Immunobiology and Pathogenesis of Viral Hepatitis

Luca G. Guidotti\textsuperscript{1,2} and Francis V. Chisari\textsuperscript{1}

\textsuperscript{1}Department of Molecular and Experimental Medicine, Scripps Research Institute, La Jolla, California 92037; email: guidotti@scripps.edu, fchisari@scripps.edu

\textsuperscript{2}Immunopathogenesis of Liver Infections Unit, San Raffaele Scientific Institute, Milan 20132, Italy

Key Words
hepatitis B virus, hepatitis C virus, cytotoxic T cells, liver cirrhosis, hepatocellular carcinoma, extrahepatic diseases

Abstract
Among the many viruses that are known to infect the human liver, hepatitis B virus (HBV) and hepatitis C virus (HCV) are unique because of their prodigious capacity to cause persistent infection, cirrhosis, and liver cancer. HBV and HCV are noncytopathic viruses and, thus, immunologically mediated events play an important role in the pathogenesis and outcome of these infections. The adaptive immune response mediates virtually all of the liver disease associated with viral hepatitis. However, it is becoming increasingly clear that antigen-nonspecific inflammatory cells exacerbate cytotoxic T lymphocyte (CTL)-induced immunopathology and that platelets enhance the accumulation of CTLs in the liver. Chronic hepatitis is characterized by an inefficient T cell response unable to completely clear HBV or HCV from the liver, which consequently sustains continuous cycles of low-level cell destruction. Over long periods of time, recurrent immune-mediated liver damage contributes to the development of cirrhosis and hepatocellular carcinoma.
Hepatitis B virus (HBV): prototypic member of the Hepadnaviridae family; a DNA virus that causes hepatitis in humans and chimpanzees

Hepatitis C virus (HCV): member of the Flaviviridae family; an RNA virus that causes hepatitis in humans and chimpanzees

Hepatocellular carcinoma (HCC): malignancy that usually develops as a consequence of persistent liver disease (most frequently during chronic viral hepatitis)

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are noncytopathic, hepatotropic members of the hepadnavirus and flavivirus families, respectively, that are spread by contact with infected blood and body fluids and cause acute and chronic necroinflammatory liver diseases (1–5).

HBV infection in immunocompetent adults results in a self-limited, transient liver disease and viral clearance in more than 95% of adults, whereas more than 90% of neonates exposed to HBV at birth become persistently infected. Persistent HBV infection is associated with varying degrees of chronic liver disease, and it often progresses to the development of cirrhosis and hepatocellular carcinoma (HCC) (1, 3). More than 350 million people are chronically infected by HBV, and approximately one million die from these late complications each year worldwide (1, 3, 5).

In contrast to the natural history of HBV, 70%–90% of acute HCV infections become persistent, and more than 170 million people worldwide are currently chronically infected by HCV and experience the same late stage complications of cirrhosis of the liver and HCC (4, 6).

Although a highly effective vaccine against HBV infection has been available for the past 20 years (7), there is no vaccine against HCV. Furthermore, many patients who are persistently infected by HBV or HCV do not respond to currently available therapies (nucleoside analogues, interferon-alpha, and ribavirin) (3–5). Improved understanding of the biology and pathogenesis of these infections is required if we are to develop an HCV vaccine and better treatments for these chronic infections.

A large body of evidence indicates that the outcome of HBV and HCV infections and the pathogenesis of the attendant liver diseases are determined by immune-mediated host-virus interactions. The experimental approaches to HBV and HCV pathogenesis have been difficult because the host range of these viruses is limited to humans and chimpanzees, and because of the lack of cell culture systems and small-animal models that are susceptible to infection. Nonetheless, a great deal of new information pertaining to the pathogenetic mechanisms that may cause liver disease during HBV and HCV infection has been obtained in recent years, thanks to the analysis of the natural history (5, 6) and immunobiology (1, 8) of HBV and HCV in humans and chimpanzees, and infections with related hepadnaviruses [e.g., woodchuck hepatitis virus (WHV) and duck hepatitis virus (DHBV)] (9) or flaviviruses [e.g., bovine viral diarrhea virus (BVDV)] (10) in susceptible species. Additional insight has been gained from multidisciplinary studies of cell lines (11–22) and mouse models that express specific viral genes or replicate the viral life cycles to varying degrees (23–36). To better understand the pathogenesis of these infections, a brief description of the tropism, entry, and replication strategies of these viruses is provided below.

Cell Tropism, Entry, and Replication of Hepatitis B Virus and Hepatitis C Virus

It is widely accepted that the parenchymal cell of the liver, i.e., the hepatocyte, is the primary site of both infections. The mechanisms by which HBV or HCV enter hepatocytes or other susceptible cells are still largely unknown, mostly because of the lack of infectible cell culture systems. Thus, the putative receptors for these viruses have not been identified, even though several studies have suggested that the initial step of HBV infection may involve the interaction of the envelope polypeptides of HBV with a variety of cell membrane proteins [including endonexin II (37), IL-6 (38), annexin V (39), apolipoprotein H (40), transferrin receptor (41), and gp180/carboxypeptidase D in the case of DHBV (42, 43)]. Similarly, binding activity of the E2 glycoprotein of HCV has
been demonstrated for CD81 (44, 45), the human scavenger receptor class B type I (SR-BI) (46), the dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) (47, 48), and the liver and lymph node-specific intercellular adhesion molecule-3-grabbing nonintegrin (L-SIGN) (47, 49). Furthermore, antibodies specific for HCV E1 and E2 glycoproteins can block the infection of susceptible cells with pseudotyped lentiviral vectors expressing these envelope proteins (45, 50, 51). The difficulty in demonstrating that HBV or HCV actually uses these proteins to infect hepatocytes, coupled with the notion that most of these putative receptors are expressed in many nonhepatic cell types (such as mononuclear leukocytes), suggests that some of these molecules may act as coreceptors and that one or more unidentified proteins must be involved in the entry of HBV or HCV into the hepatocyte.

