Immunological studies in congestive cardiomyopathy in Cameroon

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Significantly higher levels of immunoconglutinin were found in the sera of 40 Cameroonian patients aged between 15 and 80 years with congestive cardiomyopathy than in a matched group of normal Cameroonian controls. C3 values were also abnormal in the group with congestive cardiomyopathy, and a negative correlation was found between the C3 and immunoconglutinin values, indicating complement incorporation into immune complexes. Further, the levels of immunoglobulins G, M, and A were all raised, the most striking increase being of IgM, which was greater than in the normal controls and in a group with 'other cardiac disease' studied for comparison. Complement-fixing autoantibodies to cardiac muscle of the class IgG and IgM were found by immunofluorescence techniques in a significant proportion of the patients with congestive cardiomyopathy. Finally, evidence of trypanosomiasis was found by immunofluorescence tests in 27.5 per cent of patients with congestive cardiomyopathy, compared with 8.5 per cent of patients with other cardiac disease, and 1.9 per cent of normal Cameroonian controls selected randomly.

We think that some cases of congestive cardiomyopathy in Cameroon may be caused by untreated subclinical attacks of African trypanosomiasis which produce immune complexes that damage the cardiac tissue where the parasite is lodged. Secondary autoimmune carditis modifies the course of the disease, with the resulting end-stage picture.

Congestive cardiomyopathy has been defined as a disorder of heart muscle of unknown aetiology or association which is characterized by poor systolic function (Oakley, 1972). It has been variously known as idiopathic cardiomegaly (Reisinger and Blumenthal, 1941), nutritional heart disease (Gillanders, 1951), cryptogenic heart disease (South African Medical Journal, 1960), cardiovascular collagenosis (Becker, Chatgidakis, and Van Lingen, 1953), 'a cardiac disease of unknown aetiology' (Stuart and Hayes, 1963), and heart muscle disease (Eddington and Jackson, 1963).

Although mainly prevalent in the tropics, where it is an important cause of cardiac pathology, its incidence in temperate climates is probably underestimated (Hutt, 1972). Among the suggested causes are alcohol (Brigden and Robinson, 1964), the peripartum period (Walsh et al., 1965), viral infections (Bengtsson, 1972; Grist and Bell, 1969), systemic hypertension (Kristinsson, 1969; Goodwin, 1970), and parasitic infections such as filaria and trypanosomiasis (Shaper et al., 1968). Sanders (1963) and Sanders and Ritts (1965) suggest an immunological basis for the disease on the evidence of autoantibodies to cardiac tissue found in the sera of many patients with idiopathic cardiomegaly, by indirect immunofluorescence tests, and of intense staining deposits of gammaglobulin in the sarclemma and subsarcolemmal regions of ventricular muscle found on cardiac biopsy or at necropsy. Dodson et al. (1967), using tanned red cell haemagglutination techniques, found antibodies to cardiac muscle in 2 out of 5 patients with cardiomyopathy. Furthermore, sustained high levels of antiheart antibodies in the serum, induced by repeated injection of heart antigens, resulted in carditis in animals (Kaplan and Craig, 1963). These animals also had autoantibodies to autologous heart shown by immunofluorescence, complement fixation, and flocculation techniques, and deposits of bound gammaglobulin were detected in the cardiac myofibres around the focal lesions.

On the other hand, Fletcher and Wenger (1968)
found no difference in the incidence of antiheart antibodies (by immunofluorescence) in 34 patients with primary myocardial disease and in 71 control subjects. In Uganda van der Geld (1964), van der Geld et al. (1966), and Shaper et al. (1967) also found a high incidence of antiheart antibodies in both patients with idiopathic cardiomyegaly and controls. Clearly, in view of these conflicting reports, further studies are needed to elucidate the role of immune mechanisms, if any, in the pathogenesis of congestive cardiomyopathy.

Patients

Patients were selected who conformed with the WHO criteria for 'cardiomegaly of unknown origin' (World Health Organization, 1967): cardiac enlargement associated with biventricular cardiac failure; pulse of usually small volume, sinus rhythm with occasional ectopic beats; diastolic pressure either normal, or if raised to 100 to 130 mmHg (13.3 to 17.3 kPa), falling to normal after treatment of the heart failure; apex beat forceful and often associated with a left parasternal heave; thrills and murmurs of mitral and tricuspid incompetence, characteristically diminishing or disappearing on treatment; and a third sound commonly present at the apex even after correction of overt failure.

