

Immunoglobulins in chronic liver disease

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The association of high gamma globulin levels with hepatic cirrhosis is well known. Since gamma globulin levels to a great extent reflect antibody-like protein levels, the hyperglobulinaemia in chronic liver disease, which is associated with an increased turnover rate (Cohen, 1963), is likely to be part of an immunological response. Antibodies reacting with cell nuclei (Doniach, Roitt, Walker, and Sherlock, 1966), mitochondria (Walker, Doniach, Roitt, and Sherlock, 1965; Doniach *et al.*, 1966), smooth muscle (Johnson, Holborow, and Glynn, 1965), renal glomeruli (Whittingham, Mackay, and Irwin, 1966), and biliary canaliculi (Johnson, Holborow, and Glynn, 1966) are probably only a few of the pathological antibodies produced in chronic liver disease. The serum gamma globulins include the immunoglobulin components known as IgG, IgA, IgM (Bull. W.H.O., 1964), and IgD (Rowe and Fahey, 1965), and methods are now available for their distinction and measurement (Fahey and McKelvey, 1965). These components have therefore been studied both to observe the patterns of change in liver disease and to ascertain if the changes might be of diagnostic value.

Previous reports on serum immunoglobulins in small numbers of patients with chronic liver disease have suggested an association between high serum IgM and primary biliary cirrhosis (Paronetto, Schaffner, and Popper, 1964; McKelvey and Fahey, 1965; Hobbs, 1966), high serum IgA and alcoholic cirrhosis (McKelvey and Fahey, 1965; Lee, 1965), and depressed levels of IgG, IgA, and IgM in Wilson's disease (McKelvey and Fahey, 1965).

MATERIAL AND METHODS

One hundred and sixteen patients with chronic liver disease were selected from patients at the Royal Free Hospital. Diagnosis was based on clinical, histological, and biochemical findings, and consisted of chronic active hepatitis (44 cases), cryptogenic cirrhosis (19), primary biliary cirrhosis (16), alcoholic cirrhosis (14), chronic cholestasis due to large bile duct obstruction (14), Wilson's disease (6), and haemochromatosis (3). The group with large bile duct obstruction consisted of patients with obstructive jaundice of longer than four months' duration due to biliary strictures or gallstones.

As controls, 60 healthy persons consisting of 37 blood donors and 23 healthy members of the Royal Free Hospital staff were studied. With the exception of two patients (a 12-year-old child with cryptogenic cirrhosis and a 13-year-old child with chronic active hepatitis) the patients and the healthy persons studied were adults over the age of 20 years. The sera had been stored at -20°C for periods ranging from one to 18 months.

The serum immunoglobulin levels were estimated by the immunodiffusion method (Fahey and McKelvey, 1965). Sera and reference standards are placed in wells of equal size in agar plates containing specific antibody against the immunoglobulin being estimated. After incubation a precipitin ring is formed round each well, the diameter of which is related to the immunoglobulin concentration. A standard curve is drawn by plotting precipitin ring diameters of the standard solutions (on an arithmetic scale) against their concentration (on a logarithmic scale). The immunoglobulin levels in the test sera are determined by reference to the standard curve.

IgG, IgA, and IgM levels were estimated using immunoplates and standards prepared by Hyland laboratories and were expressed as mg/100 ml. The same standards were used for estimations in healthy persons and in patients with liver disease. The reproducibility of the immunoglobulin determinations was assessed by performing six separate determinations of a single serum. The maximum deviation occurring with IgG determinations was 23%; with IgA and IgM the respective figures were 18% and 14%.

Specific antiserum and standards for the measurement of IgD levels in patients with liver disease were provided by Dr. D. S. Rowe. Serum IgD levels were expressed as a percentage of a standard serum of high IgD content.

RESULTS

IMMUNOGLOBULINS IN HEALTHY PERSONS Serum IgG levels ranged between 1,000 and 2,700 mg/100 ml (mean 1,700 mg/100 ml). IgA levels were between 57 and 780 mg/100 ml (mean 334 mg/100 ml) and IgM levels were between 25 and 180 mg/100 ml (mean 75 mg/100 ml) as shown in Figure 1. Serum IgD findings in healthy persons were provided by Dr. D. S. Rowe (Fig. 2).