Following entry, the life cycles of HBV and HCV are significantly different (see also Figures 1 and 2 for schemes of the HBV and HCV life cycle). The HBV nucleocapsids are released into the cytoplasm and transported to the nuclear pore. There the relaxed circular viral DNA genome is released into the nucleus, where it is repaired by cellular polymerases into an episomal minichromosome, termed covalently closed circular (ccc) DNA, which represents the viral transcriptional template (5, 9, 52). The cccDNA molecule encodes 4 capped and polyadenylated RNAs that produce the structural and nonstructural viral proteins (5, 9, 52). One of the major HBV transcripts is a 3.5-kb greater-than-genome length RNA that is translated to produce the viral core and polymerase proteins. This transcript also serves as a pregenomic RNA that is encapsidated with the polymerase by the core protein in the cytoplasm of the hepatocyte (5, 9, 52). Viral replication occurs within these capsids by reverse transcription of the pregenomic RNA to produce a single-strand DNA copy that serves as the template for second-strand DNA synthesis that produces a circular double-stranded DNA genome (5, 9, 52). Viral capsids containing double-stranded DNA traffic either back to the nucleus to amplify the viral cccDNA genome or to the endoplasmic reticulum, where they engage the viral envelope proteins, bud into the lumen, and exit the cell as virions that can infect other cells (5, 9, 52).

In contrast to HBV, the HCV genome consists of a 9.6-kb linear, plus-stranded, uncapped RNA molecule that is translated as a single polyprotein precursor of roughly 3000 amino acid residues (53). This large polypeptide is processed by cellular and viral proteases into structural (C, E1, and E2/p7) and nonstructural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) protein subunits (54–68). Unlike HBV, the HCV life cycle and viral replication is driven by a minus-strand intermediate within a membranous compartment in the cytoplasm of the cell (53). Hence, there is a potentially important difference between HBV and HCV at this level, given that the double-stranded HBV DNA genome is completely sequestered within capsid particles, whereas the double-stranded HCV RNA genome is freely exposed in the cytoplasm of the infected cell (53). As most cell types of the innate immune system recognize incoming viruses by detecting double-stranded RNA (dsRNA) produced during the replication process, it is not surprising that, as we describe below, these two viruses induce very different early innate defense mechanisms.

EARLY INNATE DEFENSE MECHANISMS AND THEIR POTENTIAL CONTRIBUTION TO LIVER DISEASE

The host-virus relationship is a dynamic process in which the virus tries to decrease its visibility, whereas the host attempts to prevent and eradicate infection with minimal collateral damage to itself. Initially, the virus must recognize, bind and enter its target cells, and migrate to the appropriate cellular
Figure 1

Hepatitis B virus (HBV) life cycle. Entry of the HBV virion from the extracellular space into susceptible cells is a poorly defined process that is presumably receptor-mediated and leads to uncoating and transport of the capsid to the nucleus. Following capsid disassembly, the second strand of the open circular viral genome is completed, and the ends of each strand are ligated. This leads to the production of a covalently closed circular DNA (cccDNA) molecule, which is the transcriptional template of the virus. Pol II-driven transcription results in production of the viral RNAs that are transported out of the nucleus. Once in the cytoplasm, the transcripts are translated into the corresponding proteins as shown. The precore protein contains a leader sequence that transports it into the endoplasmic reticulum (ER) where it is further processed and eventually secreted as HBeAg (as shown). The X protein is a transcription factor that does not enter the nucleus; rather it interacts with cellular transcription factors in the cytoplasm that enter the nucleus and activate HBV promoter activity. The envelope proteins (Env) traverse the ER membrane as integral membrane proteins (as shown). The core and polymerase proteins assemble around the pregenomic RNA (pRNA) to form HBV RNA-containing capsids, within which the RNA is reverse transcribed to produce the first single-strand viral DNA (ssDNA, as shown). ssDNA serves as the template for second-strand DNA synthesis, thereby producing capsids containing a partially double-stranded, relaxed circular DNA molecule (rcDNA, as shown). Although the RNA-containing capsid is maturing into a DNA-containing capsid, it migrates bidirectionally within the cytoplasm. One pathway terminates at the ER membrane where it interacts with the envelope proteins that trigger an internal budding reaction. This reaction results in the formation of virions that are transported out of the cell by the default secretory pathway. The second pathway transports the maturing capsid to the nucleus to amplify the pool of cccDNA.
cytokines such as interferon (IFN) type I (designated IFN-α/β) by the infected cells (70), and (c) the triggering of effector functions of cellular components of the innate immune system [such as natural killer cells (NK) and natural killer T cells (NKT cells), etc.] (71). NK and NKT cells can be rapidly recruited to the site of virus infection and have the potential to recognize infected cells very early [i.e., before major histocompatibility complex (MHC) class I expression is significantly induced on the cell surface] (71). Activated NK and NKT cells may participate in disease pathogenesis both directly (by killing infected cells) and indirectly, by producing soluble factors (i.e., cytokines and chemokines) that have antiviral activity (71–73), recruit inflammatory cells into the infected tissue (71–73), and shape the adaptive immune response (71). Most of what is known about the early innate defense mechanisms in HBV and HCV infection has been learned in experimentally infected chimpanzees, as they can be studied from the onset of infection through the course of the associated disease. On the basis of these studies (see below), it is clear that both HBV and HCV replicate and spread throughout the liver noncytopathically, and that early innate defense mechanisms do not significantly contribute to the control of viremia or to the pathogenesis of liver disease.

Is Hepatitis B Virus or Hepatitis C Virus Directly Cytopathic for the Hepatocyte?

