Humoral immunity in cardiomyopathy

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From the Department of Cardiovascular Medicine, University of Birmingham, and Regional Immunology Laboratory, East Birmingham Hospital, Birmingham

SUMMARY Sera from patients with heart disease were examined by single sandwich indirect immunofluorescence for the presence of antibodies to human heart muscle. Endocardial biopsy specimens were also examined by direct immunofluorescence for deposition of antibodies in vivo. No consistent or specific abnormality was found in the biopsy specimens or sera of patients with congestive cardiomyopathy. The presence of anti-heart antibodies in cardiac disease appears to reflect damage to cardiac muscle whatever the cause. Immunofluorescence is an insensitive test for anti-heart antibodies.

It has been suggested that cardiac damage in congestive cardiomyopathy may be mediated by an autoimmune mechanism. The role of humoral abnormalities, in particular type II hypersensitivity, has received considerable attention, but the results and their interpretation vary widely. Previous findings in our department (using baboon heart as substrate) suggested that the role of humoral autoimmunity as a primary cause of congestive cardiomyopathy is not proven; we undertook a further study to confirm or refute the findings.

Our aims were (a) to detect serum autoantibodies to human heart muscle, (b) to detect evidence of in vivo antibody fixation by cardiac antigens, and (c) to demonstrate immune complex deposition in and around myocardial vessels.

Patients and methods

SEROLOGICAL STUDY

Sera of 95 patients were examined for the presence of antiheart antibodies. Forty three had cardiomyopathy according to the classification of Goodwin and Oakley: 28 had congestive cardiomyopathy diagnosed both clinically and by cardiac catheterisation and endomyocardial biopsy, with one exception in which the diagnosis was confirmed at necropsy and 15 had hypertrophic cardiomyopathy, in all but one of whom the diagnosis was confirmed at catheterisation. Nine of the 15 patients in the latter group had a left ventricular outflow tract obstruction detected at catheterisation. Sixteen patients had ischaemic heart disease and nine rheumatic heart disease; six subjects were healthy controls; and the remaining 21 patients comprised a miscellaneous group including five with post-infarct/post-cardiotomy syndrome (Table 1). The groups were classified symptomatically according to the New York Heart Association (NYHA) criteria at the time of venesection (Table 2): 50% of patients with congestive cardiomyopathy and 44% with ischaemic heart disease were in groups IIb, III, and IV.

Serum was separated from cells immediately after venesection and stored at −20°C. Sections from normal human left ventricle and congestive cardiomyopathic left ventricle were used as the substrate. The normal heart was obtained within 14 hours of death from a woman who died after trauma. The congestive cardiomyopathic heart was obtained within 24 hours of death from a 16 year old boy-in whom the diagnosis of congestive cardiomyopathy had been confirmed by invasive investigation and necropsy. The hearts were stored at −70°C. Sections of 7 μm were cut on a cryostat at −20°C from portions of these ventricles and dried in air for at least 10 minutes before storage at −70°C until use.

The single sandwich immunofluorescence technique was used, as previous work in this department has not shown any appreciable advantage of the double sandwich technique. Sera were tested at a dilution of 1/5 in phosphate buffered saline (PBS): 0.5 ml was incubated with sections of normal and congestive cardiomyopathic heart at room temperature for 30 minutes. The serum was removed by washing with PBS for five minutes. A drop (approximately 0.03 ml) of fluorescein isothiocyanate conjugated anti-human
Humoral immunity in cardiomyopathy

Table 1 Diagnoses in miscellaneous group of 21 patients with heart disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients examined by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serology</td>
</tr>
<tr>
<td>Post-infarct/post-cardiotomy syndrome</td>
<td>5</td>
</tr>
<tr>
<td>? Cardiomyopathy</td>
<td>5</td>
</tr>
<tr>
<td>Pericarditis or myocarditis or both</td>
<td>4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3</td>
</tr>
<tr>
<td>Constrictive pericarditis</td>
<td>1</td>
</tr>
<tr>
<td>Post-heart transplant</td>
<td></td>
</tr>
<tr>
<td>(for CCM)</td>
<td>1</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>1</td>
</tr>
<tr>
<td>Non-rheumatic mitral regurgitation</td>
<td>1</td>
</tr>
<tr>
<td>Rheumatic heart disease</td>
<td>1</td>
</tr>
<tr>
<td>Aortic incompetence</td>
<td>1</td>
</tr>
<tr>
<td>Discrete subaortic membrane</td>
<td>1</td>
</tr>
<tr>
<td>Normal</td>
<td>1</td>
</tr>
</tbody>
</table>

CCM, congestive cardiomyopathy.