Laboratory investigations were undertaken to exclude, when possible, liver, kidney, or thyroid disease; anaemia and sickle-cell disease; collagen vascular disorders; syphilis; acute rheumatic fever; and diabetes. Chest x-ray examination and 12-lead electrocardiograms were recorded routinely, but owing to lack of facilities cardiac catheter studies were not undertaken.

Of the 40 patients fulfilling these criteria 19 were men, mean age 47-5 years, and 21 were women, mean age 43-5 years. Nine (22.5\%) of these had the sickle-cell trait (haemoglobin genotype AS), the same incidence as reported previously in the 'normal' population of Yaounde (Languillon, 1957; Garnet and Labes, 1964).

Materials and methods

The sera collected from several groups of subjects were separated within a few hours and stored in aliquots at −20°C to prevent thawing and refreezing. The groups comprised (a) 40 patients with congestive cardiomyopathy; (b) 52 randomly selected Cameroonian blood donors; and (c) 26 patients with non-hypertensive cardiac disease (designated 'other cardiac diseases'). The group of 26 comprised 13 patients with acute myopericarditis—2 of trypanosomal origin and the remaining 11 of viral aetiology (Coxackie A and B, polio and Echo virus)—6 patients with rheumatic heart disease; 6 patients with hypertrophic obstructive cardiomyopathy; and 1 with endomyocardial fibrosis.

Sera from the above three groups of patients were studied for immunoglobulins, B\(_1\)/B\(_\text{H}\) globulins (C\(_3\)), immunoglobulin G, and antitrypanosomal antibodies. Antibodies to cardiac, skeletal, and smooth muscle tissue were determined using the indirect immunofluorescence techniques in sera from (1) all the patients with congestive cardiomyopathy and other cardiac diseases; (2) 31 of the 52 Cameroonian blood donors; (3) 50 randomly selected Europeans who had never lived in Africa; and (4) 20 healthy Cameroonian medical students.

Immunoglobulins

IgA, IgG, and IgM were estimated by the single radial immunodiffusion method (Mancini, Carbonara, and Heremans, 1965) using the same batch of commercial antisera (Wellcome diagnostic reagents). All determinations were performed under identical conditions of temperature and the plates were read after 48 hours. Pooled Cameroonian normal serum, previously related to WHO reference serum, was used as the working standard reference serum. Log transformation of the data was used for statistical analysis, the results being expressed as international units per millilitre (IU/ml). In the case of IgM, where many results were very high, high dilutions of the sera were used to obtain accurate readings.

B\(_1\)/B\(_\text{H}\) (C\(_3\)) globulins

These were similarly determined by the single gel diffusion precipitation technique (Mancini et al., 1965), and the values expressed as a percentage of the WHO reference serum.

Immunocconglutinin estimation

Immunocconglutinin was titrated by the method 2A of Coombs, Coombs, and Ingram (1961) incorporating the 'sedimentation pattern' technique of Lachmann (1966). The anti-Forsman serum used was raised by successive courses of immunization with Forsman hapten, and fractionated to obtain a macroglobulin through Sephadex G-200. The sedimentation patterns in these plates (using 0-6\% asexinated sheep red cells) were assessed, after allowing the reaction to proceed overnight at 4°C, by the technique of 'pouring'. The following controls were included in each run:

(a) Positive control—pooled serum from patients with trypanosomiasis.
(b) Negative control—serum known to have no demonstrable immunocconglutinin activity.
(c) Specificity control—sheep red cells were exposed to heat inactivated (56°C for 30 min) horse serum and anti-Forsman serum. The immunocoaglutination reaction was performed with these cells and the test serum, at the highest concentration used in the titration, to control for positive sedimentation other than conglutination.

Immunofluorescence

Immunofluorescence tests were performed using the sandwich technique to detect autoantibodies to heart and other muscle tissues. Tests were performed on the following tissues: (a) human fetal heart; (b) rat heart; (c) rat kidney, for smooth muscle in blood vessel wall; and (d) rat skeletal muscle. All examinations were under-
TABLE 1 Mean (± SD) levels of immunoglobulin (IU/ml) and C3 (% of WHO ref. serum) in controls, patients with congestive cardiomyopathy, and patients with other cardiac diseases together with ratios of variance of patients from controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=52)</th>
<th>Congestive myopathy (n=40)</th>
<th>Other cardiac disease (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level</td>
<td>Variance ratio</td>
<td>Level</td>
</tr>
<tr>
<td>IgG</td>
<td>170±2±57-4</td>
<td>192±2±96-3</td>
<td>173±87-2</td>
</tr>
<tr>
<td>IgA</td>
<td>120±29-2</td>
<td>149±53-6</td>
<td>121±47-7</td>
</tr>
<tr>
<td>IgM</td>
<td>182±64</td>
<td>337±215</td>
<td>228±67</td>
</tr>
<tr>
<td>C3</td>
<td>120±23-3</td>
<td>125±53-5</td>
<td>156±42-8</td>
</tr>
</tbody>
</table>

taken on 6μ cryostat sections mounted on slides and air-dried overnight.

