Immune complexes and abnormal liver function in haemophilia

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SUMMARY Abnormal $^{125}$I-Cq-binding activity was found in the sera of 94% of 55 haemophiliacs. Sera from 66% of these patients inhibited macrophage uptake of labelled aggregated human IgG in a competitive radiobioassay. These results suggest that large molecular weight immune complexes are present in these sera. Analysis of the precipitates obtained directly from the sera by addition of 4% polyethylene glycol showed either a mixture of IgG and IgM or IgM alone. There was poor correlation between the radiobioassay results and the Cq-binding activities in the whole group of 55 patients, suggesting a heterogeneous population of complexes. Nevertheless, a significant correlation was found between Cq-binding activities and the radiobioassay results in seven patients with antibodies to factor VIII, suggesting a homogeneous population of complexes. There was poor correlation between the level of immune complexes and the amount of replacement therapy the patient had received in the previous six months. Abnormal liver function tests were found in 55% of the patients studied but there was poor correlation between these levels and the Cq-binding activities and radiobioassay results. Only two patients had clinical evidence of liver disease.

Although severe haemophiliacs receive repeated transfusions of plasma products from many different donors the incidence of clinical hepatitis is surprisingly low (Biggs, 1974). Recent reports, however, suggest that many of these patients have subclinical liver disease. Mannucci et al. (1975) showed abnormalities of serum transaminase concentrations and bromsulphthalein retention in more than 40% of haemophiliacs, while Levine et al. (1976) showed raised transaminase concentrations in 68% of patients. The reason for these abnormalities is unknown and further investigations are hindered by the obvious difficulty of performing biopsies in these patients. Many forms of liver disease are associated with immunological changes such as abnormal serum immunoglobulin and complement levels (Thompson et al., 1973; Potter et al., 1973), autoantibodies (Dioniach et al., 1966; Thompson et al., 1973), increased Cq-binding and anticomplementary activity (Thomas et al., 1976), and increased C3 turnover (Potter et al., 1976). This study was undertaken to evaluate some immunological parameters in a population of haemophiliacs, many of whom, while entirely asymptomatic, have persistently abnormal liver function tests.

Patients and methods

Fifty-five patients attending the Royal Free Hospital Haemophilia Centre were investigated. The mean age was 30 years, 20 being under and 35 over the age of 20 years. Fifty-three were deficient in factor VIII (haemophilia A) and two were deficient in factor IX (haemophilia B). Thirty-four were severe cases (factor VIII or IX under 2%), 11 moderately severe (2-5%), and 10 mild (more than 5%). Seven patients had antibodies to factor VIII.

Information about the type of therapeutic material given and the total number of factor VIII or IX units infused during the six months immediately before the study was available for 45 patients. Cryoprecipitate was used alone in 27 patients, factor VIII or IX concentrate alone in five patients, while 11 patients had not received any blood product during this period. All patients, however, had been previously exposed to therapeutic materials containing factor VIII or IX. Patients were tested at least 24 hours after the last dose of factor VIII or IX. Three were also tested three hours after treatment.

Immune complexes were detected by Cq-binding activity (Cq-BA) (Zubler et al., 1976) and by radiobioassay (RBA) based on the competitive uptake by guinea-pig macrophages of labelled, aggregated IgG
and immune complexes (Mohammed et al., 1977). Estimations were performed on serum separated at room temperature and stored at -40°C. The total protein precipitated with 4% polyethylene glycol (PEG) was measured and its immunoglobulin content determined by the Mancini method. The third and fourth components of complement (C3, C4) were measured by a standard radial immunodiffusion technique. Serum immunoglobulin levels (Ig) were measured on a fluoronephelometer using Technicon antisera and standards. The differential agglutination titre was determined by the method of Bywaters and Scott (1960). Antinuclear factor and antimitochondrial and anti-smooth muscle antibodies were measured by standard fluorescent antibody techniques. Plasma bilirubin, aspartate transaminase, alkaline phosphatase, urea, serum creatinine, and urine protein were measured in each patient. Hepatitis B surface antigen (HBs Ag) and antibody (HBs Ab) were tested by radioimmunoassay (Heathcote et al., 1974). Antibody to factor VIII was measured by the method of Biggs and Bidwell (1959).

