CIRCULATING ANTI-HEART ANTIBODIES IN HEART DISEASES DETECTED USING AN IMMUNOFLUORESCENT TECHNIQUE

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Circulating anti-heart antibodies (C-AHAb) were examined in the sera of 132 patients with heart diseases and of 65 normal healthy controls using an indirect immunofluorescent technique. The patient groups consisted of 31 with dilated cardiomyopathy, 22 with hypertrophic cardiomyopathy, 38 with myocardial infarction, 15 with angina pectoris and 26 with rheumatic valvular disease. The indirect immunofluorescent staining patterns were classified into 4 types: "heterophile", "intracellular", "intercalated disc" and "nuclear" patterns. The positive incidence of C-AHAb was 81% in dilated cardiomyopathy, 77% in hypertrophic cardiomyopathy, 65% in rheumatic valvular disease, 58% in myocardial infarction, 40% in angina pectoris and 39% in normal healthy controls. A high rate of "heterophile" antibodies was found in the idiopathic cardiomyopathy group: 65% in dilated cardiomyopathy and 55% in hypertrophic cardiomyopathy. The intracellular staining pattern was present in 42% of rheumatic valvular disease. These findings suggest that positive C-AHAb and staining patterns may be useful in the assessment of the pathogenesis of certain types of heart diseases.

CIRCULATING anti-heart antibodies (C-AHAb) have been demonstrated in some diseases affecting the heart, including postmyocardial infarction syndrome, idiopathic cardiomyopathy (ICM), rheumatic valvular disease (RVD), Chagas' disease and so on. Moreover, a variety of C-AHAb have been reported using an indirect immunofluorescent (IF) technique, but the role of C-AHAb in the pathogenesis of heart diseases is not clear. Recently, Nicholson et al. have pointed out that confusion arises by using the term "C-AHAb", and stressed the importance of distinguishing between heart/muscle specific antibodies and non-tissue specific antibodies, especially the heterophile antibodies. Some investigators have reported that these antibodies are pathogenic to heart tissue, whereas others reported that these antibodies represent only a nonspecific immunologic response to the release of endogenous cardiac antigen after myocardial damage.

The purpose of the present study is to examine the different staining patterns of C-AHAb by an indirect IF technique and to determine their incidence in some categories of heart disease.

MATERIALS AND METHODS

Sera from a total of 132 patients with heart diseases and from 65 normal healthy controls (NC) were collected. There were 53 patients with idiopathic cardiomyopathy (ICM), ranging

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Key Words:
- Anti-heart antibodies
- Immunofluorescence
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- Idiopathic cardiomyopathy

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Fig. 1. Fluorescence photomicrographs of rat heart sections demonstrating positive heart-reactive antibody using an indirect immunofluorescent technique.

a: Reaction of heterophile antibody in cross section, showing the endothelia of blood vessels and the sarcolemmal-subsarcolemma staining (x400).
b: Reaction of intracellular antibody in longitudinal section, showing intense broad striational staining and/or intermyofibrillar staining (x400).
c: Longitudinal section showing the intercalated disc staining (x400).
d: Cross section showing nuclear staining (x400).

in age from 22 to 77 with a mean age of 48.2: 31 with dilated cardiomyopathy (DCM) and 22 with hypertrophic cardiomyopathy (HCM). Other patient groups included were as follows: myocardial infarction (MI), 38 patients (age range 34-74, mean 63.7); angina pectoris (AP), 15 patients (age range 37-63, mean 51.0) and rheumatic valvular disease (RVD), 26 patients (age range 30-74, mean 55.2). The control group included 65 normal healthy adults (age range 21-84, mean 42.6).

The diagnoses were made using echocardiography, phonocardiography, cardiac catheterization, coronary angiography, right ventricular endomyocardial biopsy and other clinical methods as necessary. Serum samples from patients with myocardial infarction were obtained 4 to 5 weeks after the onset of a myocardial infarction attack.

An indirect IF technique was employed to detect C-AHAb by the modified method of Choi and Reiner. The rat heart was removed immediately after sacrifice and rinsed with cold saline to remove visible traces of blood. The ventricular wall of the heart was cut into small blocks, fixed in cold 95% ethanol, and embedded in paraffin at 58°C. Sections were cut at 4 μm, placed on micro-slide glasses, and deparaffinized. The slides were washed for 15 min in 0.01 M phosphate buffered saline (pH 7.2) 3 times.