Sera were tested diluted 1/10, 1/20, and 1/40. The conjugate (Wellcome diagnostic reagents, Rabbit anti-human IgA; IgG; IgM—FITC) was diluted 1/50. The sera were applied for 20 minutes, followed by washing in phosphate-buffered saline (PBS), pH 8.0, for 30 minutes. The conjugate was then added and after 20 minutes this was rinsed off three times in phosphate-buffered saline before the slide was finally washed for 2 hours. The class of antibody was identified by purified anti-human IgG-FITC, IgM-FITC, IgA-FITC. Complement-fixing ability was investigated with purified anti-B1a/B1b globulin fluorescein conjugate preparation, after the addition of either fresh or heat-inactivated (56°C for 30 min) serum from normal European controls.

All sera were coded, a known positive and negative control included in each run, and all read by the same observer with a Nachat 200 microscope with a quartz iodide light source. The results were recorded as positive or negative at the dilution of serum employed, and the site of staining recorded as sarcolemmal/subsarcolemmal or microsomal.

Trypanosomiasis
The indirect fluorescence antibody technique has been shown to be the most sensitive method of detecting trypanosomal infection (Alvarez, Cerisola, and Rowedder, 1967). T. gambiense is antigenically similar to T. rhodesiense, and the latter was used as antigen in testing for antibodies to T. gambiense in the test sera. The indirect fluorescence was performed by the sandwich technique and serial dilutions of the test sera were used to obtain the titre of antibodies present.

Results
Immunoglobulins
The mean levels of IgG and IgM in the control group of Cameroonians were significantly higher than in appropriately matched Caucasians, a finding that has been previously reported by Turner and Voiler (1966) and Rowe et al. (1968). The mean values of IgM, IgG, and IgA in the patients with congestive cardiomyopathy were all significantly higher than those of the Cameroonian controls (F < 0.005, variance ratio test) (Table 1). In addition IgM values greater than 2 SD above the mean were found in 18 patients with congestive cardiomyopathy, and in 12 of these the values exceeded 400 IU/ml (Fig. 1). In the cases of other cardiac disease the IgG levels were significantly different by the variance ratio test from the controls (F < 0.05) and the IgA (F < 0.01). In the case of IgM, though the variance ratio test showed no significant difference from the controls analysis of the means showed a difference at the 95 per cent confidence limit.

FIG. 1 Serum IgM levels (IU/ml) in patients with congestive cardiomyopathy. Vertical interrupted line indicates geometric mean plus 2 SD above and 1 SD below mean of data from normal Cameroonian controls.
Immunoglobulin and B10/B1a (C3) globulin

Immunoglobulins are autoantibodies of the immunoglobulin classes G, M, or A which react with the complement components C3 (Lachmann and Coombs, 1965) and C4 (Lachmann, 1966) after their binding to antigen-antibody complexes (Coombs et al., 1961). The values obtained in the group of patients with congestive cardiomyopathy and in the normal Cameroonian controls is shown in Fig. 2. The difference is highly significant (P<0.005 Student’s t test). In the patients with other cardiac diseases the mean immunoglobulin titres of those with myopericarditis (4-1) and rheumatic fever (4-7) were not significantly different from those with congestive cardiomyopathy, whereas patients with hypertrophic obstructive cardiomyopathy and endomyocardial fibrosis had mean values of 1-4 and 2, respectively, similar to the normal Cameroonian controls. Interestingly, the highest immunoglobulin values (range 5 to 8 \(-\log_2\) titre) obtained in this group of patients with other cardiac diseases were found in the sera from 4 subjects with trypanosomal infection. Two of these had trypanosomal myopericarditis and the other two were from the group of 6 patients with clinical rheumatic heart disease. Trypanosomiasis is a well-known cause of high serum immunoglobulin levels (Coombs et al., 1961; Ingram et al., 1959).