Results

CIRCULATING IMMUNE COMPLEXES
By the most sensitive method, Clq-BA, complexes were present in 52 patients (94%) (Table 1). Thirty-six patients (66%) had complexes present as measured by the RBA method, all inhibiting uptake of human aggregated IgG and none giving enhanced uptake. In 34 patients (62%) protein was precipitable in the serum by the PEG method. The IgG and IgM content of these precipitates is shown in Table 2. No IgA was detected. Overall there was poor correlation (correlation coefficient r = 0.38) between percentage inhibition by RBA and percentage Clq-BA (Fig. 1), implying that the complexes were not homogeneous in character. But results of the two tests for complexes correlated significantly (r = 0.75) in the seven patients with antibodies to factor VIII (Fig. 2). There was no change in the level of circulating immune complexes in three patients studied before and three hours after the infusion of cryoprecipitate.

SERUM IMMUNOglobulINS, C3, C4
Three patients had raised serum IgM levels (Table 3), including one man with primary biliary cirrhosis whose IgM was 747 mg/dl. Only one patient had an IgM level less than 60 mg/dl, but eight had values between 60 and 90 mg/dl. The range in all patients was 42-747 mg/dl. C3 was low in six patients (13%) with a range of 88-205 mg/dl. C4 was also low in six patients with a range of 9-48 mg/dl.

Antimitochondrial antibody was detected in one patient but no other autoantibodies were found. The differential agglutination titre was negative in all patients.

LIVER FUNCTION AND RENAL FUNCTION TESTS
The plasma aspartate transaminase, alkaline phosphatase, and bilirubin concentrations are shown in Fig. 3. Aspartate transaminase levels were raised in

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<tr>
<th>Table 1</th>
<th>Circulating immune complexes in patients with haemophilia</th>
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<tr>
<td>Clq-binding activity</td>
<td>Macrophage radiobioassay</td>
</tr>
<tr>
<td>No. of patients</td>
<td>55</td>
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<tr>
<td>No. positive</td>
<td>52 (94%)</td>
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<tr>
<th>Table 2</th>
<th>Analysis of haemophilic serum proteins precipitated with 4% PEG</th>
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<tr>
<td>No. of patients tested</td>
<td>55</td>
</tr>
<tr>
<td>No. positive</td>
<td>34 (62%)</td>
</tr>
<tr>
<td>IgG + IgM</td>
<td>24</td>
</tr>
<tr>
<td>IgM alone</td>
<td>10</td>
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<tr>
<th>Table 3</th>
<th>Serum complement and immunoglobulin levels in haemophilia</th>
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<tr>
<td>Normal range (mg/dl)</td>
<td>No. of patients: Tested Low High Mean value (± SD) (mg/dl)</td>
</tr>
<tr>
<td>C3</td>
<td>120-180</td>
</tr>
<tr>
<td>C4</td>
<td>20-60</td>
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<tr>
<td>IgG</td>
<td>800-1800</td>
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<tr>
<td>IgM</td>
<td>60-250</td>
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<tr>
<td>IgA</td>
<td>90-450</td>
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30 patients (55%). Alkaline phosphatase was raised in 15 patients (27%) and bilirubin in eight (15%). Only two patients had clinical evidence of liver disease, one having primary biliary cirrhosis and the other atypical chronic active hepatitis.

In no patient was the plasma urea or serum creatinine raised and none had significant proteinuria.

**Correlation of Aspartate Transaminase and Immune Complex Levels**

There was no correlation \( r = 0.34 \) between the presence of circulating complexes and the levels of aspartate transaminase (Fig. 4) and alkaline phosphatase. Very high levels of immune complexes were present in a patient with well-documented primary biliary cirrhosis.

**Correlation of HBS Ag, HBS Ab, and Immune Complex Levels**

Only one of 48 patients tested for HBs Ag was positive. Twenty-seven out of 42 patients (64%) had detectable HBs Ab and 17 of these had antibody levels higher than could be explained by the known passive transfer of HBs Ab in our cryoprecipitate infusions (Dane and Cleghorn, 1976, personal communication). There was poor correlation between the levels of HBs Ab, immune complexes, and aspartate transaminase.