IgG (Atlantic Antibodies) was placed on each section, and after a further 30 minutes the slides were washed in PBS, mounted in buffered glycerol, and viewed under the fluorescence microscope (Leitz Ortholux). A negative control serum sample from a healthy subject and a known positive serum sample were included in each batch of slides. The sections were viewed blindly and independently by two observers. Fluorescence was graded as positive (+); weak or no fluorescence was regarded as negative (−). Each patient had serum viewed on two sections, one congestive cardiomyopathic heart and one normal heart: thus if both observers graded fluorescence as positive in both sections a maximum total of 4+ could be scored for the patient.

BIOPSY STUDY

Fifty four endomyocardial biopsy specimens were obtained at the time of cardiac catheterisation from 53 subjects. Of these, 22 had congestive cardiomyopathy, 14 hypertrophic cardiomyopathy, and five ischaemic heart disease. The diagnoses in the remaining 12 are given in Table 1. Thirteen of the first group and six of the second were also included in the serological study for anti-heart antibodies.

The biopsy specimens were obtained at cardiac catheterisation using a King’s biop tome® from the right ventricular septum in three cases and from the left ventricle in the remainder. Each specimen was placed in a screw topped ampoule, flash frozen in liquid nitrogen within 30 minutes of collection, and stored at −70°C. The specimens were mounted in OCT embedding compound (Miles Laboratories) and eight 7 μm sections were cut on to a slide at −20°C in a cryostat, air dried for at least 10 minutes, and stored at −70°C. When required, the sections were air dried and washed in PBS for 10 minutes. The conjugates were placed on the slides: fluorescein isothiocyanate conjugated anti-human IgG, IgM, and IgA (Atlantic Antibodies), complement (C3) (Kallestad), and fibrin (Hyland) were used. The sections were incubated for 30 minutes in a moist box at room temperature, rinsed, and washed in PBS for 10 minutes. The sections were mounted in 90% glycerol and viewed under the ultraviolet microscope. The sections were viewed blindly and independently by two observers (PJL and RT). The presence and site of increased fluorescence were noted.

All sections were viewed under a Leitz Ortholux II microscope with Epi illumination using a super pressure mercury source (HBO 50), a Balzer filter exciter, a red cut off filter, and a yellow barrier filter.

STATISTICAL METHOD

The significance of the intraobserver variation was measured using the χ² test. Comparisons between the patient groups were made using the χ² test for independent samples. 6

Results

SEROLOGICAL STUDY

All 95 patients had sera examined: 16 had the test repeated on at least one occasion using the same stored sera. The observers’ findings were in agreement for 73% of the sections viewed. In those cases in which the test was repeated, each observer agreed with the former assessment in 66% (PJL) and 62% (RT); this intraobserver error was significant (0.05>p>0.02). On two occasions the negative control was deemed positive, and on seven of 16 (44%) observations the positive control was classed as negative.

Observations with congestive cardiomyopathic heart as substrate were in agreement more often (85% compared with 61%) than with normal heart substrate. Repeat tests were also in agreement more often with cardiomyopathic heart (71% compared with
44%) than with normal heart, but the positive control was classed as negative on 50% of the observations with cardiomyopathic heart compared with 38% with normal heart substrate.