One mechanism whereby infected cells can limit the initial viral spread is the induction of apoptosis. The evidence that HBV or HCV can induce apoptosis is contradictory, as both pro- and antiapoptotic effects have been detected in cultured cells and transgenic mouse models expressing different viral products (74, 75). Importantly, however, during the early phase of HBV and HCV infection in chimpanzees (i.e., before virus-specific T cells enter the liver) there is no histological or biochemical evidence of hepatocyte damage (8, 76–80). Furthermore, when cellular immune responses are deficient or pharmacologically suppressed, HBV and HCV can replicate at high levels in the liver of patients [and, in the case of HBV, in transgenic mice as well (36)] in the absence of detectable pathological

Figure 2

Hepatitis C virus (HCV) life cycle. Like hepatitis B virus (HBV), entry of the HCV virion from the extracellular space into susceptible cells is a poorly defined process that is presumably receptor-mediated and possibly involves CD81 and SRB-1. Similar to other flaviviruses, it is thought that following receptor binding (1) and receptor-mediated endocytosis (2), HCV is released into the cytoplasm (3) where uncoating of the virion can occur (4). This is followed by internal ribosomal entry site (IRES)-mediated translation and polyprotein processing (5), and RNA replication in a specific membrane alteration, the membranous web (6). Packaging and assembly of new virions occurs within intracellular vesicles (7), where maturation (8) precedes vesicle fusion at the plasma membrane and virion release (9).
Viral hepatitis: necroinflammatory liver disease commonly caused in humans by one or more of the hepatotropic viruses

Collectively, these results indicate that HBV and HCV replicate noncytopathically within the primary hepatocyte in vivo and they suggest that hepatocyte damage during viral hepatitis is an immune-mediated event.

Hepatocytes Differentially Sense Hepatitis B and Hepatitis C Viral Infections

Virus replication often results in the rapid induction of IFN-α/β by the infected cell (70). Production of IFN-α/β activates a variety of intracellular antiviral mechanisms that have the potential to minimize pathogenetic processes by limiting viral production and spread (70).

Global gene expression profiling performed on liver RNA obtained at multiple time points after HCV infection in chimpanzees indicates that the expression of many genes known to be stimulated by IFN-α/β is significantly induced as HCV spreads asymptomatically throughout the liver and virus titers increase in the circulation (81–83). This induction is presumably due to the fact that HCV replicates via dsRNA intermediates in the cytoplasm, where it can readily activate the cellular dsRNA-sensing apparatus and initiate the signaling cascade (53). Remarkably, however, HCV manages to replicate within the liver despite induction of these genes, possibly because structural (e.g., E2) and nonstructural proteins of HCV (e.g., NS3 and NS5A) have the potential (at least in vitro) to block IFN-α/β induction and counteract antiviral mechanisms downstream of IFN-α/β-stimulated transcription (84–90), as we discuss later in this review.

In contrast, similar analyses in chimpanzees revealed that HBV acts like a stealth virus early after infection, remaining undetected until the onset of the adaptive immune response several weeks later (91). The relative invisibility of HBV to the innate sensing machinery of the cells likely reflects its replication strategy, which retains the transcriptional template in the nucleus, involves the production of capped and polyadenylated viral mRNAs that resemble the structure of normal cellular transcripts, and sequesters its replicating genome within viral capsid particles in the cytoplasm (5, 9, 52). Thus, whereas spreading of HBV within the liver may be facilitated by the lack of IFN-α/β induction, HCV may achieve similar results by actively counteracting the IFN-α/β response it induces (see below).

Natural Killer Cell and Natural Killer T Cell Responses to Hepatitis B and Hepatitis C Viruses

Although NK and NKT cells have been implicated in the pathogenesis of cytopathic hepatotropic viral infections like murine cytomegalovirus (MCMV) in mice (71), there is little or no evidence that these cells play a pathogenetic role during the early phase of HBV or HCV infection. Indeed, as activated NK cells and NKT cells are a rich source of IFN-γ (71) and as IFN-γ-inducible genes are not detected in the liver of chimpanzees when HBV or HCV initially spreads throughout the liver (76, 77, 81–83, 91), it is reasonable to assume that these cells are not routinely activated early in infection. The observations that (a) the interaction of the E2 protein of HCV with CD81 on NK cells inhibits their activation (90) and (b) NK cell functions can be deficient in individuals infected with HCV (92) suggest that HCV may directly contribute to evasion of the NK cell response (see below).

It is also unlikely that cells of the innate immune system contribute to the pathogenesis of viral hepatitis during the symptomatic phase of acute or chronic HBV and HCV infections because CD8+ T cell depletion prevents or delays the onset of liver injury and viral clearance in chimpanzees acutely infected by HBV (77) or HCV (93). It is, therefore, assumed from these, and other
observations that we describe below, that the adaptive immune response plays a crucial role in the pathogenesis of HBV and HCV infection.

THE ADAPTIVE IMMUNE RESPONSE TO HEPATITIS B AND C VIRUSES AND ITS CONTRIBUTION TO LIVER DISEASE

Virus-specific CD8$^+$ cytotoxic T lymphocytes (CTLs) and CD4$^+$ T-helper cells play key effector and regulatory roles, respectively, in antiviral immunity. Like innate immune cells described above, these T cells participate in viral pathogenesis either directly (by killing infected cells) or indirectly (by producing soluble factors such as cytokines and chemokines) that contribute to the inflammatory process and/or inhibit viral replication (1, 94). Because they can promote the priming, expansion, function and trafficking of cellular immune responses, T cell–derived cytokines and chemokines contribute to immunopathology (1, 94, 95). Conversely, T cell–derived antiviral cytokines can also inhibit viral replication and viral antigen expression noncytopathically; and possibly even purge viruses from viable infected cells (94). The latter effects are likely to contain tissue damage by diminishing the number of infected cells that must be killed to eradicate the infection and/or by reducing the production of antigen and, therefore, the visibility of the infected cell to the cellular immune response. T cell–derived cytokines and chemokines also participate in the shaping of antiviral antibody responses that contribute to viral clearance mainly by blocking virus entry into susceptible cells and by removing infectious virions from the circulation. Neutralizing and non-neutralizing antibodies (Abs) can also promote antiviral and pathogenetic events by activating the complement system, which has the potential to lyse antibody-coated viruses or virus-infected cells (96–98).