The serum was diluted to 1:10 with the same buffer, placed on the heart tissue section, and incubated for 50 min in a moist chamber at room temperature. After washing 3 times in the buffer, the specimens were incubated for 30 min with a 1:10 FITC conjugated rabbit anti-human IgG or IgM serum, absorbed with the liver powder of rats and mice. The sections were then washed for 45 min in the buffer 3 times, mounted in 90%
TABLE I  FREQUENCY OF CIRCULATING ANTI-HEART ANTIBODIES AND STAINING PATTERNS IN PATIENTS WITH HEART DISEASES

<table>
<thead>
<tr>
<th></th>
<th>Total No.</th>
<th>Positive</th>
<th>HE</th>
<th>IC</th>
<th>Nuc</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>ICM</td>
<td>(53)</td>
<td>(42)</td>
<td>(79.2)*</td>
<td>(32)</td>
<td>(60.4)**</td>
<td>(13)</td>
</tr>
<tr>
<td>DCM</td>
<td>31</td>
<td>25</td>
<td>80.6*</td>
<td>20</td>
<td>64.5**</td>
<td>8</td>
</tr>
<tr>
<td>HCM</td>
<td>22</td>
<td>17</td>
<td>77.3*</td>
<td>12</td>
<td>54.5**</td>
<td>5</td>
</tr>
<tr>
<td>MI</td>
<td>38</td>
<td>22</td>
<td>57.9</td>
<td>15</td>
<td>39.5</td>
<td>8</td>
</tr>
<tr>
<td>AP</td>
<td>15</td>
<td>6</td>
<td>40.0</td>
<td>4</td>
<td>26.7</td>
<td>4</td>
</tr>
<tr>
<td>RVD</td>
<td>26</td>
<td>17</td>
<td>65.4*</td>
<td>6</td>
<td>23.1</td>
<td>11</td>
</tr>
<tr>
<td>NC</td>
<td>65</td>
<td>25</td>
<td>38.5</td>
<td>17</td>
<td>26.2</td>
<td>9</td>
</tr>
</tbody>
</table>

No. = number of cases, HE = heterophile type, IC = intracellular type, Nuc = nuclear type, ID = intercalated disc type, ICM = idiopathic cardiomyopathy, DCM = dilated cardiomyopathy, HCM = hypertrophic cardiomyopathy, MI = myocardial infarction, AP = angina pectoris, RVD = rheumatic valvular disease, NC = normal healthy controls.

*significantly different from NC (p < 0.05); **significantly different from NC and other patient groups (p < 0.05)

glycerol at pH 8.4, and viewed under a Leitz fluorescent microscope at x400 magnification and photographed by 35 mm color film (Kodak Ektachrome 160). Sera containing heterophile antibodies were retested on sections of rat liver, kidney, stomach, pancreas and skeletal muscle by the methods described above for heart tissue.

The results were analyzed by the chi square test with an α of 0.05.

RESULTS

The staining patterns of C-AHAb were classified into 4 types: “heterophile”, “intracellular”, “intercalated disc” and “nuclear” patterns.

The heterophile staining pattern is shown in Fig. 1-a. The endothelium of the blood vessels and sarcolemmal-subsarcolemma were stained. The intracellular pattern is shown in Fig. 1-b. The pattern is characterized by intense broad striational staining and/or longitudinal fluorescence between the myofibrils (intermyofibrillar staining). In many cases, both patterns were observed simultaneously. The intercalated disc pattern is shown in Fig. 1-c and the nuclear pattern is shown in Fig. 1-d. The predominant type of immunoglobulin associated with this antibody was IgM.

The heterophile antibodies also produced characteristic patterns on other organ tissues. In the stomach, the parietal cells, capillaries and smooth muscle interstitium were stained (Fig. 2-a). In the kidney, the endothelium of the

Fig. 2. a: Heterophile antibody staining pattern in rat stomach. The parietal cells are stained (x400).

b: Heterophile antibody staining pattern in rat kidney. The endothelium of the glomeruli and the brush borders of the proximal tubular cells are stained (x400).
glomeruli, the brush borders of the proximal tubular cells, and parts of the distal tubules were stained (Fig. 2-b). The acinar cells of the pancreas were stained. In the liver, this antibodies reacted with Kupffer cells and the sinusoidal endothelium. In the skeletal muscle, the staining pattern was similar or identical to that of the heart.

Table I summarizes the incidence of each type of staining pattern. The occurrence rates of C-AHAb positive sera were 79.2% in ICM (80.6% in DCM, 77.3% in HCM) and 65.4% in RVD. The occurrence rates of positive sera in AP, MI and NC were all less than 60%. Statistically significant differences were present between ICM and the other subjects, including NC (p < 0.05). The occurrence rate of heterophile antibodies was 60.4% in ICM (64.5% in DCM and 54.5% in HCM). These values were significantly different from those of the other patient groups and NC (p < 0.05). The intracellular pattern was found in 42.3% of RVD, which was statistically different from that of NC (p < 0.05), but not from the other patient groups. The intercalated disc pattern was found in only RVD. There were no significant differences in the occurrence rate of nuclear pattern among the patient groups and NC.

DISCUSSION

The staining patterns of C-AHAb were examined by an indirect IF test using rat hearts fixed in cold ethanol. The patient patterns were classified into 4 types: “heterophile”, “intracellular”, “intercalated disc” and “nuclear” patterns. The intracellular staining patterns contained striational and/or intermyofibrillar pattern.

