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REVIEW
Immunological aspects of chronic active hepatitis

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INTRODUCTION

The term 'chronic active hepatitis' (CAH), first used by Saint *et al.* (1953) to describe a progressive inflammatory liver disease predominantly affecting young women and having a poor prognosis, is now used to describe a spectrum of aetiologically distinct diseases with a very similar histological picture. This is characterized by an intense portal tract mononuclear cell infiltrate spreading into the liver parenchyma and disrupting the limiting plate of periportal hepatocytes. Liver cells in this part of the lobule are generally isolated and surrounded by mononuclear cells, features described as 'piecemeal' necrosis. The parenchyma tends to be subdivided by fibrous septa accompanying the inflammation and linking portal tracts to each other or to central veins; this, together with the appearance of nodules of regenerating hepatocytes, leads to the development of cirrhosis.

Several different aetiological stimuli may be responsible for the appearance of the characteristic liver lesions of chronic active hepatitis, including non-A, non-B virus infection, Wilson's disease and alpha-1-antitrypsin deficiency. However, the three varieties of the disease most intensively investigated are the 'autoimmune' type (having features in common with the group of organ-specific autoimmune diseases), the hepatitis B virus (HBV)-related type and the drug-induced variety. After summarizing the main characteristics of these varieties, we will compare and contrast the immunological phenomena which could be responsible for the liver cell damage in these three types of CAH.

'Autoimmune' CAH

This is a disease with female preponderance (sex ratio of up to 6:1), occurring mainly in two age groups (10–30 years and post-menopausal), and includes all the cases first described as 'active chronic' (Saint *et al.*, 1953) or 'lupoid' (Joske & King, 1955) hepatitis. Hypergammaglobulinaemia is often striking (as high as 60 or 70 g/l) and predominantly IgG. High titre autoantibodies are commonly found in serum and include antinuclear antibody in 80%, anti-smooth muscle antibody in 70%, and antimitochondrial antibody in 30% of the patients. In some patients, particularly children, the dominant serum autoantibody is directed at liver and kidney microsomes (Odievie *et al.*, 1983). In about 25% of patients, other 'autoimmune' diseases are found and include thyroiditis, rheumatoid arthritis, ulcerative colitis, Sjögren's syndrome, idiopathic thrombocytopenic purpura, myasthenia gravis, pernicious anaemia, fibrosing alveolitis and Addison's disease. The disease is associated with the histocompatibility antigens HLA B8 and DR3.

HBV-related CAH

This is characterized by a striking male preponderance (sex ratio of up to 9:1) and its frequency is directly related to the carrier rate of HBsAg, being lower in Northern Europe and higher in Southern Europe, Central Africa and the Middle and Far East. Non-organ-specific autoantibodies are rarely present and in low titre. The disease has two main phases. Initially, the virus is actively

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replicating, HBeAg, HBV-DNA and viral-associated DNA-polymerase (DNA-P) are present in the serum, and HBcAg—the viral nucleocapsid antigen—is found in the nuclei of infected liver cells. After a few years there is often cessation of viral replication, generally preceded by evidence of increased liver damage, and associated with disappearance of HBeAg, HBV-DNA and DNA-P from serum. This is followed by a decrease in hepatic inflammation and improvement in liver function tests (Realdi *et al.*, 1980). Integration of the HBV genome into host DNA is thought to be responsible for the persistent HBs antigenaemia in this phase (Brechot *et al.*, 1981).

Drug-induced CAH

Three drugs have been responsible for most of the reported cases of drug-induced CAH: oxyphenisatin, alpha-methyldopa (Maddrey & Boitnott, 1977; Zimmerman, 1979) and nitrofurantoin (Sharp, Ishak & Zimmerman, 1980). Patients having oxyphenisatin-induced CAH are generally middle-aged or elderly females, with a history of ingesting the drug for at least 6 months. Non-organ specific autoantibodies are usually present, and these disappear on withdrawal of the drug. Rechallenge with small doses of oxyphenisatin can provoke abrupt increase in serum transaminases.

About 5% of patients receiving alpha-methyldopa develop an increase in serum transaminases within 1 month and about 20% of these have evidence of CAH, often associated with the appearance of LE cells and smooth muscle antibody. Nitrofurantoin-associated CAH generally occurs after ingestion of the drug for more than 6 months. Hyperglobulinaemia and the development of anti-smooth muscle and anti-nuclear antibodies are common and may persist long after withdrawal of the drug.