The mean (±SD) C3 values in the control sera were 120 IU/ml±23-3. The mean values in the group with congestive cardiomyopathy showed a wider scatter 125 IU/ml±53-5. However, there was a highly significant difference between the two groups (F<0.005 variance ratio test). The mean of the patients with other cardiac diseases was 156 IU±42-8, which again was a highly significant difference (F<0.005 variance ratio test). Interestingly, the lowest C3 levels tended to be associated with the highest immunoglobulin levels (Fig. 3), the coefficient of correlation being -0.639.

Immunofluorescence

There was a high level of antibodies to the tissues tested in both the patients with congestive cardiomyopathy and those with other cardiac disease, with no significant difference between the two groups of patients. Only a few in the control group comprising sera from randomly selected Cameroonian blood donors had antibodies to cardiac muscle, whereas antibodies to skeletal muscle were found in many (Table 2). No antibodies to skeletal or cardiac muscle were found, however, in the control sera from healthy Cameroonian medical students and Europeans who had never lived in Africa.

One notable finding was of the sarcolemmal/subsarcolemmal staining in the skeletal muscle, whereas in the fetal heart sections the staining was micromosal. The antibodies were of the immunoglobulin class G and M, and were complement binding.

Trypanosomiasis

Although *T. gambiense* is antigenically close to *T. rhodesiense* the results tended to underestmate the levels of antibodies in the samples. Nevertheless, of the 40 patients with congestive cardiomyopathy 11 (27.5%) showed evidence of trypanosomal in-

**FIG. 2** Serum immunoglobulin (IK) titres in patients with congestive cardiomyopathy (●) and in matched Cameroonian controls (○).

**FIG. 3** Serum immunoglobulin (IK) titres of patients with congestive cardiomyopathy plotted against corresponding B10/B1a (C3) values.
TABLE 2 Percentage of patients and controls showing immunofluorescent tissue antibodies at various serum dilutions.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. studied</th>
<th>Skeletal muscle</th>
<th>Heart</th>
<th>Smooth muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/10 1/20 1/40</td>
<td>1/10 1/20 1/40</td>
<td>1/10 1/20 1/40</td>
</tr>
<tr>
<td>Congestive cardiomyopathy</td>
<td>40</td>
<td>75 60 28</td>
<td>60 35 28</td>
<td>40 25 20</td>
</tr>
<tr>
<td>Other cardiac diseases</td>
<td>26</td>
<td>85 72 31</td>
<td>65 55 40</td>
<td>30 27 22</td>
</tr>
<tr>
<td>Cameroonian blood donors</td>
<td>31</td>
<td>40 35 9</td>
<td>5 3</td>
<td>Not done</td>
</tr>
<tr>
<td>Cameroonian medical students</td>
<td>20</td>
<td>— — —</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Europeans</td>
<td>50</td>
<td>— — —</td>
<td>— —</td>
<td>— —</td>
</tr>
</tbody>
</table>

fection, 3 with a titre of 1/16, and 9 with a titre of 1/64. Two patients with myopericarditis also had antibodies at a titre of 1/64, and as the trypanosomal antibodies were also found in the pericardial fluid they were diagnosed as having trypanosomal heart disease. Two further patients with acute myopericarditis, clinically diagnosed as acute rheumatic fever, were also found to have antibodies to T. rhodesiense (both at a titre of 1/64), suggesting a plurality of pathology or that they were masquerading as rheumatic heart disease. Only one (1.9%) among the sera from 52 randomly selected Cameroonian blood donors was found to have antibodies to trypanosomiasis at a titre of 1/16.

Discussion

Congestive cardiomyopathy is probably the end-stage of many different cardiac insults that act through the same mechanism to produce the cardiac damage. Our results suggest that the pathogenesis may be immunological.

Immunoglobulin concentrations are abnormally high in conditions with immunological disturbance such as systemic lupus erythematosus and progressive nephritis (Coombs et al., 1961; Ingram, 1959; Ngu and Soothill, 1969). Immunoglobulin concentrations are autoantibodies that react with some complement components after their binding to antigen-antibody complexes, and recently it has been shown that they enhance the clearance of soluble immune complexes (Ngu and Lambert, 1976). Thus, the significantly raised immunoglobulin titres in the sera of our patients with congestive cardiomyopathy compared with normal Cameroonian controls suggest that similar mechanisms were at work. This view is further substantiated by the negative correlation between the immunoglobulin levels and C3 levels in the group with congestive cardiomyopathy, indicating complement incorporation in immune complexes. Nevertheless, the raised immunoglobulin titres and congestive cardiomyopathy could possibly be unrelated and independently related to the agent or agents that precipitated the cardiac damage. The raised immunoconglutinin levels in the patients with acute rheumatic fever and trypanosomal myopericarditis are in keeping with the known immunopathogenesis of these diseases. The moderate rise in those with viral myopericarditis is consistent with the findings in other viral infections (Coomb et al., 1961), though the precise mechanism operating in such cases is still unclear.