Kaplan et al.3 using sections of the human heart and sera of patients with rheumatic fever or rheumatic heart disease, have found 3 types of staining patterns: “subsarcolemmal-sarcoplasmic”, “intermyofibrillar” and “diffuse sarcoplasmic”. Van der Geld,4 using sections of the human and rat heart and sera of patients with postpericardiotomy, rheumatic fever and other diseases have observed “subsarcolemmal-sarcoplasmic”, “intermyofibrillar” and “endoysial” staining patterns. Das et al.12 have also reported “sarcolemmal”, “subsarcolemmal” and “anti-nuclear” patterns using the human heart and sera of patients with various diseases. Tagg and McGiven6 have found 5 types of staining patterns with rat heart preparations: “sarcolemmal”, “sarcolemmal-subsarcolemmal”, “intermyofibrillar”, “band”, and “intercalated disc”.

Recently, Nicholson et al.11 have proposed a new classification for staining patterns of C-AHAb. They differentiated between heart/muscle specific AHAb and non-tissue specific antibodies. The former may give “peripheral”, “diffuse”, “striational” and “intercalated disc” patterns, while the latter included “heterophile” and “anti-mitochondrial” patterns.

Our heterophile antibodies stained the endothelium of blood vessels and sarcolemmal-subsarcolemmal regions. These antibodies were partially absorbed with sheep red blood cells and completely absorbed with guinea pig red blood cells. The antibodies also stained the rat brush borders of the proximal tubular cells and smooth muscle of the stomach, Kupffer cells and sinusoidal endothelium of the liver, and acinar cells of the pancreas. These antibodies were, thus, not tissue specific. The antibodies seemed to be identical with those described as “endoysial” by van der Geld,4 “sarcolemmal” by Tagg and McGiven6 and “heterophile” by Nicholson et al.11

Cossio et al.7 have reported an antibody reacting with the sarcolemma of myofibers and the plasma membranes of endothelial cells in Chagas' diseases using an indirect IF technique, and named this antibody the endothelial-vascular-interstitial (EVI) autoantibody. They indicated that the endocardial and the vascular pattern remained unchanged and that the interstitial pattern was no longer observed in formaldehyde-fixed sections of striated muscle, whereas positive staining appeared in or near the sarcolemma. Thus, they suggested that the gradual shift of the interstitial pattern to the sarcolemmal pattern with increasing formaldehyde fixation time was congruent with the possibility that the interstitial pattern in unfixed sections was an artifact due to a diffusion of the sarcolemmal antigen.13 The EVI factor may be identical to our heterophile antibodies because of the staining pattern by indirect IF method.

Our study did not detect the “sarcolemmal-sarcoplasmic” pattern of Kaplan et al.3 or the “peripheral” pattern of Nicholson et al.11 Kaplan and his coworkers have demonstrated that the antigenic material localized in the myofiber sarcoplasm was soluble in alcohol. Therefore, our failure to observe the antigenic material may be due to our use of alcohol to fix the rat heart.

In our study, ICM patients had a high prevalence of heterophile antibodies. In cardiomyopathy, Das et al.14 have reported that cardiomyopathic
heart tissue had abundant sarcolemmal and subsarcolemmal staining under direct IF technique. Cossio et al.
have speculated that the EVI antibody may interfere with transmembrane diffusion and transport process, that is, this antibody may have a pathogenic effect. Sterin-Borda et al.
have also shown that the EVI antibody induced morphologic and functional alterations in rat myocardial cells in vitro. These data suggest that heterophile antibodies may play a role in the pathogenesis in ICM.

At present, the antigenic stimuli responsible for the formation of heterophile antibodies in ICM still remain to be determined. Maisch et al.
using indirect IF technique, have reported that antisarcolemmal and antiendothelial staining patterns were attributable to viral myocarditis and that the patterns may be a marker of viral etiology in congestive cardiomyopathy. We would like to propose that the antigen may be released from infectious agents as in Chagas' disease or from the patient's own tissues during the pathologic process as described by Kasukawa et al.
However, this needs further investigation.

There was a high prevalence of the intracellular pattern in our RVD patients. This pattern contained the intermyofibrillar and striational staining. Nicholson et al.
have indicated that the intermyofibrillar antibody may be similar to the antimitochondrial antibody of primary biliary cirrhosis and that this antibody may appear after cardiac injury. Pinckard et al.
have demonstrated experimentally in dogs that the anti-heart mitochondria autoantibody may appear after myocardial infarction. It has been also reported that cellular and/or humoral immunological abnormalities play a role in the pathogenesis of RVD.
Recently, Sasazuki et al.
have demonstrated that the low responsiveness to streptococcal cell wall antigen was controlled by the HLA-linked immune suppression gene. These findings suggest that RVD patients may have a genetic factor that accounts for the abnormal immune reaction. In RVD, repeated streptococcal infections may produce a chain reaction starting in the myocardial damage (or even necrosis), that leads to alterations in protein structure endowing them with an antigenic quality which may be responsible for the high rate of positive intracellular patterns.

Our results suggest that positive C-AHAb and staining patterns may be useful in the assessment of the pathogenesis of certain types of heart diseases.

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