CELLULAR IMMUNITY AND MECHANISMS OF LIVER DAMAGE

The finding of a prominent lymphocytic infiltration in the portal and periportal areas of the liver of patients with CAH has prompted several studies of cellular immune reactions. Initial studies explored leucocyte sensitization to liver-derived and HBV-associated antigens in both autoimmune (Miller *et al.*, 1972; Thestrup-Pedersen, Ladefoged & Andersen, 1976) and HBV-related CAH (Gerber *et al.*, 1974; Tong *et al.*, 1975; Lee *et al.*, 1975; Realdi *et al.*, 1976; Ortona *et al.*, 1979) and, despite somewhat conflicting results (Yeung Laiwah *et al.*, 1973; de Moura, Vernace & Paronetto, 1975), most investigators claimed that cellular immunity was directed to normal hepatocyte antigens in both chronic liver diseases and, in addition, to HBsAg in the viral-induced form. However, the poor specificity of the techniques employed and the only partial purification and characterization of the antigenic preparations used did not allow any definitive conclusions. Both leucocyte migration inhibition (Miller *et al.*, 1972; Lee *et al.*, 1975; Realdi *et al.*, 1976) and blast transformation (Gerber *et al.*, 1974; Tong *et al.*, 1975; Thestrup-Pedersen *et al.*, 1976; Ortona, 1979) assays may be influenced by the presence of appreciable numbers of B lymphocytes, monocytes or polymorphs and do not allow precise evaluation of the activity of T cells, the main effectors of cellular immunity. Furthermore, most studies employed HBsAg preparations derived from the serum of infected subjects and which were therefore likely to be heavily contaminated with other viral components or host antigens. Investigations of immune responses to liver-derived antigens have been performed using a high molecular weight ($>4 \times 10^6$), macromolecular, lipid-associated complex (LSP), in the absence of purified antigens. LSP contains an undetermined number of antigens, some of which appear to be non-organ-specific, although at least one seems to be species cross-reactive and liver-specific (McFarlane, 1984). Moreover, although LSP contains vesicles and fragments of the hepatocellular plasma membrane (Jensen, Hall & Majewski, 1983), it must also contain additional, 'internal' determinants which could elicit immune reactions not relevant to the pathogenesis of liver damage *in vivo*. Due to these limitations of assays of cell-mediated immunity, attention was turned towards cell-mediated cytotoxicity experiments.

Initial investigations showed that lymphocytes from patients with autoimmune CAH were directly cytotoxic to isolated rabbit hepatocytes *in vitro* (Thomson *et al.*, 1974). The cytotoxicity was found subsequently to reside in a non-T cell population of peripheral blood lymphocytes bearing Fc

receptors (Cochrane *et al.*, 1976). By incubating isolated rabbit hepatocytes with serum from patients with autoimmune CAH, peripheral blood lymphocytes from normal subjects were shown to be capable of a cytotoxic reaction towards the liver cells which was not present when the hepatocytes were incubated with normal serum or CAH-serum previously absorbed with LSP (Gonzalez *et al.*, 1979). However, this test system, in which rabbit liver cells were incubated with human lymphocytes, was unsuitable for demonstrating T cell cytotoxicity, since its full expression depends upon histocompatibility between target and effector cells (Zinkernagel & Doherty, 1974) and, to overcome this restriction, autologous hepatocytes have been employed subsequently in similar microcytotoxicity assay systems (Mieli-Vergani *et al.*, 1979). The results of experiments using different lymphocyte subpopulations (T and non-T) and of blocking experiments with LSP have strengthened the notion that antibody (anti-LSP)-dependent cell-mediated cytotoxicity (ADCC) is the major mechanism of cytotoxicity *in vitro* in autoimmune CAH (Mieli-Vergani, 1979).

The absence of a human cell line capable of sustaining HBV replication in tissue culture constitutes a major limitation in the efforts to establish the specificity of liver cell damaging immune reactions in HBV-related CAH. An attempt to reproduce, *in vitro*, the possible cytotoxic effects of the mononuclear cells infiltrating the liver in this condition against the patient's own hepatocytes would be an alternative approach, but the very small number of mononuclear cells obtained from a diagnostic liver biopsy makes it impossible. Using, as in autoimmune CAH, patients' peripheral blood lymphocytes and autologous hepatocytes, it has been shown that, although an ADCC mediated by anti-LSP antibodies is also demonstrable (Mieli-Vergani *et al.*, 1982), the major effector mechanism is probably a T cell cytotoxicity directed at hepatitis B nucleocapsid antigen expressed on the plasma membrane of infected hepatocytes (Mondelli *et al.*, 1982; Pignatelli, Waters & Thomas, 1985). Three sets of experiments support this idea:

(a) a striking reduction in cytotoxicity has been observed following preincubation of hepatocytes with IgG containing anti-HBc antibodies, but not anti-HBs antibodies or IgG from HBV-seronegative normal human serum (Mondelli *et al.*, 1982);

(b) a similar reduction has been observed using monoclonal anti-HBc (Mondelli & Eddleston, 1984) or anti-HBe (Pignatelli *et al.*, 1985), but not anti-HBs antibodies;

(c) after preincubation with autologous T cells, a significant reduction has been found in HBcAg-expressing, but not HBsAg-containing, liver cells (Naumov *et al.*, 1984).