IMMUNOGLOBULINS IN CHRONIC LIVER DISEASE IgG levels above the normal range were recorded among patients in each of the seven diagnostic groups

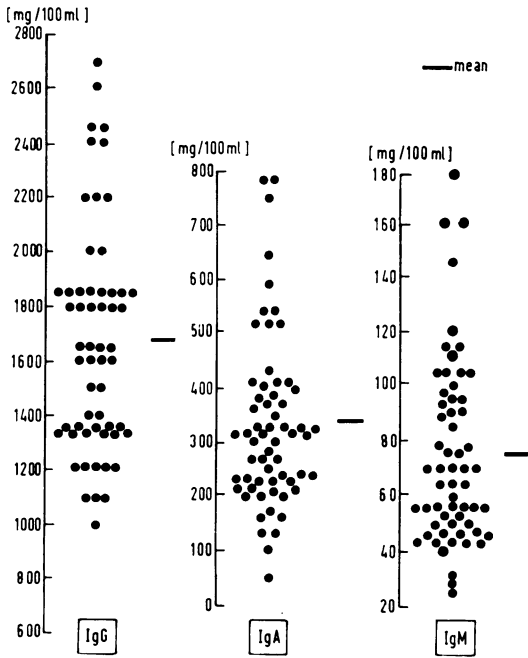


FIG. 1. Immunoglobulins in 60 healthy people.

(Fig. 3). Marked elevations were common among patients with chronic active hepatitis; such elevated levels occurred even in patients receiving prednisone.

Serum IgA levels above the normal range were recorded among all groups with the exception of the small group of patients with haemochromatosis (Fig. 4). Marked elevations of this immunoglobulin to levels of 1,000 mg/100 ml or above were by no means confined to the alcoholic cirrhosis group.

IgM elevations occurred not only among patients with primary biliary cirrhosis but among each of the seven diagnostic groups (Fig. 5).

A quarter of the patients with primary biliary cirrhosis (four out of 16) had IgM levels within the normal range. In primary biliary cirrhosis IgM levels did not correlate with duration of illness (Fig. 6).

There was a similar incidence of rises of IgG, IgA, and IgM found in chronic active hepatitis and cryptogenic cirrhosis (Fig. 7). The remaining three groups had differing patterns. The highest levels for IgG were in patients with chronic active hepatitis and cryptogenic cirrhosis, occurring in 64% and 63% respectively. Serum IgA was most commonly elevated in patients with alcoholic cirrhosis. The highest levels for IgM were in the primary biliary cirrhosis group, although the alcoholic cirrhotics did not fall far short: 81% of patients with primary biliary cirrhosis had elevated IgM levels, as also did 70% of alcoholic cirrhotics. Twenty-one per cent of patients with chronic large bile duct obstruction resembled patients with primary biliary cirrhosis in having an IgM elevation. The patterns in IgG, IgA and IgM in the sera of individual patients in the various diagnostic groups are summarized in Tables I and II.

The distribution of IgD levels in the 116 patients with liver disease shown in Fig. 2 probably did not differ significantly from that of healthy controls. A greater proportion of patients with liver disease had IgD levels of less than 1%, whereas a smaller proportion were in the 1-10% range. These findings could well be due to difficulties in detecting with accuracy very low concentrations of immunoglobulin. IgD levels above 50% of the standard serum were found in six patients, who were cases of

