Effect of Valvular Surgery on Antibody to the Group A Streptococcal Carbohydrate

By Elia M. Ayoub, M.D., Angelo Taranta, M.D., and Thomas D. Bartley, M.D.

SUMMARY

Because previous studies revealed that high levels of antibody to the group A streptococcal carbohydrate (A-antibody) persist in the serum of patients with chronic rheumatic valvular disease, the effect of valvular surgery on the level of this antibody was examined in the present study. Streptococcal antibody titers (ASO, anti-DNAse B, and A-antibody) were determined on 73 patients admitted for cardiac surgery: 36 with chronic rheumatic valvular disease and 37 with nonrheumatic cardiac or coronary disease. While the ASO and anti-DNAse B presurgical titers were similar in rheumatic and nonrheumatic individuals, the levels of A-antibody were significantly higher in rheumatic patients. The effect of blood perfusion during cardiac surgery was reflected by reversion of antibody titers towards the mean value for a normal population. A trend toward a rise in all antibodies was seen in all patients two to seven weeks after surgery. However, after an interval of eight weeks or more following surgery, a significant decline in the A-antibody occurred in rheumatic patients who had undergone excision and replacement of the affected valve, but not in those who had experienced a simple commissurotomy. These findings provide additional evidence to support a relationship between valvular disease and A-antibody level in rheumatic patients.

Additional Indexing Words:
Rheumatic valvular disease
Commissurotomy
ASO Anti-DNAse B
Congenital heart disease
Valvectomy
Coronary occlusive disease
Streptococcal antibodies

RECENT STUDIES suggest that antibody to components of the group A streptococcal cell may be involved in the pathogenesis of rheumatic heart disease. Both antibodies to M-protein or the M-associated protein, as well as antibody to the protoplast membrane, have been incriminated in the pathogenesis of myocarditis.14 The description by Goldstein, Robert, and Halpert9,10 of an immunological relationship between the group A carbohydrate and a glycopeptide extracted from cardiac valve tissue suggests that the pathogenesis of valvulitis may be related to this shared antigenicity. Studies in our laboratory have shown that elevated levels of antibody to the streptococcal group A carbohydrate (A-antibody) persisted for prolonged periods in patients with chronic rheumatic valvular heart disease.15 In contrast, observations on patients with congenital valvular disease have shown serum A-antibody levels to be in the normal range.13,14 These findings lend support to the possibility of a pathogenetic relationship between the presence of A-antibody and chronic rheumatic valvular damage. Further evidence for such a relationship was sought in the present study by examining the effect of valvuloplasty and valvectomy on the level of the serum A-antibody in rheumatic patients undergoing valvular surgery.

Materials and Methods

Seventy-three patients admitted for cardiac surgery either at the Shands Teaching Hospital of the University of Florida Medical Center or at the Bellevue Hospital of the New York University Medical School were included in this study. These consisted of patients with rheumatic valvular disease, congenital heart disease, and coronary occlusive disease (table 1). The medical records of each patient were reviewed and their assignment to a rheumatic etiology was predicated on a history compatible with a prior episode of acute rheumatic fever, with manifestations fulfilling the modified Jones Criteria,19 and the presence at surgery of findings consistent with rheumatic valvular disease.

Of the 36 patients with rheumatic valvular disease, 19 had undergone excision and replacement of a single valve with a prosthetic device, the mitral valve was replaced in 13, and the aortic valve in six patients. Fifteen patients had ex-
Table 1
Nature and Incidence of Cardiac Disease in Rheumatic Patients and Nonrheumatic Patients

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Rheumatic Valvular Disease</td>
<td></td>
</tr>
<tr>
<td>Mitral</td>
<td>22</td>
</tr>
<tr>
<td>Aortic</td>
<td>4</td>
</tr>
<tr>
<td>Mitral and Aortic</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>36</strong></td>
</tr>
<tr>
<td>II. Calcific Bicuspid Aortic Valve</td>
<td>6</td>
</tr>
<tr>
<td>III. Nonrheumatic Disease</td>
<td></td>
</tr>
<tr>
<td>Congenital</td>
<td></td>
</tr>
<tr>
<td>Aortic Stenosis</td>
<td>1</td>
</tr>
<tr>
<td>Endocardial Cushion Defect</td>
<td>4</td>
</tr>
<tr>
<td>Pulmonic Stenosis</td>
<td>2</td>
</tr>
<tr>
<td>Pulmonic Stenosis and VSD</td>
<td>2</td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>6</td>
</tr>
<tr>
<td>Atrial Septal Defect</td>
<td>3</td>
</tr>
<tr>
<td>Patent Ductus</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>19</strong></td>
</tr>
<tr>
<td>Acquired</td>
<td></td>
</tr>
<tr>
<td>Coronary Occlusive Disease</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>31</strong></td>
</tr>
</tbody>
</table>

Abbreviation: VSD = ventricular septal defect.