If T cell cytotoxicity represents the main mechanism of liver cell injury in HBV-related chronic active hepatitis, anti-HBc antibodies might 'mask' viral antigens on the hepatocyte surface and inhibit, rather than promote, cytotoxicity, thus interfering with the clearance of infected liver cells.

Two additional findings seem to support the hypothesis that cytolytic T lymphocytes could be the main effector cell population determining liver cell damage in chronic type B hepatitis. First, T cells of cytotoxic/suppressor phenotype are enriched in the liver at the site of tissue injury (Eggink *et al.*, 1982; Pape *et al.*, 1983), and second, these cells can be clonally expanded and show cytolytic properties *in vitro* (Hoffman *et al.*, 1985). Although the specificity of these T cell clones has not been established yet, HBcAg-specific T cell lines have been obtained from the liver of patients with HBsAg+ve CAH (Ferrari *et al.*, 1986).

If a nucleocapsid antigen is the target of T cell cytotoxicity in chronic HBV infection, hepatocytes containing integrated, but not free, HBV-DNA, and thus incapable of making HBcAg (Burrell *et al.*, 1982), would be protected from T cell attack. This could explain the improvement in conventional liver function tests and decrease in liver inflammation associated with termination of the phase of active viral replication, whether this occurs spontaneously or as a result of antiviral therapy. The inability of the T cells to clear all hepatocytes supporting viral replication at an earlier stage of the infection may be due to the presence of anti-HBc antibodies *in vivo*. Anti-HBc has been demonstrated on the surface of infected hepatocytes isolated from liver biopsies, and when eluted uncovers HBcAg on the membrane of these liver cells (Trevisan *et al.*, 1982). An additional possible factor decreasing the efficiency of the T cell attack is a weak expression of class I histocompatibility antigens on the membrane of hepatocytes (Montano *et al.*, 1982) or their poor association with nucleocapsid antigen, preventing the effective binding of cytotoxic T cells to the target cells.

The mechanisms provoking drug-related, immune-mediated CAH have not been investigated in

detail and are much less understood than those inducing liver damage in the autoimmune and HBV-related varieties.

HUMORAL IMMUNITY

A number of observations suggest that antibodies against surface membrane antigens expressed on hepatocytes may contribute significantly to the persistence of liver cell injury in autoimmune CAH. IgG can be detected on hepatocytes isolated from biopsy specimens (Hopf, Meyer zum Buschenfelde & Arnold, 1976; Mieli-Vergani, 1979) and the pattern of immunofluorescence is generally linear (Hopf, 1976), suggesting a reaction against antigens diffusely distributed on the cell membrane. B lymphocytes and T cells of helper/inducer phenotype predominate in the mononuclear cell infiltrate in the liver (Montano *et al.*, 1983), and antigen-presenting cells (interdigitating and dendritic cells) are also found in the periportal areas of the lobule (Bardadin & Desmet, 1984). These findings are consistent with the hypothesis that synthesis may occur within the liver of autoantibodies capable of binding to the hepatocyte surface, which may be involved in mediating hepatocyte injury.

Liver-directed autoantibodies are present in the circulation of patients with autoimmune or HBV-related CAH, as well as in acute viral or drug-induced hepatitis and in primary biliary cirrhosis (PBC), and are detectable as anti-LSP (Jensen *et al.*, 1978) or LMA (liver membrane antibodies; Tage-Jensen *et al.*, 1977), the former by radioimmunoassay and the latter by immunofluorescence using isolated rabbit hepatocytes. Although the relationship, if any, between anti-LSP and LMA is far from clear, the above findings do show the presence of liver membrane-directed autoantibodies in patients with CAH of different aetiology which, as discussed previously, can mediate an ADCC reaction *in vitro*. The role of antibody coating of hepatocytes *in vivo* is, however, still unclear, as is the mechanism underlying this humoral autoimmune response. If anti-LSP production in man is T lymphocyte-dependent (as shown in mice; Bartholomaeus, O'Donoghue & Reed, 1984), the specificity of the T cells required for this clonal B cell proliferation is most likely different in autoimmune and HBV-related CAH. Only in the former are T cells sensitized to LSP found in the circulation (Vento *et al.*, 1984), while in HBsAg+ve CAH liver-specific B cells might be activated by T lymphocytes reacting with viral antigens expressed on the surface of infected hepatocytes (Eddleston & Williams, 1974).