Table 3 shows the distribution of positive and nega-

Table 3  Distribution of positive and negative fluorescence in 95 patients with heart disease

<table>
<thead>
<tr>
<th>Diagnostic group</th>
<th>Total No. of patients</th>
<th>Fluorescence results</th>
<th>Total No. (%) positive observations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0  1+ 2+ 3+ 4+</td>
<td></td>
</tr>
<tr>
<td>Congestive cardiomyopathy</td>
<td>28</td>
<td>14 8 3 3 0</td>
<td>24/116 (21)</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>15</td>
<td>7 5 2 1 0</td>
<td>15/76 (20)</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>16</td>
<td>6 5 2 3 0</td>
<td>20/74 (27)</td>
</tr>
<tr>
<td>Rheumatic heart disease</td>
<td>9</td>
<td>4 3 2 0 0</td>
<td>8/38 (21)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>16</td>
<td>9 6 1 0 0</td>
<td>8/70 (11)</td>
</tr>
</tbody>
</table>

Table 4  Number of biopsy specimens with increased fluorescence in identified sites. (Figures in parentheses are number weakly affected)

<table>
<thead>
<tr>
<th></th>
<th>Congestive cardiomyopathy (n=22)</th>
<th>Hypertrophic cardiomyopathy (n=14)</th>
<th>Ischaemic heart disease (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrin</td>
<td>IgG C3 IgM IgA</td>
<td>Fibrin IgG C3 IgM IgA</td>
<td>Fibrin IgG C3 IgM IgA</td>
</tr>
<tr>
<td>Fibrous tissue</td>
<td>8 2</td>
<td>3 1</td>
<td>1</td>
</tr>
<tr>
<td>Endothelium</td>
<td>2 3 1 1</td>
<td>2 2 1 (1)</td>
<td>(1)</td>
</tr>
<tr>
<td>Subendothelium</td>
<td>3 3 1 1</td>
<td>2 2</td>
<td>(1)</td>
</tr>
<tr>
<td>Perimysel</td>
<td>6 7 (1) (1)</td>
<td>2 2</td>
<td></td>
</tr>
<tr>
<td>Intermuscular sepsa</td>
<td>3 2 1 2</td>
<td>1 1</td>
<td></td>
</tr>
<tr>
<td>Degenerating muscle</td>
<td>3 3 1 1</td>
<td>1 1</td>
<td></td>
</tr>
<tr>
<td>Muscle bundles</td>
<td>1 (1) (1)</td>
<td>1 1</td>
<td></td>
</tr>
<tr>
<td>Capillaries</td>
<td>1 (2) (1)</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Table 5  Results of fluorescence in serological and biopsy studies in patients with congestive and hypertrophic cardiomyopathies

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Serology (fluorescence)</th>
<th>Biopsy (fluorescence with conjugates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>Congestive cardiomyopathy</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td></td>
<td></td>
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<td>37</td>
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<td>4</td>
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<td>41</td>
<td></td>
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<tr>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Fibrin in connective tissue</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>Fibrin in subendothelium and connective tissue</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td></td>
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<tr>
<td>18</td>
<td></td>
<td></td>
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<tr>
<td>20</td>
<td></td>
<td></td>
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<tr>
<td>14</td>
<td></td>
<td></td>
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<tr>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fluorescence: -, negative; +, +++, ++++, positive.

Table 5 shows the serological and biopsy results in patients included in both parts of the study with con-

BIOSPY STUDY

Table 4 shows the results of fluorescence on the biopsy specimens. In the hypertrophic group one specimen was of poor quality and unsuitable for interpretation. In the miscellaneous group fibrin and IgG were increased in areas of fibrosis in the cases of constrictive pericarditis, hypertension, and discrete subaortic stenosis. One patient with myocarditis had increased fibrin and IgG, C3, IgM, and IgA fluorescence in the thickened endocardium and IgG and fibrin in the connective tissue. Fibrin and IgG were bound in the septa of the one normal subject.

Table 5 shows the serological and biopsy results in patients included in both parts of the study with con-
Humoral immunity in cardiomyopathy

gestive cardiomyopathy and hypertrophic card-
diomyopathy. Six of 13 patients with congestive card-
diomyopathy, three of six patients with hypertrophic card-
diomyopathy and five of the 12 in the miscellane-
ous group had some degree of immunofluorescence in the
serum. In the first group, absence of positive
fluorescence in the serum did not always infer binding
of antibody in the biopsy specimen and often fluores-
cence was seen in both sera and biopsy specimens.