Experienced repair of the valve in situ, either by annuloplasty or commissurotomy; all 15 individuals underwent repair of the mitral valve. The remaining two patients had been subjected to surgical repair of both the mitral and aortic valves; one patient underwent repair of both valves in situ while the other underwent annuloplasty of the mitral valve and replacement of the aortic valve.

Six additional patients admitted with a diagnosis of rheumatic aortic insufficiency and/or stenosis without mitral disease were found to have calcific bicuspid aortic valves. These patients, in whom the aortic valve had been replaced, did not have a prior history of a rheumatic attack and were therefore excluded from the rheumatic group and considered as a separate category.

Thirty-one nonrheumatic patients admitted for open heart surgery, either for a congenital defect (19 patients) or for coronary occlusive disease (12 patients), served as controls.

The ages of the patients with rheumatic heart disease at the time of surgery ranged from 12 to 64 years with a median age of 31 years. The group of patients undergoing valvuloplasty was younger than those undergoing valvular replacement, the median ages being 15 and 47 years, respectively. The age range of the patients with nonrheumatic heart disease was 4 to 69 years, with a median age of 33 years. The sex distribution of the patients with rheumatic heart disease was even—20 males and 22 females. Eighteen of the nonrheumatic patients were males and 13 were females; all but one of the 12 patients undergoing surgery for coronary occlusive disease were males.

Blood was obtained by venipuncture prior to surgery. Thereafter, blood samples were drawn on the first operative day and subsequently at weekly or biweekly intervals for a period of two to three months. Sporadic bleedings were obtained on some of these patients several months after surgery. The sera were separated under aseptic conditions and stored at −10°C until the time of assay. The sera from each patient were assayed simultaneously for each antibody in order to evaluate more accurately variations in the titers.

Assays for anti-streptolysin O (ASO) and anti-desoxyribonuclease B (anti-DNAse B) were performed using microtechniques previously described. The serum dilution scheme for the ASO and anti-DNAse B titers was based on a logarithmic scale, with 0.1 log separating each of the consecutive tube dilutions. Inspection of individual or group differences is based on differences in logs of titers or geometric means of titers respectively, the values obtained for the ASO and anti-DNAse B were reported as logs of antibody titers. The antibody to the streptococcal group A carbohydrate (A-antibody) was determined by the radioimmunoassay technique. The antibody levels in this test represent the fraction of the total amount of radioactive antigen precipitated by a constant amount (0.2 ml) of serum. The antibody scale for this test was from 0.00 to 1.00 "units," with the antibody levels of various sera differing by multiples of 0.01 units. All three antibody tests were assayed on the sera of each patient except in a few instances where the quantity of serum available was insufficient to determine either the ASO or anti-DNAse B titers.

**Results**

Distribution of Streptococcal Antibodies in Patients Prior to Surgery

The distribution of the streptococcal antibody titers obtained prior to surgery for the rheumatic patients and nonrheumatic controls is outlined in figure 1. The ASO titers of the control and rheumatic groups of

![Figure 1](http://circ.ahajournals.org/)

Distribution of presurgical ASO, anti-DNAse B, and A-antibody titers obtained for 31 nonrheumatic patients with congenital or acquired heart disease and 36 patients with rheumatic valvular disease. The broken lines represent the means for the values of each group.
patients are similar. The mean log titer for the anti-DNAse B titers is somewhat higher for the rheumatic group, but the difference from that of the control group is not significant \((P > 0.05)\). The distribution of the A-antibody levels reveals the same pattern observed for similar populations in previous studies.\(^{12,18}\) The mean for the nonrheumatic group \((0.44)\) is significantly lower than that obtained for the rheumatic group \((0.62)\) with a \(P\) value of < 0.01.

