The *Immunopathogenesis* of *Chronic HBV* Induced *Liver Disease*

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Introduction

The pathogenesis of hepatitis B virus induced liver disease is not well understood. Because a suitable animal model and methods for in vitro propagation of hepatitis B virus (HBV) have not been available, most current information is derived from clinical studies. Although considerable circumstantial evidence suggests the involvement of immunologic mechanisms [61] it is difficult to determine if the reported immunologic events are primary pathogenetic determinants or merely secondary to the disease.

Potentially important pathogenetic determinants include viral factors such as subtype [9] and dosage [6] mode of transmission [53] and host factors such as age, genetics [8], and sex as well as the immune response [28] to viral or autoantigens. The persistence of viral synthesis in patients without liver injury (the carrier state) suggests that the hepatitis B virus itself is not directly cytopathic for hepatocytes [29]. Nonetheless, viral persistence is frequently associated with the development of chronic hepatitis [61] indicating that the virus is at least a necessary if not a causative factor in the disease process.

Recent work suggests a link between an assortment of human diseases and alleles within the major histocompatibility gene complex (HLA). Conceivably, certain gene products might regulate HBV recognition, processing or replication as well as the qualitative features of the immune response to viral antigens. Nonetheless, despite a weak association between HBV infection and some HLA phenotypes, the most convincing evidence for a genetic role in this disease is its very high prevalence in Down's syndrome and in certain Asian and African populations, although environmental rather than genetic factors could just as easily be responsible for these observations.

A variety of humoral and cellular immune responses to viral and host antigens has been described in hepatitis B virus infection. Antibody to the coat protein,

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hepatitis B surface antigen (HBsAg), is known to neutralize viral infectivity and to contribute to the formation of the immune complexes which produce many of the extrahepatic manifestations of this disease [43, 57]. There is no evidence, however, that antibody to HBsAg is pathogenetically involved in HBV induced hepatocellular injury. Based on observations in other systems, it has been postulated [20] that anti-HBsAg might regulate HBsAg expression at the hepatocyte surface and perhaps viral genome expression as well, but this remains entirely conjectural at present. A second major antibody response is directed to the core antigen of the virus (HBcAg). The persistence of this response is one of the most sensitive serologic markers of continued hepatitis B virus replication, but it has not been implicated in the pathogenesis of hepatocellular injury. An independent group of antibody responses directed to various poorly defined normal hepatocellular antigens [47, 62] has also been identified. However, because the precise nature of the antigen is not known and because similar antibodies are found in patients with an assortment of other liver diseases, the primary pathogenetic involvement of this antigen-antibody system in viral hepatitis B is in doubt. Finally, it is reasonable to question the importance of the humoral arm of the immune response in the pathogenesis of hepatocellular injury since both acute and chronic hepatitis are seen in patients with agammaglobulinemia [44].

Considerable evidence suggests, but does not prove, that the cellular limb of the immune response might be pathogenetically important in hepatitis B virus infection as it is in many other viral diseases [77, 95]. Cellular sensitization to viral and hepatocyte antigens has been observed in the majority of patients with acute and chronic hepatitis who also display cytotoxic effector cell activities specific for target cells bearing viral and hepatocyte antigens [21, 31, 51]. Additionally, chronic active hepatitis has been produced in rabbits chronically immunized with human hepatocyte membrane antigen preparation and the histologic changes correlated well with the cellular immune response to this antigen [62].

Conceivably, therefore, hepatocellular injury in hepatitis B virus infection might be due to a cellular autoimmune response to hepatocyte membrane autoantigens [47]. Since the immune response to self-antigens is strictly controlled by an assortment of mechanisms which collectively constitute the normal state known as immunologic tolerance, one must first demonstrate the failure of one or more of these mechanisms and provide a relevant explanation for their failure before the above hypothesis can be considered tenable. In this regard, it is notable that defects in suppressor cell function putatively involved in maintenance of immunologic tolerance have been identified in patients with viral hepatitis [22, 46]. Furthermore, mechanisms capable of producing suppressor cell dysfunction have been shown to be operative in viral hepatitis [86] and may represent the primary pathogenetic event in this disease.

Based on these observations, we have developed the following hypothesis for the pathogenesis of hepatocellular injury in HBV infection (Fig. 1). HBV may infect the hepatocyte either directly or perhaps via a processing step involving the Kupffer cell which has been shown to play a role in the natural resistance to virus infections [70]. One the HBV genome is integrated into the host chromosomes, hepatocellular metabolism of normal immunoregulatory molecules is disturbed resulting in a complex imbalance, the net effect of which is the partial loss of normal suppressor
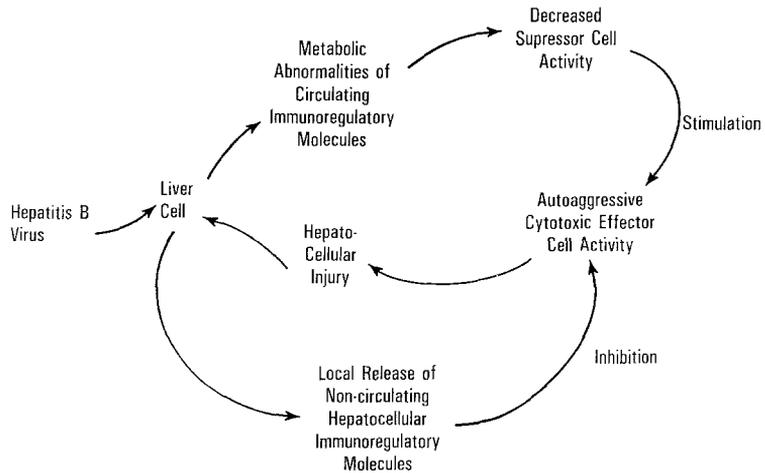
Fig. 1. An immunoregulatory hypothesis for the pathogenesis of hepatocellular injury in viral hepatitis

