerated humoral response to several cardiac, nuclear and streptococcal antigens in patients with rheumatic fever.

(*Ann Rheum Dis* 1995; 54: 740-743)

Acute rheumatic fever (ARF) and chronic rheumatic heart disease (CRHD) are characterised by the presence of autoantibodies to heart that cross react with group A streptococcal antigens.<sup>1 2</sup> The most popular theory of the disease is that molecular mimicry between bacterial and cardiac antigens initiates an autoimmune response leading to cardiovascular damage.<sup>3</sup> Although the aetiological agent of the disease has long been understood to be the group A streptococcus, the corresponding cross reactive antigen(s) has not yet been identified.

The relevant bacterial epitopes have been localised to the streptococcal M proteins and several studies have suggested myosin as the relevant cardiac antigen.<sup>4 5</sup> However, myosin antibodies are not unique to rheumatic fever and also occur in other diseases such as polymyositis and coxsackievirus B myocarditis, and in cardiac surgery patients.<sup>5 6</sup> Further-

more, cardiac autoantibodies are found in Chagas' disease, coxsackievirus B3 myocarditis, after pericardiotomy and in postmyocardial infarction syndrome, and may also be present in some normal individuals, and therefore are not unique to rheumatic fever.<sup>7</sup>

The pathogenic significance of these heart autoantibodies is difficult to establish because of the occurrence of physiological autoantibodies directed against a range of conserved self antigens, including cytoskeletal proteins such as actin and myosin.<sup>8</sup> A variety of autoantibody reactivities have been reported in rheumatic fever and this response may be consistent with either a hyperactivation of B cells or an underlying specific antigen driven cross reaction.<sup>9 10</sup>

Currently, the diagnosis of rheumatic fever is based upon the Jones Criteria (revised by the American Heart Association, 1984<sup>11</sup>). Clinical diagnosis based upon these criteria can be quite difficult and the establishment of a laboratory based diagnostic assay might therefore facilitate diagnosis. Besides attempting to understand the nature of the cross reaction(s) implicated in rheumatic fever, we aimed therefore to determine whether any set(s) of the antigens currently implicated in rheumatic fever might be of value in the establishment of such an assay.

### Patients and methods

We examined the sera of seven groups of 20 subjects each. The seven groupings comprised three groups of 'rheumatic fever sera': acute rheumatic fever (ARF) (mean age 9.1 (SD 3.6) years), children with chronic rheumatic heart disease (CRHDc) (mean age 11.7 (3.2) years), and adults with chronic rheumatic heart disease (CRHDa) (mean age 38.2 (9.7) years); two groups with other diseases: acute glomerulonephritis (AGN) (mean age 7.1 (3.6) years) and ischaemic heart disease (IHD) (mean age 56.0 (8.8) years); and two control groups: child control (mean age 10.2 (2.7) years) and adult control (mean age 34.5 (5.6) years).

Patients with rheumatic fever were examined and samples were obtained at Red Cross War Memorial Hospital and Groote Schuur Hospital in Cape Town. The diagnosis of rheumatic fever was made according to the revised Jones Criteria (American Heart Association, 1984<sup>11</sup>). All ARF patients had carditis according to the usual clinical criteria.

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Accepted for publication 15 May 1995

CRHD patients had a history of rheumatic fever with heart valve disease, had undergone no cardiac surgery, and had no medication other than penicillin prophylaxis, anticardiac failure therapy, or both. AGN and IHD patients had classical clinical symptoms of these diseases, but none had any history of rheumatic heart disease. Adult and child control subjects had no history of rheumatic fever, a clinically normal heart, no disease which might affect the immune system, and were not taking any medications at the time of the study.