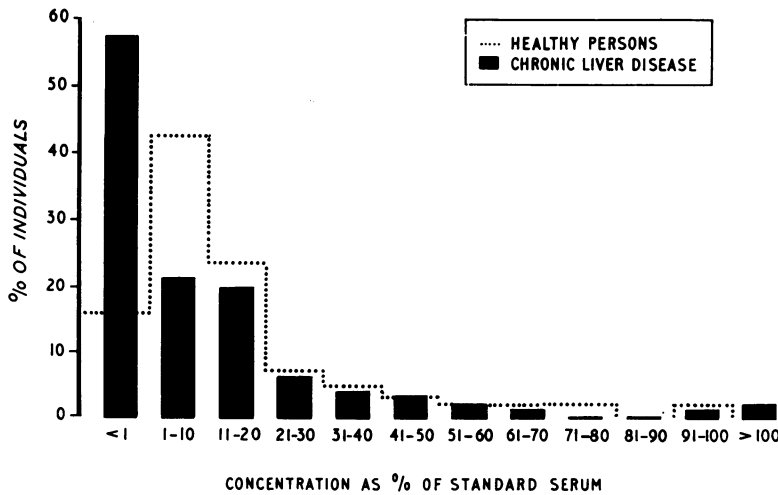


FIG. 2. Serum IgD in healthy persons and in patients with chronic liver disease.

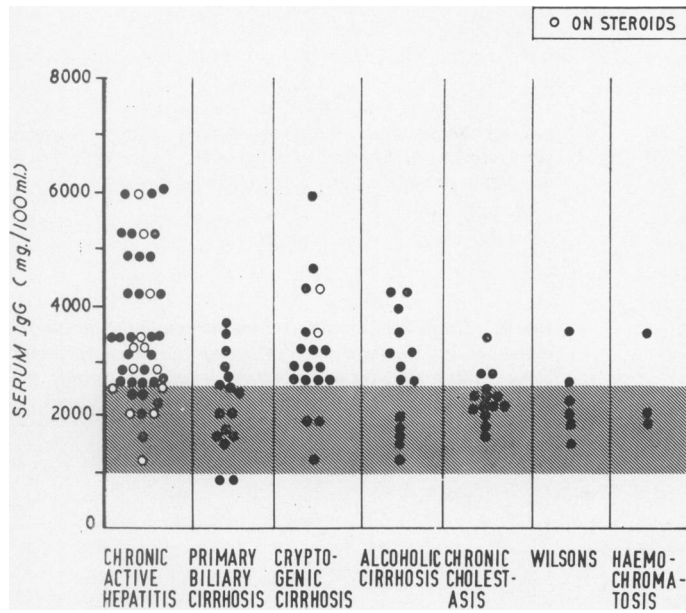


FIG. 3. Serum IgG in chronic liver disease.

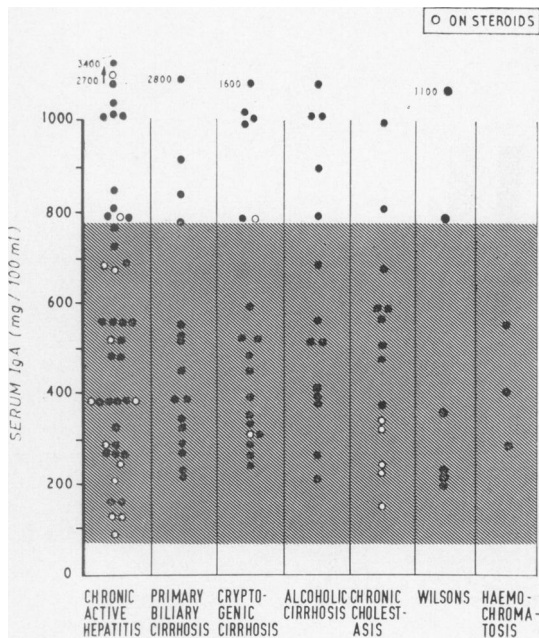


FIG. 4. Serum IgA in chronic liver disease.