cell function. This, in turn, leads to the expression of an assortment of auto-immune phenomena including the emergence and expression of autoreactive clones specific for hepatocyte membrane antigens. The ensuing cytolytic process results in the release of normally intracellular proteins that have immunosuppressive properties which down-regulate the cytotoxic effector cells locally within the liver. Normal genetic variation in the hepatocellular content of such immunoregulatory molecules may be responsible for the variable duration and intensity of this virus induced, immunologically mediated disease.

In the remainder of this review, we will discuss the information which forms the basis for the individual tenets of this hypothesis. We will begin with an overview of the cellular constituents involved in this scheme, and discuss the functional characteristics that are germane to the hypothesis. Then we will consider the target antigens to which the immune response may be directed and review the evidence that such a response does indeed occur in this disease. Finally, we will discuss the cellular and molecular immunoregulatory systems which conceivably permit the emergence of this response and consider the possibility that genetic variation in these substances may actually occur. The hypothesis will then be reiterated and new areas of potentially productive future research will be discussed.

Cellular Constituents

The major cellular constituents of the immune system are the thymus-derived (T) lymphocytes, the bone marrow-derived (B) lymphocytes, and the bone marrow-derived cells of the monocyte/macrophage series.

T Cells

T cells possess surface receptors for a glycoprotein ligand [18] on sheep red blood cells (E receptors) and also the Fc [71] portions of lgG (T γ cells) and lgM (T cells) (Table 1). T cells play an important regulatory role in the synthesis of immuno-

Table 1. Properties of the effector cells of the immune system

	T	B	NK	K	Macrophage
slg positive	–	+	–	–	–
E rosette positive	+	–	–	–	–
Esterase positive	–	–	±	–	+
Adherence	±	–	–	–	+
Phagocytosis	–	–	–	–	+
C$_3$ Receptor	–	+	+	+	+
Fc Receptor	+	–	+	+	+
Genetic restriction	+	–	–	–	–
Antigen specificity	+	–	–	–	–

+ present   T = Thymus derived lymphocyte
             B = Bone marrow derived lymphocyte
– absent   NK = Natural killer cell
             K = Killer cell

globulin (lg) by pokeweed mitogen (PWM) activated B cells. No immunoglobulin synthesis occurs in the absence of T μ cells. In the same system, T γ cells function as suppressors of immunoglobulin synthesis [71]. In addition, T cells serve a variety of antigen-specific effector functions which appear to require the positive cooperation between cell surface antigen receptors and certain products of the major histocompatibility complex (the HLA system in man) [27]. Prominent among these are lymphokine production and cellular cytotoxicity [26].

B Cells

The B cell population expresses surface membrane immunoglobulin (slg) [52], receptors for several components of the complement system and for the Fc portion of lgG (Table 1). Its major function is the synthesis and secretion of antibody molecules as a consequence of activation by an antigen for which it expresses highly specific slg receptors.

Null Cells

This class of cell lacks T cell markers (E receptors) and B cell markers (slg) but expresses Fc and C3 receptors [52]. Spontaneous cell mediated cytotoxicity (SCMC) and antibody dependent cellular cytotoxicity (ADCC) are mediated by subsets within this population [73].

Macrophages

Cells of the monocyte/macrophage series originate in the bone marrow from which they migrate either to the vascular compartment where they circulate (monocyte) or to an assortment of organs where they remain as fixed macrophages [70]. They display cytoplasmic esterase and peroxidase activities and surface membrane Fc and C3 receptors and they are adherent to a variety of surfaces [58]. They are functionally heterogeneous, e. g., phagocytosis, antigen processing and presentation, cytolytic activity, secretion of various molecular products (monokines), and they serve immunoregulatory functions as helpers and suppressors of an assortment

of B cell and T cell responses. Approximately half of the body's reserve of fixed macrophages are present in the liver in the form of Kupffer cells [79].

Functional Properties of Lymphocyte Subpopulations in Hepatitis B Virus Infection

Non Specific Parameters

Several laboratories [17, 24, 25, 68] have studied the numbers and distribution of peripheral blood lymphocytes in patients with viral hepatitis (Table 2). Most investigators have observed a decrease in the number of E rosette forming T lymphocytes in the peripheral blood of patients with AVH and CAH [19], with no change in the B lymphocyte population. Since the number of peripheral blood lymphocytes capable of binding neuraminidase treated sheep red blood cells (another specific T cell marker) is normal in hepatitis B [33] reduced E rosette formation is thought to reflect a functional alteration rather than a quantitative depletion of peripheral blood T cells in this disease.