**Discussion**

Circulating immune complexes have been reported in several diseases (Kunkel et al., 1961; Nydegger et al., 1974; Mohammed et al., 1976). They are thought to play an important pathogenic role in systemic lupus erythematosus, rheumatoid arthritis, and some cases of bacterial, viral, and parasitic infections. In this study immune complexes were detected in the sera of 94% of haemophilic patients. These complexes appear to be heterogeneous in character, as suggested by (a) the differences in positivity between the three tests employed, (b) the poor correlation between percentage inhibition in the RBA test and Clq-BA, and (c) the difference in composition of the immunoglobulins in the PEG precipitates. In the subgroup of patients with demonstrable antibodies to factor VIII, however, there was good correlation between percentage inhibition and Clq-BA, implying a degree of homogeneity in the complexes in these patients. The fact that most patients gave positive results by the Clq-BA method indicates that a proportion of
the complexes in most of them were complement fixing. However, in only a few of these patients were the levels of C3 and C4 significantly low.

There are many reports of serum complement levels in liver disease. Although low levels have been found in acute hepatitis (Thompson et al., 1973), acute hepatic necrosis (Fox et al., 1971), and cryptogenic cirrhosis (Potter et al., 1973) both low and normal levels have been reported in chronic active hepatitis and other forms of chronic liver disease (Fox et al., 1971; Potter et al., 1973; Teisberg and Gjone, 1973). Raised levels of C3 have been found in some patients with compensated primary biliary cirrhosis (Potter et al., 1973), and recently hypercatabolism of C3 has been demonstrated by Potter et al. (1976). Normal and low complement levels were present with circulating C3 breakdown products in patients with chronic active hepatitis and primary biliary cirrhosis studied by Teisberg and Gjone (1973). We did not measure C3 degradation products and are therefore unable to state whether the normal complement levels detected in our patients reflect normal complement metabolism.

Liver function tests were abnormal in over half of our patients, confirming previous reports of a high incidence of subclinical hepatitis in haemophiliacs (Mannucci et al., 1975; Levine et al., 1976). There was no correlation between these abnormalities and the levels of immune complexes, although a significant correlation has been reported in other forms of chronic liver disease (Thomas et al., 1976). Immune complexes have been detected in hepatitis B surface antigen (HBs Ag) positive hepatitis (Almeida and Waterson, 1969; Nydegger et al., 1974), and we considered the possibility that subclinical HBs Ag positive hepatitis was responsible for the abnormalities in our patients. HBs Ag was detected in only one patient by radioimmunoassay, confirming that the reported incidence of HBs Ag positivity in haemophiliacs in the United Kingdom is less than 3% (Biggs, 1974). While HBs Ag was detected in 64% of our patients, there was poor correlation between the presence of antibody and the level of circulating immune complexes.

There was no correlation between the levels of circulating complexes and the age of the patient, the type of blood product infused, or the amounts of factor VIII or IX administered. All the patients included in this study, however, had received treatment with plasma products at some time or another. In three patients studied before and after the infusion of cryoprecipitate no change in the level of complexes was seen. None of them had antibody to factor VIII.

The explanation for these circulating immune complexes in haemophilia is not clear. They are not obviously related to the infusion of blood products or to the abnormalities of liver function. None of our patients had clinical evidence of vasculitis or glomerulonephritis and the immunopathological significance of the complexes is therefore uncertain. Immune complexes are normally cleared by the reticuloendothelial system and their rate of clearance depends on their size and composition (Mannik et al., 1974). It has been shown experimentally that clearance may be impaired by prolonged exposure to circulating complexes (Wilson and Dixon, 1971; Mannik and Arend, 1971). The reported presence of palpable splenomegaly in 26% of 98 haemophiliacs (Levine et al., 1976) and of splenomegaly on 99mTc scanning in 10 of 14 haemophiliacs (Kirk, 1976, personal communication) may be a reflection of impaired reticuloendothelial function.

We are trying to determine the nature of these immune complexes and their relationship to repeated transfusions of plasma products and to the presence of antibody to factor VIII.

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