The Role of Cytotoxic T Lymphocytes in Hepatitis B and C Viral Pathogenesis

Efficient antiviral CTL responses are thought to be primed by viral antigens processed by dendritic cells and other professional antigen processing cells (APCs) in lymphoid organs (99, 100). In contrast, if T cell priming occurs within the liver, the priming is more likely to induce T cell inactivation, tolerance or apoptosis (101, 102). Although CD4$^+$ T-helper cells are normally primed by APCs that have internalized soluble viral antigens produced by other cells, priming of CTLs usually requires the processing of viral proteins that are either endogenously produced within or phagocytosed by professional APCs (99, 100). For viruses like HBV or HCV that do not appreciably infect professional APCs, tissue-derived dendritic cells that have processed apoptotic virus-infected cells and debris are likely to migrate to the regional lymph nodes to allow CTL priming to occur (99, 100). Primed CTLs clonally expand, leave the lymph nodes, migrate to the infected tissue, recognize viral antigen, and perform their effector functions. Whereas virus-specific CTLs in most virus infections can be detected in the infected tissue as early as five to seven days after exposure (103), the initial influx of T cells into the liver of chimpanzees acutely infected with HBV or HCV does not occur until two to three months after infection, despite the fact that circulating virus-specific CTLs can be detected in the peripheral blood as early as two to three weeks post exposure (8, 77, 78). This observation could reflect the absence of processed viral antigen at the surface of infected hepatocytes during active viral replication, an event that may be facilitated by the fact that MHC class I expression is minimal in hepatocytes except in the context of an inflammatory response (104).

Several lines of evidence indicate that the CTL response plays a crucial effector role in the pathogenesis of liver disease during HBV...
The onset of liver injury coincides with the entry of virus-specific CD8\(^+\) T cells into the liver of chimpanzees infected by HBV or HCV (8, 76–78) (see also Figure 3), and depletion of these cells (but not of CD4\(^+\) cells) at the peak of viremia delays the onset of biochemical, histological, and clinical evidence of hepatitis (8, 77). Second, the strong association between the magnitude of virus-specific CTL responses and liver disease severity has been shown not only in infected chimpanzees but also in many studies in patients acutely or chronically infected with HBV or HCV (1, 8). Indeed, patients with acute viral hepatitis who successfully clear HBV or HCV usually develop a relatively severe degree of hepatocyte damage that is associated with a relatively vigorous, multispecific, polyclonal CTL response to several HBV- or HCV-encoded antigens that is usually associated with (1, 8). In contrast, liver cell injury is more contained in chronically infected patients in whom the CTL response is extremely weak and narrowly focused (as always occurs during chronic HBV infection) or possibly unable to recognize infected hepatocytes owing to viral mutational escape (as occurs during chronic HCV infection) (1, 8).

Third (see also below), adoptive transfer of HBV-specific CTL lines and clones into immunologically tolerant HBV transgenic mice produces liver disease with hepatocyte necrosis and inflammation that is histologically very similar to acute viral hepatitis in humans (105, 106).

All together, these results indicate that the CTL response is required to induce most (if not all) of the liver disease that is associated with viral hepatitis. Although hepatocellular injury is clearly initiated by the cytopathic activity of virus-specific CTLs, additional functions of these cells are likely at play during HBV and possibly HCV infection. On one side, activated CTLs produce cytokines (such as IFN-\(\gamma\)) that, via the chemokines (e.g., CXCL9 and CXCL10) they locally induce, recruit into the liver large numbers of antigen-nonspecific inflammatory cells that
Mechanisms of Cytotoxic T Lymphocyte-Induced Liver Disease and Viral Clearance

As we mention above, passive transfer of virus-specific CTL lines and clones into HBV transgenic mice results in the development of a necroinflammatory liver disease that histologically resembles viral hepatitis in man (105–111). The first step in the disease process is antigen recognition by the CTLs, which rapidly induces hepatocellular apoptosis (105) (see also Figure 4, left). By transferring HBV-specific CTL clones that are genetically deficient in either FasL or Perforin into the same HBV transgenic mice, it has also

Figure 4
Sequential histopathological manifestations of liver disease after transfer of hepatitis B virus (HBV)-specific cytotoxic T lymphocyte (CTLs) into HBV-transgenic mice. Bromodeoxyuridine (BrdU)-labeled HBsAg-specific CTLs were injected into HBsAg-positive transgenic mice, and their livers were examined immunohistologically with anti-BrdU antibodies 4 h later (left). Note that the BrdU-positive CTL (arrow) is closely associated with apoptotic hepatocytes in which nuclear chromatin margination and cytoplasmic condensation are evident (indirect immunoperoxidase labeling for BrdU, lightly counterstained with H&E; original magnification X 600). By 24 h after CTL transfer, many host-derived (i.e., BrdU-negative) inflammatory cells are recruited into the liver, thereby contributing to the formation of necroinflammatory foci in which apoptotic hepatocytes and BrdU-labeled CTLs are outnumbered by host-derived mononuclear and polymorphonuclear inflammatory cells (right; indirect immunoperoxidase labeling for BrdU, lightly counterstained with H&E, original magnification X 600).
been shown that both the Perforin and Fas death pathways must be activated simultaneously for virus-specific CTLs to be cytopathic in the liver (109). CTL killing in vivo is an inefficient process that requires direct physical contact between the CTLs and the infected cells. Thus, it is not surprising that in this model the initial apoptotic process affects a small number of hepatocytes (105). As time progresses, however, many host-derived inflammatory cells are recruited into the liver, thereby contributing to the formation of necroinflammatory foci in which apoptotic hepatocytes and virus-specific CTLs are outnumbered by host-derived mononuclear and polymorphonuclear inflammatory cells (105, 107, 108) (see also Figure 4, right).