Stimulation of DNA synthesis by polyclonal activators such as phytohemaglutinin (PHA) is another measure of T lymphocyte reactivity. PHA responsiveness is reduced in both acute and chronic viral hepatitis, it returns to normal with recovery [1] and remains normal in the healthy carrier state. These observations have prompted some investigators to suggest that the ultimate outcome of HBV infection may depend on the functional integrity of the T lymphocyte population. Because these systems are modulated by assorted cellular and molecular immunoregulatory events, the functional integrity of regulatory as well as effector cell populations may determine the course of HBV related disease.

Specific Parameters

Using lymphocyte transformation, it has been shown that cellular sensitization to HBsAg occurs during acute and chronic viral hepatitis [30, 48, 55, 56, 88]. Sensitization persists with recovery but it is virtually absent in the carrier state.

Cellular sensitization to liver specific proteins, especially a liver specific hepatocyte surface membrane lipoprotein (LSP), also occurs during the acute and chronic phases of illness but unlike sensitization to HBsAg it disappears with recovery [55, 56, 67]. The latter observation is extremely interesting and suggests that powerful suppressor influences which may normally prevent the expression of this cellular autoimmune response are transiently inoperative during periods of

Table 2. Distribution of peripheral blood lymphocytes in patients with viral hepatitis B

Patients	T	B	Null
AVH-B	Decreased	Normal	Increased
CAH-B	Decreased	Normal	Increased
Carriers	Normal	Normal	Normal

AVH-B = Acute viral hepatitis type B
CAH   = Chronic active hepatitis type B

hepatocellular injury but are restored concomitant with recovery. Whether this represents a primary pathogenetic event is conjectural but waarants further consideration.

Cellular Immunoregulatory Systems

Suppressor Cell Function

Suppressor cells within the T cell and monocyte populations play a major role in the regulation of antibody production [40], cell mediated immunity, and some forms of immunologic tolerance [41, 45].

T Suppressor Cells

a) Suppression of B Cell Function. Pokeweed mitogen (PWM) can stimulate human B cells to synthesize and secrete immunoglobulin. A minor subset of T cells which bear surface membrane receptors for the Fc region of IgG (T γ cells) can suppress the synthesis of immunoglobulin by PWM activated B cells [71]. Although this activity occurs without prior mitogen treatment, it is markedly increased when T cells are first incubated with Con A [66]. These cells are exquisitely radiosensitive and comprise 10 to 15% of E rosette positive lymphocytes.

b) Suppression of T Cell Activation. T suppressor cells also inhibit the DNA synthetic response of other T cells to activation by mitogens or allogeneic cells [80]. This suppressor activity requires prior treatment with Con A for expression. Inactivation experiments have demonstrated radiosensitive and radioresistant populations within this subset.

Monocyte/Macrophage Suppressor Cells

There exists a subset within the phagocytic, adherent, esterase positive population in peripheral blood that can suppress the DNA synthetic response of allogeneic cell-stimulated T cells [54]. These suppressors are spontaneously active and radioresistant but depend on active protein synthesis for full expression of their inhibitory activity.

Suppressor Cell Function in Chronic Liver Disease

Numerous studies have demonstrated a mjor defect in suppressor T cell and suppressor monocyte activity in viral hepatitis. T cell suppressor function has been studied by Hodgson et al. [46] and Chisari et al. [22] in patients with acute and chronic active hepatitis (CAH). In CAH, the mean level of suppressor cell activity was reduced (Table 3). There was no correlation, however, between the degree of hepatic inflammation and the altered suppressor response [22, 46]. In acute hepatitis, Tavassolie et al. [86] and Chisari et al. [22] have demonstrated decreased mitogen inducible suppressor cell activity while Hodgson et al. [46] did not. However, in Hodgson's group, three patients with acute hepatitis who were studied before the peak rise in serum glutamic-oxaloacetic transaminase (SGOT) displayed

Table 3. Suppressor cell function in viral hepatitis

Patients	Suppressor cell activity	
Patients	T Cell	Monocyte/Macrophage
AVH-B	Decreased//Normal	Decreased
CAH-B	Normal	Deceased
Convalescent	Normal	Normal

AVH-B = Acute viral hepatitis, type B
CAH-B = Chronic active hepatitis, type B

reduced suppressor cell activity which returned to normal with resolution of the disease [46].

We [15, 22] have demonstrated that monocyte/mediated suppressor cell and mitogen induced (T cell) suppressor cell activity are decreased in acute and chronic hepatitis but return to normal with recovery. As in Hodgson's study, no correlation was observed between suppressor dysfunction and disease activity. Additionally, random association was observed between the two suppressor cell types in terms of functional integrity, suggesting that more than one mechanism was responsible for suppressor cell dysfunction in these patients.

The exact mechanisms responsible for suppressor cell dysfunction are unclear. However, Tavassolie et al. [86] have demonstrated that a factor present in the sera of patients with acute and chronic active liver disease inhibits the function of normal suppressor T cells in vitro. Additionally, we have observed (Fig. 2) that serum from patients with acute viral hepatitis B inhibits monocyte mediated suppressor cell activity. In a study of 48 hepatitis patients and 10 normal controls monocyte suppressor activity was reduced to 60% of reference levels when the assay was performed in the presence of 20% patient's serum. In contrast, normal control serum had no effect on suppressor cell function (F. V. Chisari, -unpublished observations). Thus, it is possible that at least a portion of the defects in suppressor cell activity in viral hepatitis may be due to abnormalities of the network of the immunoregulatory molecules normally present in serum. Since many of these molecules are synthesized in the liver [20], it is conceivable that virus-induced alterations in hepatocellular metabolism may initiate the loss of suppressor cell function by virtue of the induction of circulating inhibitors. Precedent for this hypothesis exists and will be discussed and developed in depth in a later section.