#### IMMUNOCYTOCHEMISTRY AND IMMUNOFLUORESCENCE: HEART ANTIBODIES

Paraffin sections of normal human heart obtained at surgery were incubated with rheumatic fever and control sera (1:50 dilution) and specific binding was detected with a biotin-streptavidin-peroxidase system. Each serum was tested three times and scored by a blinded observer on a scale of increasing visual intensity from 1 to 4. Immunofluorescence staining of sera against frozen human heart tissue sections was performed as described by Zabriskie and Freimer.<sup>2</sup>

Mean (SD) serum concentrations of immunoglobulins IgG, IgA, and IgM for the different groups of sera

	ARF (n = 20)	AGN (n = 20)	CRHDc (n = 20)	CRHDa (n = 20)	IHD (n = 20)	Control c (n = 20)	Control a (n = 20)
IgG (g/l)	22.1 (6.0)	20 (8.2)	19.1 (4.7)	22.1 (6.0)	15.2 (4.0)	16.6 (3.8)	13.1 (2.0)
IgA (g/l)	3.2 (1.7)	1.8 (0.9)	3.5 (1.3)	4.8 (2.6)	4 (1.8)	2.1 (0.8)	2.4 (0.9)
IgM (g/l)	2.8 (1.4)	2.4 (1.5)	2.9 (1.4)	2.6 (1.4)	1.9 (1.1)	2.3 (1.0)	2.1 (1.1)

n = Number of sera in each group tested. ARF = Acute rheumatic fever; AGN = acute glomerulonephritis; CRHD = chronic rheumatic heart disease (c = child, a = adult); IHD = ischaemic heart disease; Controls: c = child, a = adult.

#### PREPARATION OF SOLUBLE HUMAN HEART ANTIGEN

Normal heart tissue obtained at postmortem examination was pulverised under liquid nitrogen, homogenised in a buffer containing protease inhibitors, centrifuged, and the pellet solubilised in 3-(3-cholamidopropyl)-dimethylammonio]-1-propane sulphonate (CHAPS) detergent. The extraction product was stored in aliquots at  $-70^{\circ}\text{C}$ .

#### ENZYME-LINKED IMMUNOSORBENT ASSAYS (ELISA)

Antibodies to heart, myosin, actin, and phosphorylase b were determined by coating polyvinyl chloride microtitration plates overnight at optimal concentrations of the antigens in bicarbonate buffer pH 9.8, and detecting specific binding using a peroxidase conjugated IgG second antibody system.

#### OTHER ANTIBODIES

Antibodies to the following antigens were measured essentially as described in the given references: type II collagen,<sup>12</sup> cardiolipin,<sup>13</sup> ribonucleoprotein (RNP) and Smith (Sm) extractable antigens,<sup>14</sup> DNA.<sup>14</sup> Antinuclear antibodies were measured at dilutions of 1:40 by indirect immunofluorescence against the human epithelial cell line HEp-2 as substrate (AFT Hep System, Behring Diagnostics). Streptolysin O antibody titres were measured using the Streptolysin O Reagent system (Technicon). DNase B antibodies were measured using the Streptokinase-B kit supplied by Wampole Laboratories.