The leukocyte recruitment process is a chemokine-dependent event, as blocking the IFN-γ-inducible chemokines CXCL9 and CXCL10 reduces both the migration of antigen-nonspecific mononuclear cells into the liver and the severity of liver disease after CTL injection (107). Importantly, this effect occurs without affecting the migratory or antigen-recognition potential of the virus-specific CTLs (107). The association of reduced liver disease with reduced recruitment of antigen-nonspecific mononuclear cells implies that these cells can amplify the liver damage initiated by the CTLs. Furthermore, as most of the CTL-dependent antiviral activity depends on the IFN-γ that they locally produce after antigen recognition (see Reference 107 and also below), the notion that neutralization of CXCL9 and CXCL10 is associated with maintenance of antiviral effects but diminished tissue damage may have implications for the development of immunotherapeutic approaches for the treatment of chronic HBV infection.

Recent studies also showed that the severity of CTL-induced liver disease in this model is ameliorated by the depletion of Gr-1<sup>+</sup> cells [Gr-1 is an antigen highly expressed by polymorphonuclear neutrophils (PMNs)], which secondarily abolishes the intrahepatic recruitment of all antigen-nonspecific Gr-1<sup>−</sup> mononuclear cells (i.e., NK and NKT cells, T and B lymphocytes, monocytes, macrophages, dendritic cells) despite the strong induction of chemokine gene expression (110). Those results suggest that CTL-induced functions in addition to chemokine expression are necessary for mononuclear cell recruitment to occur. These functions likely include the production of matrix metalloproteinases (MMPs) by PMNs (such as MMP-8 and MMP-9) because (a) these enzymes are rapidly activated in the liver after CTL transfer and (b) inhibition of these enzymes reduces the intrahepatic recruitment of antigen-nonspecific mononuclear cells and much of the attendant liver disease (111). These results are compatible with the observation that PMNs are the first cell type to be recruited into the liver following antigen recognition by the CTLs. According to this hypothesis, the production of MMPs by PMNs (and perhaps other cells) may cleave components of the extracellular matrix or perform yet undefined functions that facilitate the recruitment of mononuclear cells into the liver parenchyma in response to chemoattractants. A cartoon summarizing the results we describe above is provided in Figure 5.

Mechanisms related to those we describe above may contribute to the pathogenesis of viral hepatitis in man, where, as in the HBV transgenic mice, the number of HBV or HCV-specific CTLs detected in the liver is outnumbered by recruited, virus-nonspecific T cells (112, 113) and other inflammatory cells (114). Furthermore, the IFN-γ-dependent secondary processes that lead to the intrahepatic recruitment of inflammatory cells during natural infection are likely to be quite similar to those observed in the HBV transgenic mice, regardless of the nature of the cell type that originally produces this cytokine or the virus that infects the liver. Accordingly, it is noteworthy that intrahepatic expression of CXCL9 and CXCL10 is induced during chronic HCV infection in humans and that this is associated
Mechanisms of cytotoxic T-lymphocyte (CTL)-induced liver disease and viral clearance. After antigen recognition, hepatitis B virus (HBV)-specific CTLs kill a small number of hepatocytes (1) via Fas L– and Perforin-mediated pathways and produce antiviral cytokines (2) that inhibit HBV replication noncytopathically in a greater number of cells. The same cytokines can activate parenchymal and nonparenchymal cells of the liver to produce chemokines (3) that recruit antigen-nonspecific polymorphonuclear cells (e.g., neutrophils) into the organ. Production of matrix metalloproteinases by these cells (4) in addition to chemokine induction (5) may contribute to the migration of antigen-nonspecific lymphomononuclear cells (e.g., natural killer cells, T cells, and macrophages) into the liver and the amplification of the liver disease initiated by the CTL (6). MMPs, matrix metalloproteinases.

with increased numbers of liver-infiltrating inflammatory cells and enhanced liver disease (115, 116).

The pathogenetic mechanisms whereby antigen-nonspecific inflammatory cells induce liver damage are not well understood and may involve the local production of proinflammatory and cytotoxic mediators (including TNF-α, proteases such as Perforin, hydrogen peroxide, superoxide anion, and nitric oxide). Furthermore, some of these cell subtypes (in particular NK cells, NKT cells, and T-helper cells), as well as platelets, express Fas-L (117–119), a glycoprotein known to induce apoptosis in Fas-positive cells such as hepatocytes (120).

New studies in our laboratory also demonstrate that platelets play a previously unrecognized role in the pathogenesis of CTL-induced liver disease (236). These studies show that platelets are detectable within intrahepatic necroinflammatory foci following transfer of virus-specific CTLs into HBV transgenic mice or following infection of normal inbred mice with replication-deficient adenoviruses. Selective platelet depletion significantly reduces the intrahepatic accumulation of virus-specific CTLs and the resulting liver damage without impairing CTL effector functions. Furthermore, transfusion of platelets into thrombocytopenic animals restores CTL homing and liver disease severity,
but not when platelet activation is inhibited in vivo. Accordingly, platelet activation is required to promote CTL/platelet interactions under flow conditions in vitro. On the basis of these results, one can envision that an initial immune-induced inflammatory response results in changes of the vessel wall that promotes platelet activation, which in turn favors the local accumulation of virus-specific CTLs through specific interactions with activated platelets.