Cellular Cytotoxicity

If cellular immune mechanisms are responsible for hepatocellular injury in viral hepatitis, one would expect that with the variety of cytotoxicity assay systems currently available distinct differences between hepatitis patients and normal controls should be detectable. Several such studies have been performed using peripheral blood lymphocytes from patients with hepatitis B viral infection as effectors against a variety of surrogate hepatocyte target [3, 21, 31, 37, 51, 89–93]. The inability to culture adult human hepatocytes has necessitated the use of other
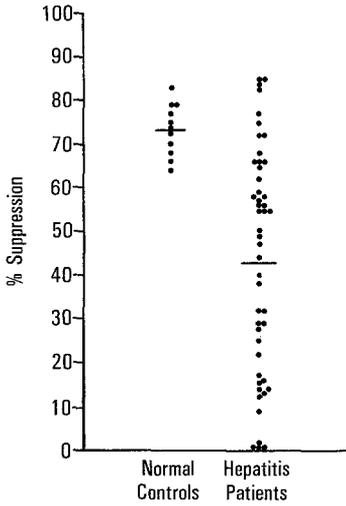
Fig. 2. Effect of serum on monocyte mediated suppressor cell function. The 48 patients studied who had acute hepatitis all had clinical and biochemical evidence of disease for less than 12 weeks. Monocyte suppressor function was fully expressed (70% suppression) in the presence of normal control serum. In contrast, hepatitis sera reduced suppressor cell activity by approximately 40%

target cells [4, 10, 38] in these assay systems: for example, isolated rabbit hepatocytes, autologous human hepatocytes, a human liver derived continuous cell line (Chang), the HBV producing PLC-PRF/5 cell line, the liver specific protein containing SK-HEP 1 hepatoma cell line, hepatitis B surface antigen coated avian red blood cells, and liver specific protein coated pigeon red blood cells. In most of these assay systems, chromium-labeled target cells are employed. However, because unacceptably high levels of spontaneous cell death occur in human autologous hepatocytes, chromium release assays have not been feasible and, therefore, other parameters (adherence to plastic) have been employed to examine target cell viability in these studies [23, 31, 32, 37, 94].

Spontaneous Cell Mediated Cytotoxicity (SCMC)

SCMC is mediated by the natural killer (NK) cell which has the null cell phenotype [89]. NK cell activity is enhanced by interferon and is thought to be important in immune surveillance of cancer and as a pre-immune defense mechanism against an assortment of microbial pathogens. No prior sensitization is required and a wide variety of target cells are susceptible to NK mediated lysis. Genetic restriction does not appear to play a major role in NK cell function.

Antigen Specific Cellular Cytotoxicity (ASCC)

This system is mediated by specifically sensitized T killer cells and thus is a measure of antigen specific cytolysis. The target cells must express cell surface antigen to which the effector cells have been sensitized and for which they have specific receptors. Additionally, T cell mediated cytotoxicity occurs only when the effector cells and target cells share certain histocompatibility antigens [95]. Thus, unlike the other in vitro systems discussed in this section this system requires the generation of two distinct recognition signals before it can be operative: one which is provided by

specific target antigen, and the second which is provided by the shared HLA determined self antigen.

Mitogen Induced Cellular Cytotoxicity (MICC)

This system is mediated by mitogen activated T cells but more closely resembles NK cell function rather than TK cell functions since it is neither antigen specific nor is it genetically restricted [73]. Nonetheless, it provides a functional yardstick for the terminal, effector phase of T cytolytic activity.

Antibody Dependent Cellular Cytotoxicity (ADCC)

Both the humoral and cellular limbs of the immune response are involved in this system. Antigen specificity is determined by the antibody combining site (Fab) of an immunoglobulin molecule, the Fc region of which serves to complete a bridge between a target cell and an Fc receptor-bearing non-T lymphocyte. The effector cell in this system has the null cell phenotype and is designated simply as a killer (K) cell [73].

Target Antigens

If immune mediated cytolysis is an important mechanism in the pathogenesis of viral hepatitis, specific antigens or determinants on the surface of infected hepatocytes must act as targets for one or more of the various effector cells. With the inability to maintain human hepatocytes in vitro it has been impossible to define these entities with any degree of precision. Two major antigens have been implicated as possible candidates. The hepatitis virus or some component of it is a suitable candidate if deposited on the surface of the infected hepatocyte and, therefore, accessible to the immune system. Alternatively, hepatocyte membrane auto-antigens may serve as targets either if they are in some way structurally or conformationally altered by the infection or if tolerance is abrogated as a consequence of decreased suppressor cell function.