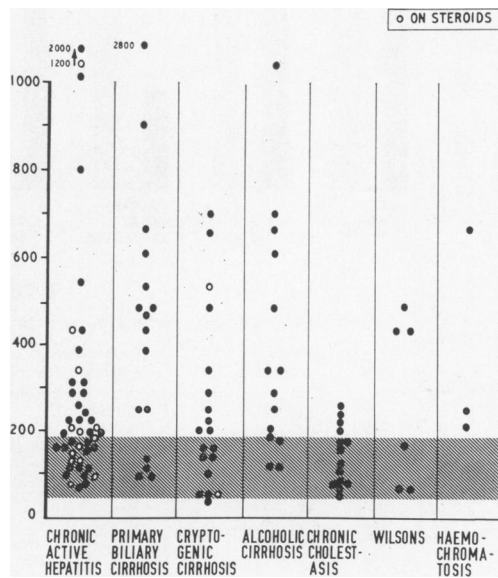


FIG. 5. Serum IgM in chronic liver disease.

PRIMARY BILIARY CIRRHOSIS

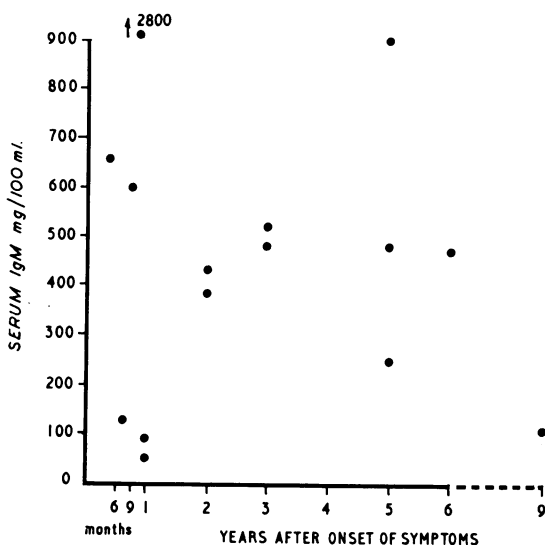


FIG. 6. Serum IgM levels in primary biliary cirrhosis correlated with duration of symptoms. These IgM levels are single determinations in 14 different patients.

FIG. 7. Incidence of raised immunoglobulin levels in active chronic hepatitis, cryptogenic cirrhosis, primary biliary cirrhosis, alcoholic cirrhosis, and in chronic obstructive jaundice due to biliary strictures or stones.

FIG. 6

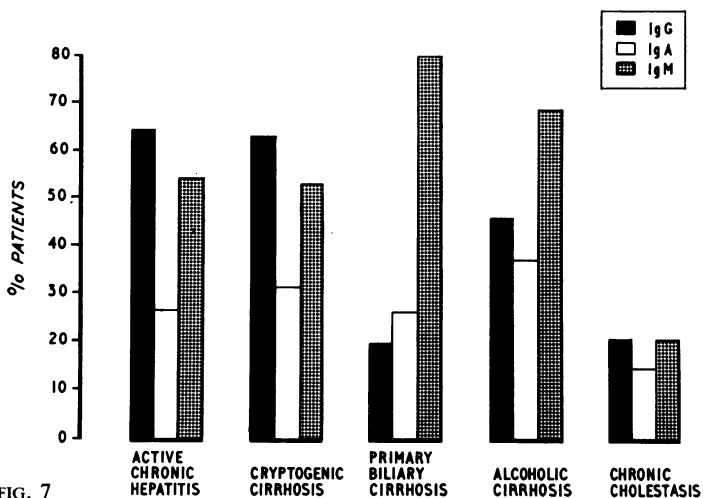


FIG. 7

TABLE I

IMMUNOGLOBULIN PATTERNS IN INDIVIDUAL PATIENTS WITH CHRONIC LIVER DISEASE

IgG	IgA	IgM	Chronic Active Hepatitis	Cryptogenic Cirrhosis	Primary Biliary Cirrhosis	Alcoholic Cirrhosis	Chronic Cholestasis
-	-	-	4 (9%)	5 (26%)	3 (19%)	2 (14%)	7 (50%)
+	-	-	10 (23%)	3 (16%)	0	2 (14%)	2 (14%)
+	+	-	2 (5%)	1 (5%)	0	0	0
+	+	+	6 (14%)	5 (26%)	1 (6%)	1 (7%)	1 (7%)
+	-	+	12 (27%)	4 (21%)	2 (13%)	4 (29%)	0
-	+	-	3 (7%)	0	1 (6%)	1 (7%)	0
-	+	+	2 (5%)	0	2 (13%)	3 (21%)	1 (7%)
-	-	+	5 (11%)	1 (5%)	7 (44%)	1 (7%)	3 (21%)
Totals			44	19	16	14	14

