# Antibodies and immunoglobulins in liver disease

Raised levels of serum gamma globulins occur in many acute and chronic hepatic disorders. In acute inflammatory disease, *eg*, virus hepatitis, septic cholangitis, infectious mononucleosis, a moderate increase is seen seven to 14 days after onset but returns to normal within a few months[1] and probably represents antibody formation against the infecting organism. The persistence of high globulin levels may indicate transition to chronic liver disease[2] where hypergammaglobulinaemia is generally more pronounced. In cirrhosis, serum protein electrophoresis may show $\beta$-$\gamma$ fusion due to an increase in the faster globulins ($\gamma_1$) whereas a more narrow, distinct gamma peak is characteristic of active chronic hepatitis (juvenile cirrhosis)[3] and consists essentially of the slower $\gamma_2$ globulins.[4, 5] The electrophoretic $\beta$ and $\gamma$ regions are known to contain the five immunoglobulin classes IgG, IgA, IgM, IgD, and IgE so far identified.[6] It is now possible to estimate these globulins, except IgE, quantitatively by single radial immunodiffusion,[7, 8] and a number of authors have performed these tests in liver diseases.[4, 9-13] A further study of this nature appeared in the previous issue of this journal.[14] Extreme hypergammaglobulinaemia is characteristically seen in the active phase of juvenile cirrhosis[3] and most workers agree that there is frequently a substantial increase in serum IgG concentration. IgA elevation has been described as typical of portal cirrhosis, particularly in association with chronic alcoholism. In primary biliary cirrhosis the IgM fraction is usually raised and it has been claimed that isolated elevation of this immunoglobulin may be used in differentiating this condition from secondary biliary and other types of cirrhosis.[13] In the recent study,[14] however, it was shown that in all these diseases the changes were rarely selective and varying degrees of elevation were frequently found in each of the three major immunoglobulin classes IgG, IgA, and IgM. Thus although IgM levels were raised in 81 % of cases of primary biliary cirrhosis, they were also high in 21 % of patients with main bile-duct obstruction, and this is in agreement with the high IgM values found in three out of seven cases of secondary biliary cirrhosis in another series.[9] Thus the diagnostic value of quantitative immunoglobulin determinations is limited. IgD levels appear to be normal in all the liver disorders so far studied.[13, 14] The most recently described immunoglobulin, IgE, which is of particular clinical importance with reference to allergy since it contains the antibodies of immediate-type reactivity, *ie* the reagins,[15] has not so far been sought for in hepatic disorders. In most patients with liver disease and hyperglobulinaemia, the globulins are polyclonal with chemically distinct molecules showing the entire range of different light chains on starch-gel electrophoresis in urea medium. However, in isolated cases of cirrhosis M-bands with single light-chains, characteristic of monoclonal gammopathy, have been described.[5]

Immunoglobulins are not produced in the normal liver but in chronic hepatic disease they have been identified in the lymphoid and plasma cells infiltrating portal areas and parenchyma. IgG appears to be the predominant immunoglobulin synthesized by mesenchymal cells in active chronic

hepatitis,[16] while lymphoid aggregates around damaged bile ductules in primary biliary cirrhosis produce mainly IgM.[9]