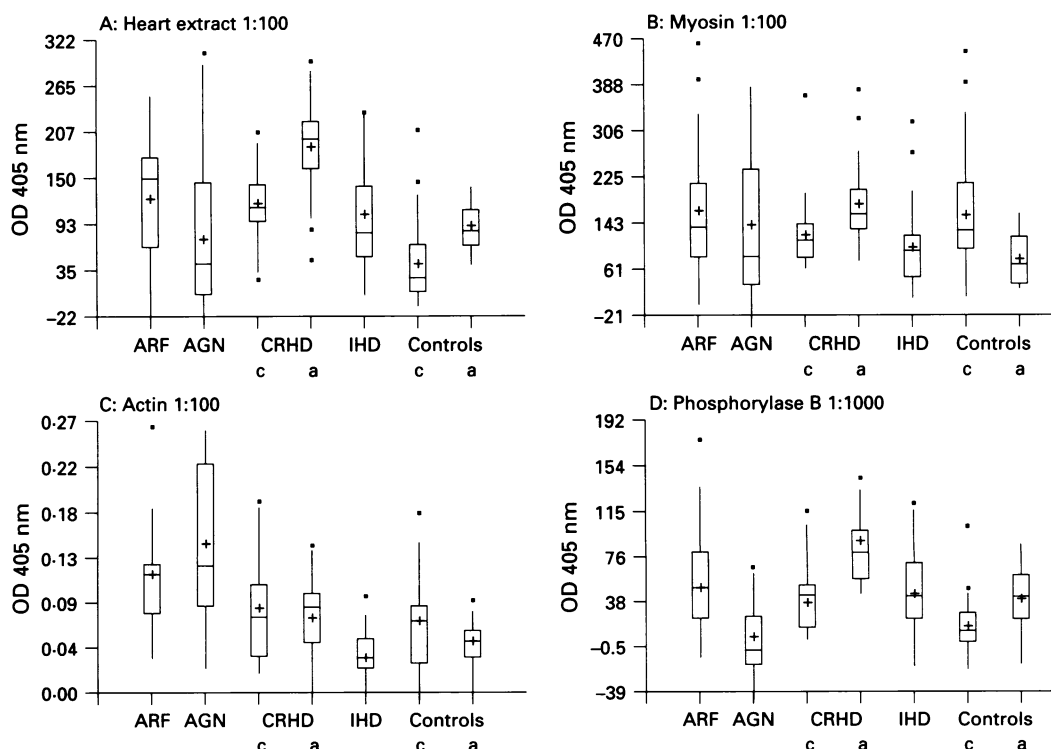


Figure 1 Antibodies to soluble heart antigens: results of binding of serum dilutions of 1:100 heart extract (A), myosin (B), actin (C), or 1:1000 phosphorylase D (D). Box plots with statistical means (+), medians (horizontal bar), interquartile ranges (boxed areas comprising values falling within the 25th and 75th percentiles), and outlying values (\*).

## STATISTICAL ANALYSIS

Differences in the various antibody measurements between the seven groups of sera were sought using the Mann-Whitney *U* test and Fischer's exact probability test. For correlations between measurements within the same group of sera, Spearman's rank correlation test was applied.

## Results

## IgA AND IgG IN RHEUMATIC FEVER SERA

The table shows serum immunoglobulin mean concentrations (IgA, IgG, and IgM) in the seven groups of sera. IgG concentrations were significantly greater in the ARF and CRHDA groups compared with controls (ARF,  $p = 0.001$ ; CRHDA,  $p = 0.0007$ ). IgA concentrations were significantly greater in all rheumatic fever groups, but were also increased in IHD sera (ARF,  $p = 0.04$ ; CRHDC,  $p = 0.0002$ ; CRHDA,  $p = 0.003$ ; IHD,  $p = 0.008$ ).

## HEART ANTIBODIES

Figure 1 illustrates a trend of higher reactivity towards certain cardiac antigens among the rheumatic fever groups of sera, especially in the CRHDA and ARF groups. However, some of the control groups clearly also contained antibodies to some of these antigens; for example AGN sera bound actin. Autoantibodies to type II collagen and cardiolipin were not increased in rheumatic fever sera. Immunoperoxidase staining of human heart sections showed that 80% of rheumatic fever sera and 25–65% of CRHD sera stained positively at intensities of 2+ and greater (results not shown).

## ANTINUCLEAR ANTIBODIES

Three rheumatic fever sera displayed a speckled positive immunofluorescence staining pattern against HEP-2 cell nuclei, indicative of antinuclear antibodies. Two ARF sera contained antibodies against the Sm and RNP extractable antigens, but none of the sera contained DNA antibodies.

## STREPTOCOCCAL ANTIBODIES

Antibody binding to streptococcal proteins M5, M6, and M19 (fig 2) and to M1, M3, and M24 (not shown) indicated a trend of increased binding in the ARF and CRHD groups of sera. A similar trend was seen in the binding to the heart antigens (fig 1). ARF patients exhibited increased levels of antibody to streptolysin O and DNase B antigens but, unexpectedly, the control groups and IHD group also showed some binding to these antigens.

## Discussion

The results do not demonstrate any obvious single set of antibodies to cardiac tissue and streptococcal antigens underlying a cross reaction between the antigens. Autoantibodies

and streptococcal antibodies against certain antigens were increased in the rheumatic fever groups, but also in some control groups of sera and were thus not unique to rheumatic fever sera. The latter appeared, however, to have significantly increased levels of antibody to a greater number of streptococcal and cardiac antigens, in addition to greater concentrations of IgA and IgG, indicating that the humoral response may be somewhat exaggerated in rheumatic fever.