Viral Antigens

The coat protein of the hepatitis B virus (HBsAg) is present within the cytoplasm and at the surface membrane of infected hepatocytes. Excess hepatitis B surface protein circulates as 22 nanometer particles with spherical and filamentous morphology. An internal viral core antigen, HBcAg, is found almost exclusively within hepatocyte nuclei. HBcAg is assembled into 27 nanometer particles in the nucleus from whence it moves to the cytoplasm where it is enveloped with surface protein and is transported into the vascular compartment where it circulates as a 44 nanometer Dane particle. The e antigens [59] are a group of poorly delineated molecules which were initially suggested to represent antibodies of restricted heterogeneity that bind Dane particles. Neurath and Strick [74] have proposed that the antigenic determinants of e represent idiotypic determinants of what may be an IgG4 immunoglobulin and thus anti-e would then represent anti-idiotype antibody. Other workers have provided compelling evidence that HBeAg is a structural

component of the viral core [69]. Nonetheless, the e antigen complex has not been identified at the cell surface and it is, therefore, unlikely that e is a viable target antigen in this system. HBsAg is expressed at the cell surface during the acute phase of type B viral hepatitis. In more chronic forms, it appears to be confined primarily to the cytoplasm and is not often expressed at the cell membrane [2]. Unlike HBsAg, which is characteristically located within hepatocyte cytoplasm and at the cell surface where it is accessible as a potential target antigen, the core antigen has never been identified on the hepatocyte surface and is only rarely present in the cytoplasm [75]. Therefore, HBcAg is probably not a potential target antigen in this disease. Since Warnatz et al. [93] and Alberti et al. [3] have demonstrated cellular cytotoxicity to HBsAg coated target cells in patients with CAH, this or some other related, hitherto unknown viral antigen might be a pathogenetically important target in this disease. Of great interest is the reported progression of HBsAg positive acute hepatitis to HBsAg negative chronic hepatitis [78] in which currently identifiable antigens cannot be implicated. Such evidence suggests that host determined antigens may prove to be of paramount importance as pathogenetic determinants regardless of the HBsAg status of the patient.

Liver Specific Antigens

Organ-specific hepatocyte surface membrane antigens [60, 63] might also serve as target antigens under appropriate circumstances. Among these the most likely candidate is liver specific protein (LSP), a hepatocyte membrane lipoprotein [7, 14], which has been demonstrated by immunofluorescence on the surface of rabbit and human hepatocytes. The potential importance of LSP was initially demonstrated by Meyer Zum Buschenfelde [62] and colleagues who chronically immunized rabbits with human LPS. Following an intensive sequence of injections, the rabbits developed histologic evidence of chronic active hepatitis [64] which correlated very well with parameters of cellular immunity to both human and rabbit LSP. More recently, several investigators have detected antibodies to LPS [49] in the serum of patients with HBsAg positive acute and chronic hepatitis. Cellular sensitization to liver specific antigens also occurs in viral hepatitis and has been demonstrated in patients with acute and chronic hepatitis, but most notably it disappears with recovery [55, 65, 84]. Thus, sensitization to LSP appears better correlated with hepatocellular injury than sensitization to HBsAg which persists after recovery. The loss of sensitization also correlates in separate studies with recovery of normal suppressor cell function and suggests that sensitization to LSP may have an auto-immune basis (Table 4). In cytoxicity studies using rabbit or autologous human hepatocytes as targets, nearly all patients displayed LSP specific cytotoxic effector cell activity during the acute and chronic phases of viral hepatitis which disappeared coincident with recovery as above, further supporting the auto-immune hypothesis.

Cytotoxic Effector Function in Viral Hepatitis

The prominent intrahepatic mononuclear inflammatory cell infiltrate characteristic of acute and chronic viral hepatitis B suggests that the associated hepatocytolysis

Table 4. Comparison of suppressor cell function and cellular immune reactivity to liver specific protein in viral hepatitis

| | Stage of disease | | |
	Acute	Convalescent	Chronic
Suppressor cell activity	Decreased	Normal	Decreased
LSP sensitization	Present	Absent	Present
LSP specific cytotoxicity	Present	Absent	Present

may be mediated by cellular immune mechanisms. This impression is supported by reports describing the presence of relevant cytotoxic effector lymphocytes capable of killing autologous [76, 92] and xenogeneic [23, 31, 39, 94] hepatocytes in the peripheral blood mononuclear cell population of patients with hepatitis B virus infection. Since the precise nature of the target in these liver cell mixtures is unknown and because serial observations and examinations of the appropriate normal control groups are not possible, these studies although extremely provocative, are of uncertain significance. Vogten et al. [90] recently described a phagocytic cytotoxic effector cell for pigeon red blood cells coated with liver specific lipoprotein (LPS) in patients with chronic active hepatitis. Alberti and colleagues [3] found cytolytic T lymphocytes specific for hepatitis B surface antigen in the peripheral blood mononuclear cell population in patients with acute and chronic hepatitis. However, the significance of these observations is unclear because, in spite of the apparent antigen specificity in these systems, the expression of antigens chemically coupled to xenogeneic cells from irrelevant organs certainly differs from their natural expression in autologous or allogeneic hepatocytes.

Cytolytically active non-T cells specific for rabbit hepatocytes have been described in patients with chronic active hepatitis [23, 31, 94]. Additionally, rat hepatocyte targets have been shown to be susceptible to injury by peripheral blood mononuclear cells from patients with chronic active hepatitis [39]. Whether these interesting findings using animal targets are pertinent to human hepatocellular injury remains to be seen.