The data were examined to determine if differences in antibody titer distribution were associated with a specific type of rheumatic valvular involvement. Of the 36 patients with rheumatic valvular disease, 28 had predominant mitral disease, with six of the 28 showing evidence of minimal aortic or tricuspid insufficiency. In six rheumatic patients with predominant aortic disease, two of the patients had evidence of mild mitral insufficiency. The remaining two patients had severe involvement of both aortic and mitral valves. Distribution of the ASO, anti-DNAse B, and A-antibody titers for the patients with predominant rheumatic mitral valvular disease are shown in figure 2, and contrasted with the distributions of antibody titers obtained on the rheumatic patients with predominant aortic valvular disease. The distributions of titers for the six patients with calcific bicuspid aortic valvular disease are also included for comparison. The means of titers for these groups showed no significant difference for the ASO and anti-DNAse B titers. The distribution and mean of the A-antibody levels for the patients with rheumatic mitral and aortic disease are not significantly different \((\text{mean titers, } 0.59 \text{ and } 0.73; \ P = 0.3)\). Despite the small number of determinations, the A-antibody levels were significantly higher in patients with rheumatic aortic disease than in patients with congenital aortic disease \((\text{mean titers, } 0.73 \text{ and } 0.39; \ P = 0.02)\).

Effect of Blood Exchange on Antibody Level

The effect of the exchange transfusion which occurs during open heart surgery on the level of the antibody titers was investigated. There were 50 patients — 22 rheumatic and 28 nonrheumatic — from whom serum samples had been obtained prior to surgery and again on the first postoperative day. A comparison of the antibody titers obtained on the preoperative and postoperative sera demonstrated a trend for the titers obtained on the first postoperative day to revert toward the mean value for a normal population. Analysis of the data showed that the means for the antibody titers obtained for the nonrheumatic population were 120 for the ASO, 120 for the anti-DNAse B, and 0.44 for the A-antibody. As shown in table 2, a significant rise in antibody titer on the first postoperative day occurred primarily in those patients whose preoperative titers were below the mean, while a decline in titer occurred mostly in patients whose antibody titers were higher than the mean titer. This trend was seen for all three antibody tests. When the changes which occurred in the rheumatic patients were compared with those of the nonrheumatic patients, both groups were found to be similar.

Effect of Cardiac Surgery on the ASO and Anti-DNAse B Titers

The effect of cardiac surgery on the levels of the various antibodies was examined. Changes from the

![Figure 2](https://example.com/f2.png)

**Figure 2**

Distribution of presurgical antibody titers \((\text{ASO, anti-DNAse B and } A\text{-antibody})\) for 30 patients with predominant rheumatic mitral disease, six patients with predominant rheumatic aortic disease, and six patients with nonrheumatic calcific bicuspid aortic valves. The broken lines represent the means for the values for each group.

**Table 2**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Presurgical antibody titer</th>
<th>Number of patients</th>
<th>Number of patients showing significant changes*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;=120</td>
<td>18</td>
<td>9 9 9 0</td>
</tr>
<tr>
<td></td>
<td>=120</td>
<td>9</td>
<td>0 8 1</td>
</tr>
<tr>
<td></td>
<td>&gt;120</td>
<td>22</td>
<td>0 13 9</td>
</tr>
<tr>
<td>Anti-DNAse B</td>
<td>&lt;=120</td>
<td>19</td>
<td>9 10 0</td>
</tr>
<tr>
<td></td>
<td>=120</td>
<td>4</td>
<td>0 4 0</td>
</tr>
<tr>
<td></td>
<td>&gt;120</td>
<td>27</td>
<td>2 15 10</td>
</tr>
<tr>
<td>A-Antibody</td>
<td>&lt;=0.44</td>
<td>20</td>
<td>17 2 1</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>1</td>
<td>1 0 0</td>
</tr>
<tr>
<td></td>
<td>&gt;0.44</td>
<td>29</td>
<td>3 9 17</td>
</tr>
</tbody>
</table>

*Significant change \(= 0.2 \log\) for ASO and anti-DNAse B; \(= 0.05\) for the A-antibody level.
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preoperative ASO, anti-DNAse B titers and A-antibody levels were determined at various intervals during the postoperative period. No striking difference was observed when the nonrheumatic patients were compared with the rheumatic patients as a whole group. Further analysis of the data was carried out by dividing the rheumatic patients into two groups: those patients who had annuloplasty or a commissurotomy without removal of the valve and those patients whose affected valves were resected and replaced by protheses. The results of this analysis are graphed in figures 3-5; the mean changes in antibodies from the preoperative level are shown at varying intervals during the postoperative period for the three patient categories. To assess the significance of these changes, the 95% confidence limit (± 2 standard errors of the mean multiplied by the correction factor for small numbers at the 95% confidence limit19) was calculated for each mean value. Changes exceeding the 95% confidence limit were considered significant.