+ = Immunoglobulin level above normal range.
 - = Immunoglobulin level within normal range

TABLE II

IMMUNOGLOBULIN PATTERNS IN WILSON'S DISEASE AND IN HAEMOCHROMATOSIS

IgG	IgA	IgM	No. of Patients
<i>Wilson's disease (6 patients)</i>			
—	+	+	1
+	+	+	1
—	—	+	1
—	—	—	3
<i>Haemochromatosis (3 patients)</i>			
+	—	+	1
—	—	+	2

chronic active hepatitis (2), cryptogenic cirrhosis (2), and Wilson's disease (2).

DISCUSSION

The normal range for IgG and IgA is considerably higher than that in two other published series (Fahey and McKelvey, 1965; Stiehm and Fudenberg, 1966), whereas the range for IgM is in reasonable agreement with them. The discrepancy is explained by differences in reference (standard) sera, so in order to have a valid comparison between healthy persons and the patients the same standards were used throughout this survey.

No single immunoglobulin pattern was diagnostic of any of the seven chronic liver diseases studied. It may be difficult to diagnose chronic large bile duct obstruction and primary biliary cirrhosis; in the former surgical intervention is indicated whereas in the latter it is contraindicated. On the basis of serum IgM rises in primary biliary cirrhosis, it has been suggested that serum IgM levels might make the distinction from chronic bile duct obstruction (Hobbs, 1966), but the finding of normal IgM levels in 19% of patients with primary biliary cirrhosis and of raised IgM levels in 21% of patients with chronic large bile duct obstruction makes the serum IgM level a much less reliable criterion than the mitochondrial antibody test (Walker *et al.*, 1965; Doniach *et al.*, 1966). Paronetto *et al.* (1964) also recorded high macroglobulin levels in nine cases of primary biliary cirrhosis (one of them had a normal level at the time of initial testing) and in three out of seven patients with secondary biliary cirrhosis. The high incidence of IgA elevation in alcoholic cirrhosis has been confirmed but elevation was not uncommon in other chronic liver diseases and this reduced its value as a diagnostic test.

Serum immunoglobulin levels were not reduced in patients with Wilson's disease involving the liver. The numbers tested, however, were small. Patients with Wilson's disease may have very high total serum gamma globulins (Sherlock, 1966), and the

commonest immunoglobulin change observed in the present study was elevation of serum IgM (Table II).

The similarity in the patterns of immunoglobulin change in chronic active hepatitis and cryptogenic cirrhosis is striking, and may represent a response to the same or similar antigenic stimuli in the two diseases. The distinction between the two conditions, based on the total serum gamma globulin level and the degree of plasma cell infiltration and cell necrosis in the liver biopsy, can sometimes be difficult; these criteria may merely be separating individuals with differing degrees of immunological response. Many patients with chronic active hepatitis ultimately develop cirrhosis. These observations suggest that cryptogenic cirrhosis might be the late stage of a subclinical chronic active hepatitis.

Markedly raised immunoglobulin levels were observed among some patients receiving prednisone, the dosage of which ranged between 5 and 30 mg per day (Figs. 3, 4, and 5). Many of these patients had higher total gamma globulin levels before the initiation of steroid therapy. Therefore, it is possible that their immunoglobulin levels had been reduced to some extent by steroid therapy.

SUMMARY

Using the immunodiffusion method the serum levels of immunoglobulins IgG, IgA, IgM, and IgD were determined in 116 patients with a variety of chronic liver diseases. The main groups were chronic active hepatitis, cryptogenic cirrhosis, alcoholic cirrhosis, primary biliary cirrhosis, and chronic obstructive jaundice due to biliary strictures or stones. A small group of patients with haemochromatosis and Wilson's disease were included.

Markedly raised levels of IgG, IgA, and IgM were observed among patients in almost all the diagnostic groups.

