The role of immunological mechanisms in acute and chronic hepatitis

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Summary

Studies of asymptomatic carriers of hepatitis B antigen (HB Ag) have suggested that the hepatitis B virus may not be directly damaging to liver cells and it is possible that the hepatocellular necrosis which accompanies acute hepatitis may be induced by immunological reactions directed at viral antigenic determinants on the surface of infected cells. Immunological reactions may also be implicated in the pathogenesis of active chronic hepatitis. Although antibodies which are commonly present in the serum are probably not of primary importance in the immunopathology, recent studies have demonstrated liver-specific immune responses, both humoral and cellular, which may be more directly related to the pathogenesis.

There is increasing evidence that immunological reactions, potentially damaging to liver cells, may play an important part in the pathogenesis of both acute and chronic hepatitis.

Acute hepatitis

A rapid increase in our knowledge of the immunopathology of acute hepatitis has followed the discovery of the hepatitis B antigen. Screening of blood samples on a large scale to detect this marker for the presence of the hepatitis B virus soon established the presence of persistent carriers of the antigen in the population. Two types of carrier have been recognized: those who are apparently in good health, usually detected by screening of blood donor populations, and patients with some degree of immunological deficiency, usually secondary to some other condition (Fig. 1).

Clinical investigation of healthy carriers has shown that a proportion of them have asymptomatic liver disease. In one study from Copenhagen (Reinicke et al., 1972), liver biopsies were normal in thirteen of twenty-four carriers examined, but showed chronic persistent hepatitis in ten (41%) and active cirrhosis in one (5%). On the other hand, all of twenty-two young Greek soldiers who were carriers of the antigen had normal liver histology or only mild non-specific changes (Hadziyannis et al., 1972) and there seems little doubt that some healthy carriers have absolutely no evidence of liver disease.

The increased frequency of the carrier state with immunological deficiency is well-documented. It has been described with Down’s syndrome, lepromatous leprosy, diabetes, uraemia, and in patients receiving immunosuppressive therapy following renal transplantation or as cytotoxic treatment of leukaemia and lymphoma. These carriers differ from the healthy carriers in having a greater incidence of liver disease. Sixty-three per cent of carriers with Down’s syndrome had histological abnormalities on liver biopsy (Gerstley et al., 1972) and 75% of carriers with treated Hodgkin’s disease had chronic persistent hepatitis (Grange et al., 1973), while untreated Hodgkin’s patients were all antigen-negative.

Why some carriers should exist in apparently harmless symbiosis with the hepatitis virus, while others develop chronic liver disease has been the subject of recent speculation (Dudley, Fox and Sherlock, 1972). The antigen is certainly present in the liver, for when liver biopsies from healthy carriers are examined for the presence of HB Ag by immuno-fluorescence, many of the hepatocytes show cytoplasmic fluorescence (Fig. 2). It is likely that these cells also contain virus, for although it is possible in theory for HB Ag to be present in the absence of mature virus particles, blood from these carriers is known to be capable of transmitting acute hepatitis. The fact that a symbiotic union can exist implies
that the virus itself is not damaging to liver cells, and also suggests that the hepatocellular damage in patients with acute hepatitis is associated with some indirect mechanism triggered by the presence of the virus.

In other infections with viruses which are not cytopathic, the immune response to infection has been implicated in the production of tissue damage. Thus, in experimental lymphocytic choriomeningitis in mice the neurological lesions are thought to be mediated by a cellular immune reaction directed at viral determinants on the surface of infected cells. The disease can be almost completely suppressed by either induction of tolerance, irradiation or immunosuppression with drugs (Hotchin, 1971). Application of such a theory of immunopathology to hepatitis B virus infection would lead to the prediction of a total lack of immune response to HB Ag in healthy carriers (Table 1), and the findings, although preliminary, are in agreement with this. No antigen/antibody complexes were detected in the blood of one asymptomatic carrier (Almeida and Waterson, 1969) and in others there has been no evidence of any cellular immunity (Yeung Leiwah, 1971; Dudley, Giustino and Sherlock, 1972). We have followed one completely healthy carrier for more than 1 year and have found neither antibody to HB Ag by radioimmunoassay nor cell-mediated immunity to the antigen using the leucocyte migration test. This failure of the immune system to react to HB Ag may be genetically determined, as Blumberg's studies suggested (Blumberg, London and Sutnick, 1971) and it is of interest that such specific immunological unresponsiveness in animals has been linked to histocompatibility determinants (Benaceraff and McDevitt, 1972).