Other groups have examined the functional capabilities of the known cytotoxic effector cells of patients with acute and chronic hepatitis with somewhat conflicting results. Utilizing Chang cells as targets, Wands and Isselbacher [91] and Kakumu et al. [51] demonstrated increased spontaneous cell mediated cytotoxicity in patients with acute and chronic active hepatitis. In contrast, Vierling et al. [89] found no abnormalities in spontaneous cell mediated cytotoxicity, mitogen induced cellular cytotoxicity, or antibody dependent cellular cytotoxicity in systems defined to measure the activity of natural killer (NK), T killer, and K cells, respectively, in patients with chronic liver disease.

We have recently performed a study [21] to determine whether human target cells which naturally express liver specific protein (LSP) and hepatitis B surface antigen (HBsAg) on their surface membrane are preferentially killed by peripheral blood cytotoxic effector cells from patients with acute and chronic hepatitis B. By so doing, we hope to determine whether one or both surface proteins are relevant

antigens in hepatitis B virus induced hepatocellular injury. Chang cells (which lack LSP and HBsAg) were used as specificity controls and also for comparison with the published reports of others. Spontaneous and mitogen induced cytotoxic effector cell activity towards human hepatocyte cell lines which naturally express surface membrane LSP and HBsAg were not observably increased in any patient group in our study. This was surprising in view of published reports that patients with hepatitis B virus associated chronic active liver disease possess phagocytic cytotoxic effector cells active against LSP coated pigeon red blood cells in patients with acute and chronic HBV infections [90] and cytotoxic T cells specific for HBsAg coupled to chicken red cells [3]. This discrepancy is not easily explained except on the basis of the nature of the target cells used in the different studies. Conceivably, prior biochemical purification and conjugation of HBsAg and LSP to non-mammalian cell surface membranes may have exposed antigenic determinants not present on the HBsAg and LSP positive targets used in the present study. Whether a cytotoxic response to such altered antigens is relevant to the putative pathophysiologic response in vivo is not known. Alternatively, although serologically detectable LSP and HBsAg determinants exist on the human hepatoma cell lines we employed, it is conceivable that these molecules express serologically undetectable lymphocyte defined antigens which are either buried in the target cell membrane or genetically deleted from these cell lines.

The data available herein are open to several interpretations. First, contrary to our hypothesis, hepatocyte injury in the course of viral hepatitis may not result from cellular immune effector mechanisms. However, lacking proof of another cause, we consider this interpretation premature. Second, cellular immune mechanisms may be operative but the specific effector cell either does not circulate or is preferentially removed from the peripheral blood compartment by binding to hepatocyte surface antigens. Although this interpretation is testable by examining the cytotoxic effector capability of intrahepatic lymphoid cells, substantial technical difficulties make the execution of these experiments impractical at present. Therefore, this approach is restricted to animal model systems with the assumption that the pathogenetic mechanisms are similar in human and animal species. Third, cellular immune mechanisms may be operative but the target cells employed in the current studies do not express the target antigens recognized in vivo. Alternatively, the relevant antigens may have been present on these target cells but certain biologic requirements for the expression of a cytotoxic response to these antigens may not have been fulfilled in our assay systems including the expression of a latent, antigen-specific cytotoxic response. In this respect, the observations of Zinkernagel and Doherty [95] demonstrating that effector and target cells must share certain histocompatibility antigens as a prerequisite for the expression of a virus specific cytotoxic effector cell response are particularly germane [26].

Molecular Immunoregulatory Systems

A heterogenous assortment of immunoregulatory molecules [11–13, 16, 34–36, 42, 72, 87] has been identified in human plasma (Table 5). A substantial proportion of these molecules is known to be metabolized in the liver. Most of these molecules

Table 5. Plasma immunoregulatory molecules

Lipoproteins
    Rosette inhibitory factor (RIF)
    Chylomicrons
    Very low density lipoproteins
    Intermediate density lipoproteins
    Low density lipoprotein inhibitor (LDL-In)
    Low density lipoprotein
C-reactive protein
Alpha fetoprotein
Fibrinogen degradation products
Immunoregulatory peptide

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inhibit lymphocyte activation; some of them inhibit initiation of the immune response and some also suppress the primary induction of cytotoxic effector cells. Others have been shown to inhibit T suppressor cell activity.

Rosette Inhibitory Factor (RIF)

This molecule is found only in patients with viral hepatitis [19] and was the first of the immunoregulatory lipoproteins to be discovered.
It is responsible for the defect in human T lymphocyte E rosette function seen in over half of the patients with acute viral hepatitis but disappears with clinical recovery [17]. RIF is a unique, abnormal serum lipoprotein [16] with distinct biophysical and biologic properties (Table 6). It inhibits E rosette formation in an active metabolic fashion and does not merely interfere with the binding of the T lymphocyte and the sheep red blood cell on a steric hindrance basis. Thus, RIF represents a prototype abnormality in the molecular immunoregulatory system which occurs in patients with viral hepatitis. Other abnormalities are likewise known to occur in this disease: a variety of major structural abnormalities in the plasma lipoproteins have been reported in viral hepatitis and other liver diseases [81] and alpha fetoprotein levels increase in association with hepatocyte regeneration in hepatitis [82]. It is reasonable to postulate, therefore, that certain manifestations of lymphocyte dysfunction observed in patients with viral hepatitis might be related to either hepatic induction of new abnormal regulatory molecules such as RIF or the quantitative and qualitative abnormalities in the hepatic metabolism of other normal molecules. The net effect of altered immunologic reactivity would then depend on the selective induction of specific abnormalities in the biosynthesis of individual immunoregulatory molecules and one would expect these to vary from patient to patient.