Importantly, the IFN-γ produced by activated CTLs following antigen recognition is also responsible for most of the antiviral potential of the CTLs (94, 106, 121). Indeed, in the HBV transgenic mouse model, IFN-γ prevents the assembly of replication-competent HBV RNA-containing capsids in the hepatocyte (122) in a proteasome- (123) and kinase-dependent (124) manner. During this remarkable process, the viral nucleocapsids disappear from the cytoplasm of the hepatocytes (106, 122), and the viral RNAs are destabilized by a SSB/La-dependent mechanism in the nucleus (125–128), whereas the hepatocytes remain perfectly healthy (106, 125, 129).

This notion that IFN-γ produced by activated CTLs can play a direct role in viral clearance was corroborated by studies in chimpanzees acutely infected with HBV (76, 77). It was shown in these animals that most of the viral DNA disappeared from the liver before the peak of liver disease and concomitant with the initial intrahepatic appearance of IFN-γ (76, 77). Moreover, neither intrahepatic IFN-γ induction nor viral clearance occurred in HBV-infected chimpanzees that were depleted of CTLs at the peak of infection (77). Similarly, CTL-mediated destruction of infected cells is probably not the only way to eliminate HCV as well because (a) viral clearance in chimpanzees can occur in the absence of liver disease as long as antiviral CTLs are present and produce IFN-γ (130) and (b) IFN-γ has been shown to inhibit replication of HCV replicons in vitro (15, 131–133).

### The Role of T-Helper Cells in Hepatitis B and C Virus Pathogenesis

On the basis of their central role as regulators of the immune response in other viral infections (134), it is likely that CD4+ T-helper cells contribute to the control of HBV or HCV infection mainly by facilitating the induction and maintenance of virus-specific CD8+ T cells. In keeping with this, relatively vigorous HBV- or HCV-specific CD4+ T cell responses are always associated with quantitatively and qualitatively significant CD8+ T cell responses in humans and chimpanzees that resolve HBV or HCV infection (1, 8). Although it is thought that most patients who become chronically infected by HBV probably never mount significant CD4+ and CD8+ T cell responses (1), this hypothesis is not certain because of (a) the relative rarity of chronic progression in acutely infected adults, and (b) the difficulty of identifying acutely infected patients for analysis before they become symptomatic. In contrast, recent observations indicate that virus-specific cellular immune responses can occur during the acute phase of HCV infection in patients or chimpanzees that ultimately become persistently infected (8, 93, 135). Although associated with temporary control of viremia, these CD4+ and CD8+ T cell responses are usually transient in nature (8, 93, 135), functionally defective (8, 93, 135), and associated with virus escape mutations in MHC class I epitopes (8, 93, 135). These results suggest that, when it occurs, the initial priming and expansion of HCV-specific CD4+ and CD8+ T cells does not automatically guarantee clearance of HCV infection. Rather, permanent HCV control appears to require functional CD4+ and CD8+ T cells that are sustained over relatively long periods of time (8, 93, 135).

Despite the fact that T-helper cells have been shown to have cytolytic activity in vitro, the role of this process in the pathogenesis of viral hepatitis is entirely unclear. However,
recent observations in an acutely HBV-infected chimpanzee that was depleted of CD4+ T cells at the peak of infection fail to support this notion because the liver disease was comparable in this animal and immunologically unmanipulated controls (77).

Collectively, these results indicate that virus-specific CD4+ T helper cells are not likely to directly mediate liver disease during HBV or HCV infection; rather they probably play a crucial immunoregulatory role in viral hepatitis, particularly at the level of CTL induction and/or the establishment or maintenance of a memory cell pool. Recent work in HCV-infected chimpanzees depleted of CD4+ or memory CD8+ T cells corroborated the notions that (a) virus-specific CTLs are required to control the virus and (b) they depend on CD4+ T cell help (8, 93, 135).

The Role of Antibodies in Hepatitis B and C Virus Pathogenesis

As we mention above, neutralizing antibodies (Abs) can block viral entry into susceptible cells (98). The role of neutralizing Abs in the resolution or prevention of HBV or HCV infection is poorly understood. Evidence that Abs with neutralizing activity emerge following a self-limited HBV infection is supported by the observation that chimpanzees that resolved a previous infection are completely protected from rechallenge (136). Like other infections with noncytopathic viruses such as lymphocytic choriomeningitis virus (LCMV) (137), the appearance of neutralizing Abs is thought to occur relatively late after HBV exposure and, thus, is unlikely to play a role in the early phase of viral clearance during acute infection (1). The evidence that neutralizing Abs can control long-term noncytopathic infections that are not completely cleared [e.g., LCMV (138)] coupled with the observation that complete viral clearance (viral sterilization) following clinical recovery from HBV infection may never occur (139, 140) suggest that a sustained neutralizing Ab response may prevent the reemergence of HBV in patients that resolved the infection.

Recent work, using infectious pseudotyped viruses bearing HCV envelope glycoproteins, indicates that most patients acutely infected with HCV do not develop neutralizing proteins and, when they do appear, they do not correlate with viral clearance (141), similar to HBV infection. In contrast, using the pseudotyped virus neutralization assay, neutralizing Abs to HCV can be detected in most chronically HCV-infected patients (141, 142). Although this is consistent with the fact that passive transfer of serum from chronically HCV-infected patients can protect chimpanzees challenged with the same virus (143), it also indicates that the antibodies are ineffective at terminating an ongoing infection, presumably because the viruses that originally elicited the antibodies have been counterselected, and the antibodies are not neutralizing for the concurrent quasispecies. Thus, in contrast to chronic HBV infection, neutralizing Abs are present in patients who are chronically infected with HCV, presumably reflecting sensitization to viruses that were counterselected and no longer present.