As shown in figure 3, a significant rise in ASO titers occurred at 2-4 weeks in the nonrheumatic patients. In this group of patients, the ASO titers showed a tendency to remain elevated throughout the follow-up period, in contrast to the rheumatic population where the general tendency for the ASO was to decline. Similarly, the anti-DNAse B showed a significant rise at 2-4 weeks, as well as at the 5-7 week postoperative period for the nonrheumatic patients (fig. 4). In both rheumatic categories, an initial decline in the anti-DNAse B was noted during the first week followed by

**Figure 3**

Changes in ASO titers following surgery expressed as the mean of the difference for the determinations (number in brackets) performed at each interval. The vertical lines represent the 95% confidence limits for the values. Mean differences are considered significant when the 95% confidence limit does not reach or exceed the baseline (zero line).

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**Figure 4**

Changes in anti-DNAse B titers, from the presurgical levels in nonrheumatic and rheumatic patients following cardiac surgery. (See figure 3 for explanation.)

a rise during the subsequent 2-7 week interval, then by a reversion to the presurgical levels.

Changes in A-Antibody Level Following Surgery

The changes in the A-antibody levels for the three patient categories are shown in figure 5. The nonrheumatic controls and rheumatic patients undergoing commissurotomy showed a tendency toward a rise in the A-antibody levels throughout the 2-month post-surgical period. In contrast, rheumatic patients undergoing valvectomy showed a trend toward a postoperative decline in the antibody level which was significant eight weeks and later after surgery. Because antibody titers observed at eight weeks

**Figure 5**

Changes in A-antibody level, from the presurgical levels in nonrheumatic and rheumatic patients following cardiac surgery. (See figure 3 for explanation.)
should reflect primarily the host's own response, further evaluations were made to validate this difference in antibody levels between the two groups in the rheumatic category.

A comparison of the presurgical titers and the follow-up titers obtained at eight weeks or later in these two categories of patients was carried out. Included in this comparison were 15 patients who underwent commissurotomy and 12 patients who had valvectomy, from whom sera had been obtained prior to surgery and also eight weeks or later following surgery. A comparison of the presurgical A-antibody levels obtained on these two groups of patients showed that they were not significantly different (means, 0.71 and 0.61; \( P \) value > 0.2). The changes in titer from the presurgical to the postsurgical level were analyzed for each group, confirming that the change for the commissurotomy group was not significant (\( P = 0.1 \)) but that the change for the valvectomy group was significant (\( P = 0.01 \)). To confirm this difference, further analysis was carried out on the individual differences between the presurgical and the postsurgical antibody levels obtained for each patient. The values obtained, which are plotted on figure 6, were found to be significantly different (\( P < 0.001 \)). In addition, it can be seen that the majority of the patients undergoing commissurotomy showed a rise in the A-antibody level, while almost all patients undergoing valvectomy experienced a decline in their A-antibody level. An analysis of the frequency with which the patients in each group showed a rise or a decline in titer, i.e., values greater or lower than the 'O' baseline, yielded a \( \chi^2 \) of 7.2 (\( P < 0.01 \)).

![Figure 6](attachment:image.png)

**Figure 6**

Distribution of individual differences between the presurgical A-antibody levels and levels obtained eight weeks or later after surgery for rheumatic patients undergoing commissurotomy and those undergoing valve resection.

**Discussion**

The description by Goldstein and his coworkers\(^9\)-\(^11\) of an immunological relationship between the group A streptococcal carbohydrate and the cardiac valvular glycoprotein has raised speculation regarding the role of antibody to this streptococcal antigen in the pathogenesis of rheumatic valvular heart disease. These investigators showed that group A streptococcal antiserum reacted with glycopeptides extracted from bovine or human heart valves. Recent work by Kasp-Grochowska et al.\(^10\),\(^20\) failed to confirm a cross-reaction between bovine valve glycopeptides and the streptococcal group A antiserum. These latter investigators suggested that the precipitin reaction obtained by Goldstein et al. might be nonspecific or related to the use of Freund's adjuvant. No studies on human valve glycopeptides were performed by Kasp-Grochowska and coworkers, and the possible relationship of the group A streptococcus to the human valve antigens awaits confirmation.

Previous studies in our laboratory have shown persistence of antibody to the streptococcal group A carbohydrate in patients with rheumatic valvular disease.\(^12\) Because these studies suggested a relationship between this antibody and rheumatic valvular disease, the present study was carried out to determine the effect of valvular surgery in rheumatic patients on the A-antibody level. The data obtained initially showed variable changes in the antibody to the various streptococcal antigens with no particular difference in the changes observed between the rheumatic and non-rheumatic patients undergoing open heart surgery. Closer examination revealed some significant differences, however. These differences, some of which were common to all patients and otherspecific to one group, pertained to apparent changes in antibody levels at various intervals following cardiac surgery, as well as to changes within the rheumatic group which related to whether the valve was resected or left in situ.