Studies on humoral immune reactions to hepatocytes in drug-related liver damage have concentrated on halothane-induced hepatitis. This hepatotoxic substance can provoke massive hepatic necrosis, but in only two cases reported so far has it been suspected of being responsible for inducing CAH (Thomas, 1974; Kronborg, 1983). By exposing rabbits to halothane and using their hepatocytes, it has been shown that antibodies to halothane-altered cells are present in patients with severe halothane hepatitis (Vergani *et al.*, 1980); a current hypothesis is that a metabolite of halothane is expressed on the surface of liver cells, presumably in association with 'self' proteins, and this complex provides both an immunogen and target for an antibody-mediated attack (possibly through an ADCC reaction) (Neuberger & Kenna, 1987).

T CELL-MEDIATED IMMUNOREGULATION

The mechanisms permitting the persistence of liver-directed autoimmune reactions in all subgroups of chronic active hepatitis and of HBV in the HBsAg+ve variety of the disease are of obvious relevance to pathogenesis. Both in autoimmune and in HBV-related CAH, defects in immunoregulation have been implicated. Early studies concentrated on the evaluation of non-antigen specific suppressor T cell (Ts) function using Con A-stimulated suppressor cells to inhibit proliferation of allogeneic and autologous T cells or proliferation and immunoglobulin production of PWM-stimulated B cells. These studies have shown that a defect in Ts cell function is present in patients with CAH, but not in patients with chronic persistent hepatitis or inactive cirrhosis (Kakumu, Kasuaki & Kashio, 1980; Kashio, Hotta & Kakumu, 1981; Nouri-Aria *et al.*, 1982); although this

might suggest that impaired suppressor cell activity was secondary to hepatic inflammation, this is uncertain, since defective suppressor cell function has also been found, in a small study (Nouri-Aria *et al.*, 1985), in healthy first-degree relatives of patients with autoimmune CAH. However, defective non-antigen specific Ts cell function has been observed in several other non-organ and organ-specific autoimmune diseases and in the latter it is hard to conceive that such an unspecific defect can lead to diseases of highly restricted organ-specificity. Additional antigen-specific defects are likely to be more relevant and of crucial importance for the appearance and maintenance of these diseases.

Recently, an indirect T lymphocyte migration inhibitory factor (T-LIF) assay in agarose microdroplets has been developed and applied to studies of T cell-mediated responses to liver-derived and HBV antigens, and of their specific immunoregulatory control in patients with autoimmune and HBsAg+ve CAH (Vento *et al.*, 1984; 1985). In the autoimmune variety of the disease, T cells from peripheral blood release T-LIF when incubated for 48 h with LSP, and T lymphocytes from normal, unrelated subjects, added in low ratio, can specifically suppress this *in vitro* response (Vento *et al.*, 1984). Recent, preliminary experiments (S. Vento, C. J. O'Brien, A. L. W. F. Eddleston, unpublished) have shown that circulating CD4+ve T cells present in normal subjects and acting in a non-HLA restricted mode, through patients' CD8+ve T lymphocytes, on T-LIF releasing CD4+ve T cells of patients with autoimmune CAH are responsible for this suppressive effect. These results are potentially of considerable relevance for the pathogenesis of autoimmune CAH, as they imply that CD4+ve T-inducers of suppressor T lymphocytes specific for liver-derived antigens are present in the circulation of normal healthy subjects and defective in the patient population. Although CD4+ve T suppressor inducer cells are known to be non-HLA-restricted (Heijnen *et al.*, 1979; Ballieux *et al.*, 1979; Heijnen, Pot & Ballieux, 1982), they generally require a six-day culture period before becoming 'active' (Heijnen, 1979); the rapid effect observed in the T-LIF assay seems to indicate that CD4+ve T suppressor inducer cells specific for liver-derived antigens are probably activated *in vivo* in normal subjects, and may play an important role in controlling liver-directed autoreactivity.

The liver-specific T suppressor inducer defect could be genetically determined, although non-HLA-linked, as it has also been found in a high proportion of healthy relatives of patients with autoimmune CAH (O'Brien *et al.*, 1986).