Serum IgG levels were raised most commonly in active chronic hepatitis and cryptogenic cirrhosis, occurring in 64% and in 63% respectively; in the three other main groups they were elevated in 20 to 46% of cases. Forty-six per cent of patients with cirrhosis of the alcoholic had elevated IgA levels but they were high in 15 to 36% of the other groups of patients. A very high incidence (81%) of raised IgM levels was found in patients with primary biliary cirrhosis and in patients with cirrhosis of the alcoholic 70% had high IgM levels. In the remaining groups high levels of IgM were observed in 21 to 56% of patients.

High levels of IgD were observed in two out of 44 patients with active chronic hepatitis, two out of 19 with cryptogenic cirrhosis, and in two out of six with Wilson's disease.

There was a similarity in the incidence of immunoglobulin rises in chronic active hepatitis and in cryptogenic cirrhosis.

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REFERENCES

- Bulletin of the World Health Organisation* (1964). Nomenclature for human immunoglobulins. **30**, 447-450.
- Cohen, S. (1963). γ -Globulin metabolism. *Brit. med. Bull.*, **19**, 206-206.
- Doniach, D., Roitt, I. M., Walker, J. G., and Sherlock, S. (1966). Tissue antibodies in primary biliary cirrhosis, active chronic (lupoid) hepatitis, cryptogenic cirrhosis and other liver diseases and their clinical implications. *Clin. exp. Immunol.*, **1**, 237-262.
- Fahey, J. L., and McKelvey, E. M. (1965). Quantitative determination of serum immunoglobulins in Antibody-Agar plates. *J. Immunol.*, **94**, 84-90.
- Johnson, G. D., Holborow, E. J., and Glynn, L. E. (1965). Antibody to smooth muscle in patients with liver disease. *Lancet*, **2**, 878-879.
- , —, — (1966). Antibody to liver in lupoid hepatitis. *Ibid.*, **2**, 416-418.
- Hobbs, J. R. (1966). Primary biliary cirrhosis: positive antibody tests associated with increased immunoglobulin IgM. *Proc. roy. Soc. Med.*, **59**, 568.
- Lee, F. I. (1965). Immunoglobulins in viral hepatitis and active alcoholic liver disease. *Lancet*, **2**, 1043-1046.
- McKelvey, E. M., and Fahey, J. L. (1965). Immunoglobulin changes in disease: quantitation on the basis of heavy polypeptide chains, IgG (γ G), IgA (γ A), and IgM (γ M), and of light polypeptide chains, type K (I) and type L (II). *J. clin. Invest.*, **44**, 1778-1787.
- Paronetto, F., Schaffner, F., and Popper, H. (1964). Immunocytochemical and serologic observations in primary biliary cirrhosis. *New Engl. Med.*, **271**, 1123-1128.
- Rowe, D. S., and Fahey, J. L. (1965). A new class of human immunoglobulins II. Normal serum IgD. *J. exp. Med.*, **121**, 185-199.
- Sherlock, S. (1966). *Diseases of the Liver and Biliary System*, 3rd ed. p. 384. Blackwell, Oxford.
- Stiehm, E. R., and Fudenberg, H. H. (1966). Serum levels of immune globulins in health and disease: a survey. *Pediatrics*, **37**, 715-727.
- Walker, J. G., Doniach, D., Roitt, I. M., and Sherlock, S. (1965). Serological tests in diagnosis of primary biliary cirrhosis. *Lancet*, **1**, 827-831.
- Whittingham, S., Mackay, I. R., and Irwin, J. (1966). Autoimmune hepatitis: immunofluorescence reactions with cytoplasm of smooth muscle and renal glomerular cells. *Ibid.*, **1**, 1333-1335.

ADDENDUM

Preliminary studies with ^{125}I labelled autologous IgG in a patient with chronic active hepatitis and similarly labelled autologous IgM in a patient with primary biliary cirrhosis suggest that the high levels of these immunoglobulins are due to increased synthesis (Bradley and Feizi, 1968, unpublished observations).