The first change observed immediately postoperatively occurred in all patient categories studied. A decline or rise which was related to the presurgical antibody titer occurred in the various antibodies. As shown, this change represented the effect of exchange transfusion concurrent with the use of the pump oxygenator and replacement of the patient's blood with blood from normal donors. While this change was most prominent during the first 24 hours after surgery for all three antibodies tested, the trend persisted partially through the first postoperative week for the ASO and anti-DNase B titers.

The second observation, which was less uniform, was the change in antibody titers during the following
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4–6 weeks. A significant rise in the ASO and anti-DNAse B as well as a trend for a change in the same direction for the A-antibody was observed in the non-rheumatic population. In the rheumatic patients undergoing commissurotomy, the ASO change was variable, while the anti-DNAse B and A-antibody levels tended to show a rise. These changes are similar to those observed by other investigators. Stoller et al. reported a significant rise in streptococcal antibody titers in 26% of rheumatic patients who underwent commissurotomy; 10 of the 14 patients who showed a rise in antibody manifested the rise in ASO within one month of surgery. This rise occurred despite the fact that all these patients were receiving antibiotics during that period of time. Parallel to this observation and to our present observation on the rise in A-antibody in patients undergoing commissurotomy is the report by Kaplan on the occurrence of circulating antibody reactive with myocardial subsarcolemma as well as the reporting by Zabriskie et al. of the presence of heart-reactive antibody in the sera of all the postcardiotomy patients studied. In his study, Kaplan reports that while the antibody was present in the serum of 15% of patients with rheumatic heart disease, it was found in 69% of rheumatic patients after valvuloplasty. Of particular interest is the fact that studies on some of those patients showed absence of these antibodies five days after surgery, followed by the appearance of this antibody 8–21 days after valvuloplasty.

In our present study the controls, consisting of non-rheumatic patients who had undergone open heart surgery, showed a postoperative rise in all antibodies similar to that observed for the anti-DNAse B and the A-antibody in the rheumatic patients undergoing commissurotomy. That this postoperative rise in antibody, which occurs both in the nonrheumatic and the rheumatic undergoing commissurotomy, could represent a nonspecific response is suggested by prior studies. In his review on the nature of specific and nonspecific immunologic stimuli, Freund characterizes the nonspecific antibody response as showing an initial rise, followed by a fall and another rise. These changes, which occur within the first three weeks in immunized animals, could apply to the variations seen for the ASO and anti-DNAse B titers in the control and commissurotomy groups. Further evidence that this rise could also reflect a nonspecific delayed effect of exchange transfusion is suggested by the recent studies of Branda et al. and Greenblatt et al. Branda et al. found that during a primary response to bovine serum albumin in rabbits, exchange transfusion was followed by an antibody rebound to about twice the pre-exchange levels. Working with rabbits immunized with group A and C streptococci, Greenblatt et al. showed that following exchange transfusions with normal blood the level of antibody to the streptococcal polysaccharide returned to near pre-exchange levels within 48-72 hours, and, in fact, in a few instances the antibody level exceeded the pretransfusion level.

The change in antibody titers in the rheumatic patients who underwent excision of the affected valve are of particular interest. While the changes in ASO and anti-DNAse B, which were not statistically significant, are somewhat similar to those observed for the rheumatic patients undergoing commissurotomy, the changes in A-antibody levels are unique and contrast with those seen for the commissurotomy group and for the nonrheumatic group. A decline in this antibody was observed in patients undergoing valve resection which became significant eight weeks after surgery. This decrease in antibody is of particular importance as it occurs at a time which exceeds the half-life of administered gammaglobulin and, hence, cannot be ascribed to passively administered antibody. This change, therefore, should reflect an intrinsic alteration in the host's own antibody response.

The data in this study appear to provide additional evidence to support a relationship between the A-antibody level and rheumatic valvular disease. The present findings complement the previous observation on the persistence of this antibody in patients with chronic rheumatic valvular disease. The observed decline in A-antibody following valvular excision but not following valvuloplasty, suggests that the stimulus responsible for the maintenance of the high presurgical A-antibody levels has been removed in the former group of patients. Whether this stimulus is derived from cross-reactive valvular glycoprotein, as suggested by the work of Goldstein et al., or from the slow degradation and persistence of the streptococcal carbohydrate within the diseased valvular tissue, as suggested by other investigators, remains to be clarified.

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