Neutralizing and nonneutralizing antiviral Abs can also accelerate the removal of virions from the circulation by the interaction of the Fc portion of virus-bound antibodies with Fc receptors present on the surface of phagocytic cells (96, 97). Through the same Fc-dependent interactions, antiviral Abs may facilitate virus-uptake by professional APCs in secondary lymphoid organs and therefore enhance the presentation of viral antigens to T and B cells (144). Finally, antiviral Abs can promote complement activation and induce complement-dependent lytic activities against viruses or virus-infected cells (96, 97). Although complement activation enhances the neutralization function of HCV-specific Abs against pseudotyped viruses in vitro (145), direct experimental evidence that any of these Ab-mediated functions occurs during natural HBV or HCV infection is lacking.
As we describe below in greater depth, in addition to liver injury, HBV and HCV infections are associated with a variety of extrahepatic diseases that can cause a significant increase in morbidity and even mortality (146–149). Deposition of circulating immune complexes (containing mostly IgM, IgG, and HBsAg) is believed to play a pathogenetic role in the development of manifestations such as skin rash, arthritis, arthralgia, glomerulonephritis, polyarteritis nodosa, and papular acrodermatitis that can occur during acute and, especially, chronic HBV infection (147, 148). Similarly, immune complexes have been linked to cryoglobulinemia, glomerulonephritis, porphyria cutanea tarda, and necrotizing cutaneous vasculitis during chronic HCV infection (146, 149).

**Evasion of the Innate and Adaptive Immune Response**

On the basis of the studies summarized above, it is clear that the immune response to HBV or HCV plays a crucial role in viral clearance and liver disease. It is therefore reasonable to assume that viral persistence following these infections requires that innate and/or adaptive immune responses must be either not induced or deficient, or, if present, they must be overwhelmed, counteracted or evaded.

**Virus Factors Affecting Innate Immune Responses**

As we describe above, because the initial spread of HCV throughout the liver occurs despite the transcriptional activation of both a large number of IFN-inducible genes (including some with known antiviral activity such as 2′-5′-oligoadenylate synthetase and MxA (81–83)) and the downstream genes that they themselves induce (81–83), the evidence suggests that HCV-infected hepatocytes readily sense and respond to the infection, but that this response fails to control viral replication. The apparent resistance of HCV to these early innate defense mechanisms has been ascribed to the capacity of the HCV NS3/4A protease to block signaling from toll-like receptor-3 (TLR-3) and a recently discovered dsRNA-binding protein (RIG-I) that triggers IRF-3 activation and IFN-β gene expression (237–240).

In addition, several structural and nonstructural proteins of HCV inhibit nonoverlapping functions of the innate immune response. For example, the E2 and NS5A proteins of HCV have been shown to bind to the kinase domain of dsRNA-dependent protein kinase (PKR) and inhibit interferon regulatory factor (IRF)-1 phosphorylation (84–86). In addition, NS3 has been shown to prevent the phosphorylation, dimerization, nuclear translocation and, therefore, the transactivating function of IRF-3 (87). Although activated IRF-1 and IRF-3 are both known to induce IFN-β gene expression and stimulate expression of genes downstream of IFN-β (150, 151), in view of the ability of E2, NS3, and NS5A to inhibit the activation of IFN-regulated genes one would not expect IFN-regulated genes to be strongly induced in the liver during HCV infection. Nonetheless, genomic analysis of the liver of many HCV-infected chimpanzees has shown a strong correlation between the level of HCV viremia and the intrahepatic expression of a large number of IFN-regulated genes (81–83). Thus, despite its ability to block IFN induction in vitro, HCV strongly induces these antiviral genes in vivo but appears to be resistant to their effects. Although more studies are needed to understand the basis for these apparently contradictory observations, at present we favor the notion that HCV circumvents the IFN signaling cascade by inducing it in either uninfected liver cells or infected cells, but inhibiting the antiviral effector functions of IFN-induced genes downstream of IFN induction. In keeping with this hypothesis, previous reports showed that IFN-α/β induction by Newcastle disease virus (NDV) is completely normal in IRF-1 deficient cells (152, 153). These results indicate that IRF-1 does not mediate the induction of IFN-α/β.
by viral infection, and they support the notion that, if IRF-1 blockade occurs at all in HCV-infected cells, the effect is likely to be at the target gene level rather than at the level of IFN induction. Furthermore, reports that NDV-induced IFN-α/β mRNA expression is profoundly reduced in IRF-3-deficient cells (154) suggest that IRF-3 is required for IFN induction following viral infection. However, if IFN-induced genes are strongly upregulated during HCV infection, the negative impact of NS3 on IRF-3 activation in the liver would appear to be minimal. In summary, much more work must be done before we understand the mechanisms that make it possible for HCV to flourish in the face of active IFN induction in the infected liver. Indeed, the roles of IRF-1 and IRF-3 in this process, if any, remain to be determined.

In contrast to the strong induction of IFN-regulated genes during HCV infection, as we discuss above, early innate defense mechanisms are not induced in the liver during HBV infection (91). Although we do not know the reason for these surprising differences, we suspect that they are due to the different replication strategies of the two viruses as described above. Whatever the reason for these differences, the fact that HBV has not needed to develop evasion or subversion strategies to escape the innate immune response may explain why it is so readily controlled when confronted by the cellular and molecular machinery of the adaptive immune response, and, as we discuss in the next section, why it has evolved mechanisms apparently intended to blunt the induction and impact of that response.