In most normal individuals an immune response to HB Ag would follow infection with the hepatitis B virus and in this situation infected cells would be destroyed producing the clinical features of acute

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hepatitis (Table 1). The humoral antibody response to HB Ag is usually undetectable by conventional tests but, by using a radioimmunoassay, specific antibody is now being detected in most patients during recovery from acute hepatitis B infection and recently, several studies have demonstrated the presence of cellular immunity to the antigen (Yeung Leiwah, 1971; Dudley \textit{et al.}, 1972). Whether these immune responses are damaging to infected hepatocytes remains to be determined. In carriers with liver damage it is tempting to speculate that the immunological response might be qualitatively or quantitatively defective (Table 1), inadequate to eliminate the virus but sufficient to produce varying degrees of immunologically-mediated damage to hepatocytes. Dudley \textit{et al.} (1972) have produced some evidence in support of this hypothesis but further studies are clearly needed.

**Active chronic hepatitis**

In this country, 17\% of patients with active chronic hepatitis are HB Ag positive (Reed \textit{et al.}, 1973) and while the immunological reactions described above may be implicated in the production of liver cell damage in these patients, other studies have suggested that auto-immune responses are often present and that some of them may be of pathogenetic significance. Antibodies reacting with nuclear, mitochondrial and smooth muscle antigens are often found in the serum but these are not organ-specific, so it is unlikely that they are directly involved in the pathogenesis of a disease largely confined to the liver. Recently, liver-specific immunological reactions have been detected in patients with active chronic hepatitis and these could be more important in the production of liver damage. Meyer zum Buschenfelde, using indirect immunofluorescence, demonstrated liver-specific antibodies in 10\% of his patients with active chronic hepatitis and went on to isolate the liver-specific antigens from human liver to which these antibodies were directed (Meyer zum Buschenfelde and Kossling, 1971). A finding of great interest was that when these human antigens were injected repeatedly into rabbits, liver lesions were produced which closely resembled chronic aggressive hepatitis in man (Fig. 3).

Our own studies of the immunological reactions which accompany rejection of some human liver transplants (Eddleston \textit{et al.}, 1971) focused our attention on cell-mediated immunity. Using the leucocyte migration test as an assay of this type of immune response, we were able to demonstrate in about half our cases of active chronic hepatitis that there was evidence of hypersensitivity to antigens in

![Fig. 3. The histological changes in the liver of a rabbit which had received multiple injections of human liver-specific antigens. There is a dense mononuclear cell infiltrate in the portal areas with extensive piece meal necrosis of surrounding hepatocytes, features similar to those seen in patients with active chronic hepatitis. (By courtesy of Dr K. H. Meyer zum Buschenfelde.)](http://pmj.bmj.com/)
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a human liver homogenate (Smith et al., 1972). As with humoral antibodies, this sensitization would be of more relevance to the pathogenesis if it could be shown to be liver-specific. We have recently isolated from human liver one of the liver-specific antigens of Meyer zum Buschenfelde, a lipoprotein thought to be a normal constituent of the hepatocyte plasma membrane (Hopf, Meyer zum Buschenfelde and Freundenberg, 1974) and have used it as antigen in the leucocyte migration test. In an initial study sensitization to the lipoprotein was found in eleven of sixteen patients with active chronic hepatitis (Miller et al., 1972) and in an extension of this work these early findings have been confirmed (Fig. 4). Fifty-one cases of active chronic hepatitis have been tested and thirty of these (59%) show evidence of cell-mediated immunity to the liver-specific antigen.

In contrast, sensitization was detected in none of forty-two control subjects and in only one of fourteen patients with haemochromatosis or alcoholic cirrhosis. Many of the patients with active chronic hepatitis were being treated with immunosuppressive drugs and if untreated cases alone were considered the incidence of sensitization was much higher, of nine such patients in the present series eight showed evidence of cell-mediated immunity to the liver-specific lipoprotein.