Antigen-specific immunoregulatory abnormalities could be of relevance also in HBV-related CAH. The absence of detectable T and B cell responses to HBsAg could constitute an important factor preventing clearance of the virus, and there is evidence that specific Ts cells present in the circulation of patients with HBsAg+ve CAH prevent both anti-HBs production *in vitro* (Dusheiko *et al.*, 1983) and T-LIF release by T lymphocytes sensitized to HBsAg (Vento *et al.*, 1985). Whether these Ts cells appear early in the course of the infection and contribute to the development of the HBV chronic carrier state or are secondary to the HBsAg overload present in chronically infected subjects is unknown.

Immunoregulation has been almost ignored in studies of drug-induced CAH. However, it has long been suspected that alpha-methyldopa could induce an immunoregulatory defect, due to the other numerous 'autoimmune' features associated with methyldopa therapy (haemolytic anaemia, thrombocytopenic purpura, neutropenia, development of a positive Coombs test), and it has been reported that the drug induces a non-antigen specific suppressor cell defect in patients with haemolytic anaemia (Kirtland, Mohler & Horwitz, 1980).

IMMUNOGENETICS

This review cannot cover in detail the complex relations between major histocompatibility complex genes and autoimmune diseases, which have recently been reviewed by other authors (Shoenfeld & Schwartz, 1984; McDevitt, 1985). Like numerous other autoimmune diseases, 'autoimmune' chronic active hepatitis is associated with the HLA antigens B8 and DR3 (Mackay & Tait, 1980). The disease is also associated with the immunoglobulin allotype Gm a+ x+ (Whittingham *et al.*, 1981). However, the occurrence of the illness in subjects with neither HLA B8 DR3 nor Gm a+ x+ implies either that the susceptibility alleles may be only in partial linkage disequilibrium with HLA

B8 and Gm a+ x+, or that the disease group is heterogeneous with respect to genetic predisposition. In contrast, no clear cut associations have been detected between HBsAg+ve CAH and particular histocompatibility antigens, and genetic factors which may contribute to defective clearance of the hepatitis B virus have not yet been identified.

CONCLUSIONS

Despite over 15 years of investigations, the immunopathogenesis of chronic active hepatitis is still obscure, and problems remain as to the interpretation of the results and especially to the relevance of the findings for the sequence of events initiating and maintaining liver cell damage *in vivo*. Three important questions remain unanswered.

(1) Why is the pattern of liver cell damage periportal?
(2) Which are the relevant target antigens for liver-directed autoimmune or drug-induced reactions?
(3) Is there any role for cells or factors other than suppressor T lymphocytes in the regulation and control of liver-directed immune reactions?

T cell killing of HBV-infected hepatocytes cannot account entirely for the pattern of liver cell damage seen in HBsAg+ve CAH, as infected hepatocytes are found throughout the lobule and are not particularly concentrated in the periportal areas (Kojima *et al.*, 1977). This suggests that immune reactions directed at normal autoantigens are responsible for the 'piecemeal' necrosis observed in CAH of different aetiology, but it is still obscure how and why lymphocytes gain access to the periportal area and no studies have been performed on lymphocyte-endothelial cell interactions, which could be of major importance for the pathogenesis of liver damage in chronic active hepatitis.

The target antigens for damaging immune reactions on the liver cell surface are still unidentified. Recently, some progress has been made towards the identification of autoantigens responsible for liver-directed immune responses in 'autoimmune' CAH; the hepatic asialoglycoprotein receptor ('hepatic lectin'), a well-defined, cell membrane-expressed, liver-specific antigen contained in LSP preparations (McFarlane *et al.*, 1984), has been shown to elicit both cellular (Vento *et al.*, 1986) and humoral immunity (McFarlane *et al.*, 1986) in patients with autoimmune CAH. However, cellular immunity to hepatic lectin is found also in a proportion of patients with primary biliary cirrhosis (Vento, 1986) and antibodies to this receptor protein are present also in patients with HBV-related CAH, acute viral hepatitis and primary biliary cirrhosis (McFarlane *et al.*, 1986). Hence, hepatic lectin may be one of numerous antigens to which autoimmune reactions are directed once unresponsiveness to self antigens on the liver cells is abrogated. Purification of other hepatocyte membrane antigens and use of lymphocyte cloning procedures are needed in order to further our understanding of liver-directed immune reactions in chronic active hepatitis.

Finally, mechanisms different from suppressor T cell circuits could play a role in the control of damaging liver-directed immune reactions in chronic active hepatitis. Idiotype/anti-idiotype interactions could be one such mechanism (Male, 1986) and very recent experimental work has given some support to this possibility (Tsubouchi, Yoshioka & Kakumu, 1985), opening a new, promising area of research.

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