Liver Extract (LEx)

Another immunoregulatory molecule has recently been isolated from human livers [13]. This molecule is a protein with a molecular weight of approximately 65 000 daltons and has been shown capable of inhibiting the DNA synthetic response of peripheral blood lymphocytes to mitogens and allogeneic cell stimuli. In addition, it inhibits mitogen activated and basal protein synthesis and reduces immunoglobulin

Table 6. Biologic and biophysical properties of rosette inhibitory factor

Abnormal low density lipoprotein
Restricted to hepatitis A, B, and nonA/nonB virus infections
Apolipopeptides A_{II}, B, C_{III}
Binds to specific T lymphocyte receptors (2900/cell)
Extremely high binding affinity $(1 \times 10^{13}$ L/M)
Molecular weight 2.5×10^6 daltons
Inhibits E rosette function and the mixed lymphocyte response by an active metabolic process
Biologic half-life = 1.5 h

synthesis by pokeweed mitogen activated human peripheral blood lymphocytes. It also inhibits ongoing DNA synthesis indicating that it can modulate biologic events which are already underway.

If one postulates that LEx is released into the extracellular environment as a consequence of hepatocellular injury, it could modulate lymphcyte effector function in the immediate vicinity of adjacent target hepatocytes and thus limit the magnitude and extent of injury according to its concentration in situ. Since the yield of LEx from different livers all obtained and purified under identical conditions has varied over a 20–fold range [13], we suspect that intracellular concentrations of LEx may vary from individual to individual. If this is true, it is possible that the variable severity and focal nature of hepatocellular necrosis in viral hepatitis may be related to local and quantitative differences in the content and the concentration of LEx released during the initial phase of hepatocellular injury.

In recent experiments we have evaluated the effect of LEx on spontaneous and mitogen induced cellular cytotoxicity to test our hypothesis that the local release of LEx within the liver can affect the cytotoxic effector function of resident cytolytically active lymphocytes. LEx was found to inhibit SCMC and MICC at all effector: target cell ratios studied (Table 7). This supports our contention that the effector phase of cell mediated cytotoxicity is susceptible to modulation by hepatic immunoregulatory molecules.

Experimental Viral Hepatitis

The cells of the reticuloendothelial system have largely been neglected as potentially important factors in the pathogenesis of hepatocellular injury in hepatitis B virus infection. It is reasonable to suspect however, that the macrophage and in particular the Kupffer cell, may be involved for two reasons.
a) The macrophage is generally known to be vitally important in natural resistance to virus infections [70, 79].
b) The macrophage is known to determine the outcome of murine hepatitis virus infection in various inbred strains of mice.

The murine hepatitis viruses (MHV) are highly contagious RNA coronaviruses, and produce mild, usually asymptomatic hepatitis, in the wild. However, variant strains produce extensive hepatocellular necrosis in selected inbred mice. Weiser and Bang [5] have demonstrated that hepatocellular injury is linked to the

Table 7. Effect of liver extract on cytotoxic effector cell function

Spontaneous cell mediated cytotoxicity

Effector/Target	Control	Liver extract	% inhibition
	(% Cytotoxicity		
5	28	18	35.7
20	36	26	27.8
40	48	24	50.0

Mitogen induced cellular cytotoxicity

Effector/Target	Control	Liver extract	% Inhibition
	(% Cytotoxicity)		
5	40	22	45.0
20	65	54	17.0
40	69	59	14.5

Liver extract added to effector cells at 25 µg/ml for 1 h prior to addition of target cells (Chang cells). In the MICC assay phytohemagglutinin at a final concentration of 4 µg/ml was added to the effector cells simultaneous with the target cells. Results are expressed as percent chromium-51 released after 18 h incubation. Percent inhibition is calculated as follows:

$$1 - \frac{\text{Liver extract cpm}}{\text{control cpm}} \times 100$$

inheritance of a dominant gene that codes for macrophage resistance to viral replication [83]. Although the biochemical basis for variable macrophage susceptibility to infection has not been fully determined, we have identified the production of a serine protease by MHV-infected peripheral blood monocytes in susceptible strains but not in resistant strains of mice (G. Levy, unpublished observation).

The analogy between the variable susceptibility of murine and human populations to MHV- and HBV-induced hepatocellular injury is striking and suggests that similar pathogenic mechanisms may be operative. Because Kupffer cell hyperplasia is a prominent feature of acute viral hepatitis in man, the concept that phagocytic cells within the liver may be involved in the processing of hepatitis B virus cannot be ignored. Nonetheless, considerable differences also exist between MHV and HBV infection and over interpretation of the analogy must be avoided. However, further investigation into the role of the macrophage in human hepatitis B virus infection appears warranted.

Discussion

It should be obvious from the foregoing that the pathogenesis of hepatocellular injury in viral hepatitis is unclear. There is a considerable body of knowledge that indicates that assorted aberrations of the immune response are characteristic accompaniments of hepatitis B virus infection and hepatocellular injury. However, the precise role played by these immune parameters is entirely indeterminant at

present because of our current lack of knowledge about the biology of the virus and the cellular biology of the host-virus relationship.