In an attempt to relate the various immunological findings to the disease process, we proposed, some time ago, a simple working hypothesis (Fig. 5). We envisaged an exogenous stimulus, perhaps a virus or a drug, damaging the liver and releasing an altered liver specific protein. An immune response to this altered antigen might then cross react with the normal protein leading to increasing liver cell destruction. If the immune system were abnormal (perhaps genetically predetermined) this autoimmune reaction could be vigorous and long-lasting. Release of intracellular antigens might secondarily stimulate production of the various autoantibodies which are such a feature of active chronic hepatitis. The recent studies with the liver-specific cell surface lipoprotein are certainly consistent with this model although direct evidence of the cytotoxicity of this cell-mediated reaction has not yet been produced.

Fig. 4. Results of the leucocyte migration test, with liver-specific lipoprotein as antigen, in some chronic liver diseases. ■ haemochromatosis; ×, alcoholic cirrhosis, final column only.

Fig. 5. A proposed model of active chronic hepatitis to show how the various immunological findings may relate to the disease process. (From Smith, Eddleston and Williams, 1971.)
There is also some evidence in support of the postulated abnormality in the immune system. Family studies have shown a significantly increased frequency of autoantibodies in the relatives of our patients with active chronic hepatitis (Galbraith et al., 1974) and Mackay and Morris (1972) have reported an increased frequency of the histocompatibility antigens HL-AI and HL-A8 in this condition. Very little is known about the role of possible initiating agents. The finding of HB Ag in the serum of 17% of our cases of active chronic hepatitis raises the possibility that in this small group of cases the immunopathology may be different and that immune responses directed at viral antigens may be of particular importance. However, we have not been able to detect any differences between HB Ag positive and HB Ag negative cases with respect to clinical manifestations, immunoglobulins, autoantibodies, cell-mediated immunity or prognosis (Reed et al., 1973) and it is not yet clear what role the hepatitis B virus plays in the progression of this chronic liver disease.

Acknowledgment

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References


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Discussion

Dr Yvonne Cossart asked 'Why don't you think the virus damages liver cells?' Dr Eddleston: Our main argument is that chronic carriers do exist who have absolutely no liver disease although they may carry the virus as well as the antigen. Another point is that in Dr Zuckerman's studies, using tissue culture techniques, the only change seen is by immunofluorescence. There is no cytopathic change in this situation.

Commenting on this, Dr I. Dymock, Manchester, stated that he questioned the absence of liver damage in the asymptomatic hepatitis B antigen carrier. In a study of blood donors in the Manchester area liver biopsies had been done in approximately thirty. Four showed either chronic active hepatitis or cirrhosis, eleven had chronic persistent hepatitis, while most of the remainder had minimal cellular damage with some focal cellular necrosis and an associated inflammatory exudate around this and in only three of the subjects was there no evidence of liver damage. In several of the patients progression of the lesions had been noted during a period of up to
18 months. Thus longitudinal studies were vital in such subjects. Dr Eddleston: I think there are two points. One is the problem of trying to define exactly, normal liver histology. Pathologists don't get to look too frequently at liver biopsies from asymptomatic subjects. The other point I wish to make is that the incidence of histological abnormality on liver biopsy in hepatitis B antigen carriers varies a great deal from one part of the world to another. For example, the Copenhagen group found biopsies to be abnormal in 40% of cases while Hadziyannis in Athens found no abnormality in twenty-two asymptomatic carriers. Clearly, more information is needed but I am quite convinced that there are some carriers who have absolutely no liver disease.

Dr McSween of Glasgow asked, 'Is there occasionally an inappropriate predetermined immunological response to the viral A antigen, with resultant chronic liver disease? Dr Eddleston replied that one does not know about viral hepatitis A but with hepatitis B antigen, Blumberg's genetic studies suggested familial or genetic predisposition to carry the agent. They would thus appear to have a specific inability to recognize the antigen. There were animal studies which linked unresponsiveness to antigens with the genetic status of the animals. Dr Eddleston stated that he had studied lymphocyte sensitization to liver specific lipoprotein in patients with acute viral hepatitis with positive results in 50%.'
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