These results confirm findings of previous investigators that sera of ARF patients contain antibodies to heart tissue and to certain streptococcal antigens.<sup>1 15</sup> In addition, our results demonstrate that ARF and CRHD sera also contain antibodies to some nuclear antigens. Such antinuclear antibodies have been noted in other autoimmune diseases such as systemic lupus erythematosus. Boonpucknavig *et al*<sup>10</sup> have reported evidence of anti-mitochondrial and smooth muscle antibodies in patients with rheumatic fever and CRHD. The significance of antibodies to nuclear antigens in rheumatic fever remains unclear, however.

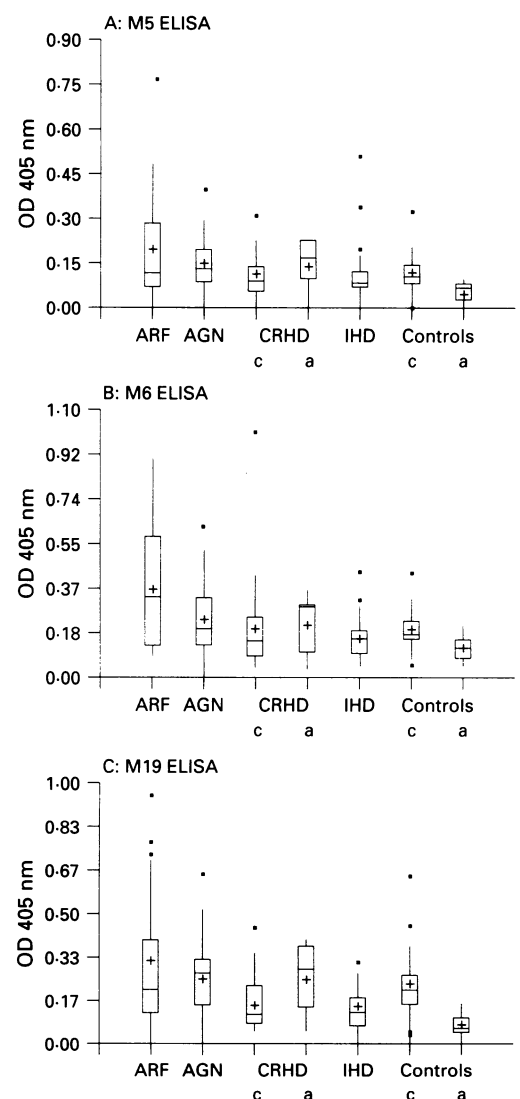


Figure 2 Antibody reactivity against group A streptococcal M proteins at serum dilutions of 1:500. A = M5; B = M6; C = M19. Statistical parameters as in figure 1.

Some recent investigations have focused on the role of streptococcal proteins as superantigens involved in rheumatic fever.<sup>16</sup> Other research also supports a role for cell mediated immunity in the pathogenesis of rheumatic fever, and it is thus probable that both arms of the immune system are involved. In this study, however, we have focused on only the humoral response by attempting to define antigen(s) peculiarly reactive with rheumatic fever sera. Although patients with rheumatic fever demonstrate increased levels of antibodies to a variety of cardiac, nuclear, and streptococcal antigens, solid phase antibody binding techniques are not sufficiently discriminatory for the evaluation of cross reactivity in this disease. We could demonstrate no unique set of cross reactive antigens, but our results do not exclude the possibility of a cross reactive epitope underlying the pathogenesis of the disease. Finer analyses may provide evidence of an epitope shared between cardiac and streptococcal antigens.

We thank the following for their assistance: Dr James Dale, University of Tennessee, Memphis (USA), for measuring antibodies to streptococcal M proteins; Rose Cooper, Margaret Cooper, and Renee Allin for performing ELISAs; Ms Jane Hall for the statistical analyses; and Mrs Vicki Melly for secretarial assistance. The study was supported by the South African Arthritis Foundation, the South African Heart Foundation, and the Institute of Child Health, University of Cape Town.

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