Immunoglobulins are produced in response to a variety of stimuli. The higher levels of immunoglobulins in the patients with congestive cardiomyopathy and other cardiac diseases compared with controls might in part be due to autoantibodies to cardiac tissue. However, the striking increase in IgM levels was seen only in patients with congestive cardiomyopathy and consequently must derive from a different stimulus. Persistent parasitization is known to be associated with increased IgM synthesis (Lambert and Houba, 1974), and possibly a difference of specific parasitic infection among the groups tested rather than any difference in host response to the infection is responsible for this.

Antibodies to cardiac tissue have been reported in a variety of heart diseases, but except in rheumatic fever, in which they may play a pathogenic role (Goldstein, Halpern, and Robert, 1967), their significance is far from clear (Kaplan and Frenqley, 1969). The production of carditis in animals after a prolonged circulation of antibodies to cardiac tissue suggests a causal role (Kaplan and Craig, 1963). Though this might also be the case in congestive cardiomyopathy, the alternative view that these antibodies are produced in response to cardiac damage by other agents cannot be refuted (Gery, Davies, and Ehrenfield 1960). We found cardiac antibodies of the G and M classes. That they participate in the formation of immune complexes was suggested by their complement-fixing ability and the abnormal C3 and immunoconglutinin values in the
sera with the highest IgM and IgG levels. The high incidence of autoantibodies in the patients with other cardiac diseases is to be expected since they are known to occur in rheumatic fever and peri-carditis (Kaplan and Frengley, 1969), which indeed accounted for all but one of the positive cases in that group of patients. Our results are at variance with those of Shaper et al. (1967), van der Geld (1964), and van der Geld et al. (1966) in that cardiac auto-antibodies were found in the sera of only a minority of blood donors (Table 2). Their absence in the sera of healthy Cameroonian medical students suggests that they are indeed indicative of cardiac damage and that their presence in a few of the blood donors may have reflected undiagnosed disease. The high incidence of antibodies to skeletal muscle in the blood donors is difficult to explain.

Aetiology

Although T. cruzi is a well-known cause of heart disease in South America, in Africa, until recently, more attention has been paid to the effects of trypanosomiasis on the central nervous system. African trypanosomiasis certainly causes a pannymyocarditis (Armengaud and Biram, 1960; Francis, 1972), including in some cases a valvulitis (Poltera, Cox, and Owor, 1975), with raised IgM and IgA levels (Armengaud and Biram, 1960). Lambert and Houba (1974) have found evidence for an immunopathogenic basis of the lesions in mice caused by T. brucei, as shown by the alterations of the complement components, granular deposits of immunoglobulins detected along the heart fibres by immunofluorescence, and a disseminated vasculitis with a preponderance of lesions in the heart and brain. These authors think that a similar mechanism operates in African trypanosomiasis.

T. gambiense is endemic in at least four areas of Cameroon, so that its incidence in the population varies with the geographic origin of the people concerned. Though the area in which this study was undertaken is not one where trypanosomiasis is endemic it is within easy reach of two such areas. Our findings of antibodies to T. rhodesiense in 27.5 per cent of patients with congestive cardiomyopathy in contrast to 8.5 per cent of patients with other cardiac diseases—excluding the two patients with trypanosomal myopericarditis—and 1.9 per cent of randomly selected blood donors suggests a causal relation. Indeed, trypanosomiasis is one of the most potent stimulators of immunocoaglutinins (Coombs et al., 1961; Ingram et al., 1959) and IgM. That high levels of these antibodies were found in our patients with congestive cardiomyopathy, a significant proportion of whom had evidence of trypanosomal infection, is therefore not surprising. Furthermore, Cossio et al. (1974) found in cases of Chagas's disease a circulating immunoglobulin which reacted on indirect immunofluorescence with the endocardium, skeletal muscle, and vascular structures of other organs—findings similar to those in our patients with congestive cardiomyopathy.

We conclude that in untreated subclinical attacks of African trypanosomiasis immune complexes are produced which damage the cardiac tissue in which the parasite is lodged. Secondary autoimmune carditis may modify the course of the disease, with a resulting end-stage picture of congestive cardiomyopathy.

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References


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