Discussion

The specificity of the immunofluorescence test for
anti-heart antibodies in serum was 93% but the sen-
sitivity was only 56%. The test is therefore specific for
these antibodies as few false positives occur, and this
is borne out in the control group, but the percentage
of false negatives is so high that results cannot give a
true reflection of the incidence of anti-heart anti-
bodies. Our results show that the antibodies appear to
be present to some extent in all forms of heart disease,
that they do not differ significantly between groups,
and that anti-heart antibodies probably reflect non-
specific cardiac damage. This is in agreement with
other studies.3 9 Although sera from all the patients in
the post-infarct/post-cardiotomy group showed fluores-
cence on at least one occasion, only 50% of all the
observations in this group were positive, reflecting
the low sensitivity of the test. As reported elsewhere10
the results for the patients with more severe symp-
toms did not differ significantly either within their
groups or between the groups and do not support the
previous finding in our laboratory5 or Bolt and
Grothey's findings.2

There were wide interobserver and intraobserver
variations: only 73% of observations were in agree-
ment, and on repeat testing the observer agreed with
his former assessment on only 66% (PJL) and 62%
(RT) of occasions (0.05>p>0.02). Assessment of
fluorescence is subjective and there is no accepted
method of quantifying the amount of fluorescence.
It is important that tests are performed blind and with
more than one observer, otherwise inconsistencies are
bound to arise.11 12

In our previous study5 fresh baboon heart was used
as substrate; in the present study fresh normal human
heart and fresh cardiomyopathic human heart were
used and no difference in results was obtained.

There was no consistent pattern of fluorescence in
any group: the sites of fluorescence and the propor-
tion of biopsy specimens showing fluorescence did not
differ between the groups. Increased fibrin staining
usually reflected fibrosis regardless of the aetiology
and is therefore secondary to damage. Increased
fluorescence with C3 was rarely seen, and then in no
consistent site, suggesting that complement mediated
damage does not occur in congestive (or hypertrophic)
cardiomyopathy. IgG was seen in apparently normal
muscle bundles in only three patients (slightly in one
other) in the congestive cardiomyopathy group and in
one patient in the hypertrophic cardiomyopathy group;
thus it can be concluded that the primary
mechanism of muscle damage in congestive cardio-
myopathy is not by autoantibodies with or without
complement. In only occasional cases in all groups did
fluorescence staining occur in the capillary walls; thus
immune complexes do not appear to be the cause of
tissue destruction. These findings confirm earlier
work in our department13 and contrast with the
results of other workers14 whose studies were not
performed blind. We believe that binding of
immunoglobulin is not specific to cardiomyopathy15
and certainly cannot be incriminated as the primary
site of damage, as suggested by Sanders and Ritts.14
Das et al.16 postulated that anti-heart antibodies are
not present in the serum because they are bound by
antigen in the heart: the findings of the present study
and former work13 do not support this view.

The immunofluorescence test, although specific for
anti-heart antibodies, has low sensitivity and the true
incidence of these antibodies in patients with conges-
tive cardiomyopathy (or any other heart disease) can-
ot be assessed by this method. Our results do suggest
that patients with this disease do not differ substan-
tially from patients with other forms of heart disease
when studied in this way. A primary role for humoral
immunity in congestive cardiomyopathy cannot be
entirely dismissed as the relevant antibody may not
have been detected by this technique or the appropri-
ate antigen may be inaccessible or absent in a cardiac
biopsy specimen. We have, however, failed to prove
an important role for humoral immunity in the
pathogenesis of congestive cardiomyopathy. We have
been unable to detect circulating antibodies specific to
this disease or specific antigen in cardiomyopathic
heart binding antibody, and in the biopsy specimens
obtained no immune complex deposition was seen.
The anti-heart antibodies detected by immuno-
fluorescence probably reflect damage to cardiac
muscle regardless of the cause.

PJL is supported by a British Heart Foundation
research grant.

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immunological processes. In: Riecker G, Weber A,
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**Notice**

**British Cardiac Society**

The Autumn Meeting will be held at Wembley on 21 and 22 November 1983, and the closing date for abstracts was 26 July 1983.

The Annual General Meeting for 1984 will take place in Leicester on 11 and 12 April 1984, and the closing date for receipt of abstracts will be 3 January 1984.

The Autumn Meeting in 1984 will be held on 3 and 4 December 1984, and the closing date for receipt of abstracts will be 15 August 1984.
Humoral immunity in cardiomyopathy.

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