Despite these handicaps it is possible to construct a hypothesis for hepatitis B virus infection and hepatocellular injury based on the assembly of a large variety of independent observations from separate laboratories over the last few years. This hypothesis is highly conjectural and should merely form the basis for additional experimentation rather than serve as a dogmatic interpretation of available information.

The events leading to a hepatitis B virus induced disease should be considered at two levels: First, the events responsible for parasitization of the hepatocyte by HBV; second, the events which lead to hepatocellular injury.

Following entry into the body the virus must gain access to the hepatocyte before replication and hepatocellular injury can occur. The reticuloendothelial system, and in particular the intrahepatic fixed macrophages (Kupfer cells), may conceivably play a pivotal role in removal of infectious *virions* from the circulation before they gain access to hepatocytes. Indeed, it is likely that in most instances of HBV infection the reticuloendothelial system adequately destroys the virus and protects the hepatocyte from viral parasitization and subsequent disease. This suggestion is compatible with the relatively high frequency of subclinical or inapparent hepatitis B virus infection in most human populations. If the viral inoculum is sufficiently large to overwhelm the reticuloendothelial system or if the macrophage is unable to adequately neutralize the virus, then hepatocyte infection and disease may follow.

Alternatively, macrophage functions other than phagocytosis may also influence the infection process. Based on the genetically-determined macrophage-mediated variation in susceptibility to murine hepatitis virus infection and the identification of a biochemical correlate of this genetically determined macrophage heterogeneity, it is conceivable that macrophage activation products, such as serine proteases, might alter the hepatocyte surface membrane so as to render it either more or less capable of binding and internalizing infectious viral particles. Additionally, other macrophage secretory products might conceivably be directly toxic to hepatocytes such that viral induced macrophage activation might result in hepatocellular injury and necrosis irrespective of the coexistent presence of the viral genome and gene products within neighboring hepatocytes.

If the mechanisms which produce hepatocellular injury are initiated because of viral genome incorporation within hepatocytes rather than by non-specific activation of neighboring macrophages, then a complex sequence of events may be responsible for the ultimate production of hepatocytolysis. Hepatocellular injury may result from cellular attack systems which are specific for surface membrane autoantigens such as LSP or for viral antigens if and when they are expressed at the hepatocyte surface. The specific cytolytic effector systems which may be responsible for hepatocytolysis cannot currently be identified. Indeed, the specific target antigen is unknown and the requirements for effective cytolytic activity in vivo are unclear. If T cell mediated cytotoxicity is important in this process, the tenets of the genetic restriction hypothesis would have to be fulfilled. Identification of this pathogenetically important phenomenon with in vitro assay systems must therefore await the

development of a target cell which expresses both the relevant target antigens and shares the necessary histocompatibility loci with the effector cells.

The emergence of anti-hepatocyte, cell-mediate immune reactivity appears to be normally precluded by powerful suppressor influences which serve to maintain the state of tolerance towards hepatocyte antigens. It appears as if activation of normally suppressed cytolytic effector functions may be a consequence of a dysfunctional state of immunologic homeostasis due to an imbalance in immuno-regulatory molecules normally synthesized by the liver. Since the biosynthesis of many of these molecules depends upon normal hepatocellular function and since qualitative and quantitative abnormalities of the molecular immunoregulatory system are known to occur during viral hepatitis (e. g. RIF), it is reasonable to postulate that viral genome incorporation into hepatocyte chromosomes results in dysfunctional immunoregulatory metabolism and secondary inhibition of normal suppressor cell function. Indeed we have shown (Fig. 2) that molecules which inhibit suppressor monocyte activity are detectable in the circulation of patients with acute viral hepatitis. Conceivably, they might allow the emergence of a normally suppressed cellular immune response to host or viral antigens on the hepatocyte surface resulting in hepatocellular injury.

Two mechanisms might be responsible for either the resolution of the disease process or its continuation in a chronic form. First, hitherto unrecognized events may suppress viral genome expression permitting the resolution of dysfunctional hepatocellular biosynthetic activity restoring hepatic metabolism of immunoregu-latory molecules to normal. This would then the reimposition of normal immuno-logic homeostasis with the reconstitution of the suppressor cell population. Precedent for viral genome suppression is found in the model systems of measles and herpes virus infection developed by Joseph and Oldstone [50] and Stevens and Cook [85].

The second mechanism is based on our observation that hepatic LEx concentrations appear to vary from person to person [13]. If LEx is released into the extracellular intrahepatic microenvironment, as a consequence of cell mediated hepatocellular injury, it could inhibit local cytotoxic effector cell activity. Depending on the original hepatocellular concentration of LEx, cytotoxic effector cells at the site of injury would be inhibited to varying degrees. This might result in the range of well-known clinical syndromes characteristic of hepatitis B virus infection. Additionally, inhibition of cytotoxic effector cell activity within the liver might result in the focal distribution of injury also characteristic of viral hepatitis.

Conclusion

In conclusion, until the cellular biology of hepatitis B virus infection is understood and until the technology for the development of suitable virus infected autologous target cells is available, it will not be possible to definitively establish the mechanism involved in the pathogenesis of hepatitis B virus induced hepatocellular injury. Clearly, considerable effort must now be focused on the basic biology of virus infection, replication, and effects on hepatocellular metabolism before it will be possible to perform the definitive experiments to elucidate the role played by the immune system in the pathogenesis of this disease.

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