In some cases of chronic liver disease the rise in serum immunoglobulins is associated with detectable circulating tissue autoantibodies. Early studies of immune reactions in liver disease employed complement fixation with liver and other tissue extracts in an attempt to develop a diagnostic test for infective hepatitis.[17, 18] Positive reactions were obtained over a wide spectrum of hepatic disease and it became apparent that these were unrelated to the hepatitis virus, but resulted from interactions of tissue components with serum autoantibodies. High complement-fixation titres, however, are virtually confined to three conditions, namely, active chronic hepatitis, cryptogenic cirrhosis, and primary biliary cirrhosis.[19] This test is nevertheless non-organ specific and no antibody reacting exclusively with liver has so far been identified. The latex-FII fixation test, which has been widely used in liver disorders,[20, 21] detects rheumatoid factors which are antibodies directed against IgG.[22] This test is also positive in many liver disorders. More recently immunofluorescent tests have been employed in the detection of a number of serum antibodies. High titres occur in the same three diseases giving strongly positive complement-fixation reactions.[19] Antinuclear antibodies of both diffuse and speckled varieties have been detected in 75% of cases of active chronic hepatitis,[23] in 46% of primary biliary cirrhosis cases, and in 38% of patients with cryptogenic cirrhosis.[19] In the active chronic hepatitis group there is a correlation between antinuclear antibody titres and serum gamma globulin levels.[19] A cytoplasmic antibody reacting with bile ductules has been described in a high proportion (75%) of patients with primary biliary cirrhosis.[24] Although these structures bear the brunt of the liver injury in this condition,[25] it is difficult to assign an aetiological role to the antibody as it is present in as many as 67% of patients with viral hepatitis and in 10% of a normal population.[24] A different immunofluorescent reaction is that with smooth muscle fibres[26] found in about 70% of cases of active chronic and lupoid hepatitis.[19, 26, 27] Other reactions described in this disease produce staining of renal glomerular cells[27] and 'bile canaliculi'.[28] The presence of these antibodies will probably be of use in diagnosis when interpreted in conjunction with other tests. Their evaluation in larger series is still in progress. Greater diagnostic help is afforded by the mitochondrial antibody test[29] which is positive in 79% to 94% of patients with primary biliary cirrhosis and in only 0% to 3% of cases of main bile duct obstruction.[19, 30-34] These small variations in incidence can be accounted for by differences in the methods employed. Since this antibody is found only infrequently and in low titres in other non-cirrhotic forms of cholestasis, *eg*, virus hepatitis, cholestatic drug jaundice, biliary atresia, sclerosing cholangitis, pericholangitis associated with colitis, and benign recurrent cholestasis, a strongly positive reaction is of great help in distinguishing primary biliary cirrhosis from these disorders which may simulate it. In the ill-defined group of patients with cirrhosis of uncertain aetiology usually designated as 'cryptogenic' a varying incidence of between 4% and 25% has been reported. This may be due to differences in diagnostic criteria and geographical distribution of cirrhosis.[35] Features such as jaundice, pruritus, and markedly raised serum alkaline phosphatase levels in many of the positive reactors in our personal series suggest that at least some of these have a disease related to primary biliary cirrhosis.[31] In active chronic

hepatitis, as in cryptogenic cirrhosis, the reported incidence of mitochondrial fluorescence varies considerably due to differences in diagnostic criteria.

It is of considerable importance that only 0·5% of more than 8,000 mixed hospital patients without overt liver disease have positive mitochondrial fluorescence. Detailed study of these reactors showed raised serum alkaline phosphatase levels and abnormal bromsulphthalein retention in about one fifth of the patients and liver biopsies revealed mild inflammatory changes in portal zones.[36] These patients usually presented with atypical arthralgias. Others, without evidence of abnormal liver function, had only low titres of mitochondrial fluorescence but showed strong complement fixation, related to the presence of less well-defined cytoplasmic antibodies already known to exist in the collagenoses.[37] The same mixed reactions probably account for the discrepancy between mitochondrial and tissue complement-fixation titres in active chronic hepatitis.[19] The use of a purified mitochondrial antigen for complement fixation will help in further defining these multiple antibodies. Biochemical studies show that the antibodies characteristic of primary biliary cirrhosis react selectively with a component of the mitochondrial membranes.[38, 39] The enzymes of the electron transport chain do not appear to be implicated in the antigenic determinant.[40] It is not yet understood why a disease process affecting bile ductules should be so constantly associated with antibodies which react with mitochondria in all tissues. The analogy of syphilis where an antibody reacts with cardiolipin, another specific mitochondrial inner-membrane component, might suggest a cross-reacting antibody in primary biliary cirrhosis directed against an unknown infective agent.

The tissue antibodies reviewed by no means account for the elevations of immunoglobulins in the chronic liver diseases. In alcoholic cirrhosis, haemochromatosis, and Wilson's disease, where antibody tests have been consistently negative, serum immunoglobulins may be raised[14] while no correlation can be demonstrated between serum gamma globulin or IgM levels and mitochondrial antibody or antinuclear antibody titres in primary biliary cirrhosis. It seems likely that a generalized hyperreactivity of the lymphoreticular system[41] is responsible for the increased synthesis of immunoglobulins in cirrhosis[42] and there is also evidence of increased immune responses to bacterial antigens.[18, 43] The nature of the stimulus to the lymphoreticular system is unclear but may be related in some way to hepatic injury or to the cirrhotic process itself. A non-specific response, however, cannot fully explain the appearance of specific autoantibodies nor their predilection for certain disorders. These antibodies cannot be related simply to release of antigen following liver injury as in experimental animals[44] where the auto-immunization is transient and the antibodies distinct from those seen in the human diseases. Severe hepatocellular damage in man, moreover, is only rarely associated with a progressive autoimmune state.

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