**Virus Factors Affecting Adaptive Immune Responses**

The HBV precore protein (HBeAg) is a secreted, truncated form of the HBV core protein (HBcAg) that is not required for viral assembly, replication, or infection (155–158). Thanks to its capacity to cross the placenta and induce neonatal tolerance in HBV transgenic mice (159), it has been proposed that the precore protein may perform a similar function in humans and facilitate viral persistence following neonatal infection. Furthermore, the precore protein has been recently shown to inhibit induction of the T cell response to a cross-reactive epitope in HBCAg in adult T cell receptor transgenic mice. This inhibition of the immune response is mediated either by deletion or anergy of HBCAg/HBeAg cross-reactive T cells, depending on their functional avidity for the tolerogenic epitope (160). Thus, the HBV precore protein may contribute to the outcome and pathogenesis of HBV infection by inducing tolerance in HBCAg-specific T cells and reducing their potential to kill cells. In keeping with these observations, clinical evidence indicates that HBeAg-negative variants of HBV are often cleared following neonatal exposure and are usually associated with more severe and even fulminant course of hepatitis when they infect adults (5, 161). The observation that the hepatitis B surface antigen (HBsAg)-specific CD8+ T cell response is blunted and exhibits altered HLA/peptide tetramer-binding properties in patients chronically infected with HBV with high serum-HBsAg titers (162) suggests that HBsAg may function as a high-dose tolerogen during HBV infection. Lastly, it has been shown that the HBV X protein can reduce cellular proteasome activity when it is overexpressed in vitro (163). If this also occurs during natural infection, the X protein may affect HBV pathogenesis by inhibiting antigen processing and presentation, thereby decreasing the visibility of infected hepatocytes to the immune system.

Finally, mutational inactivation of B cell and T cell epitopes facilitate viral persistence in both HBV and HCV infections, although the inactivation plays a more prominent role in HCV infection because of HCV’s higher mutation rate (164–170). Although the most convincing evidence of mutational escape from the T cell response has been demonstrated in HCV-infected chimpanzees (169, 170), mutations involving epitope residues...
that abrogate (168), energize (165, 171–173), or antagonize (165, 168, 174–176) recognition by the T cell receptor, binding to MHC (168), proteasome-dependent processing (177), and ATP-dependent transport (178) have also been reported in humans infected by HBV or HCV.

Host Factors Affecting Immune Responses

The observation that immune tolerance to HBV is likely responsible for viral persistence in most neonatal HBV infections, coupled with the fact that a vigorous, multispecific, and polyclonal cellular immune response is usually associated with the clearance of HBV and HCV in immunocompetent adults, suggests that host factors play a crucial role in the outcome of these infections (1, 179). This hypothesis is strongly supported by the de novo induction of expression of a large number of T cell, cytokine and cytokine-induced genes in the liver of chimpanzees during the clearance of HBV and HCV (77, 78, 136, 180). The key remaining question is why the T cell response is either quantitatively weak or qualitatively inadequate to terminate infection in adult-onset chronic HBV and HCV infections. The literature suggests that several nonexclusive mechanisms may be responsible. For example, viral persistence has been associated with primary failure to efficiently mount virus-specific CD4+ and CD8+ T cell responses in both HCV-infected chimpanzees and in a patient who was studied prospectively following accidental needlestick exposure to HCV (8, 78, 130). Failure to induce a cellular immune response could reflect virus-induced deficiencies in antigen presentation (reviewed in Reference 181), genetically determined restriction of the virus-specific T cell repertoire (reviewed in Reference 182) or antigen overload during immunological priming (reviewed in References 183 and 184). Alternatively, the induction of anergy and/or exhaustion of an initially vigorous T cell response as a result of antigen overload and excessive T cell stimulation, the induction of regulatory T cells, and, at least theoretically, the delivery of negative regulatory signals [e.g., via CTL antigen-4 (CTLA-4), B and T lymphocyte attenuator (BTLA), and programmed death 1 (PD1)] to activated T cells by infected hepatocytes could also play a role in the secondary T cell unresponsiveness that has been observed in patients chronically infected with HBV or HCV. In support of this, it has been shown recently that acute HCV infections that progress to chronicity are often characterized by relatively strong virus-specific CD4+ and CD8+ T cell responses that are not sustained over time (185, 186). Excessive antigen-driven T cell stimulation during persistent infection may also negatively influence the capacity of HCV-specific CTLs to produce antiviral cytokines, as has been suggested from a study where the production of IFN-γ by these cells was found to be compromised following in vitro exposure to antigen (130, 187). Finally, the observation that antiviral treatment in patients chronically infected with HBV can rescue virus-specific CTL responses that were not detectable before therapy (188) suggests that those CTL responses were present in these subjects but suppressed. All together, these results indicate that both primary and secondary immunological unresponsiveness to both viruses occur and, presumably, contribute to the establishment of persistent HBV and HCV infection.

The Response of the Liver to Immune-Mediated Injury

The response of the liver to immune-mediated injury includes hepatocellular regeneration and the activation of reparative inflammatory/fibrogenic processes that resemble those of wound-healing (see below). Regenerative mechanisms may also play a direct antiviral role if HBV or HCV genomes do not survive cell division and the daughter cells are protected from reinfection. As HBV or HCV-infected hepatocytes are
eliminated during self-limited infection, the secondary regenerative and reparative events also subside until viral clearance is achieved and full anatomical and functional integrity of the organ is restored. In contrast, the inefficient immune response to HBV and HCV in chronically infected patients sets up continuous cycles of low-level liver cell destruction that (over long periods of time) lead to fibrosis, cirrhosis, steatosis, and HCC. The pathogenesis of these secondary events involves virus- and/or host-dependent factors that perturb normal liver repair functions, lipid metabolism, and antiapoptotic and/or growth promoting activities, as we discuss below.

Liver Cell Regeneration

Although mature hepatocytes are terminally differentiated cells, they retain extraordinary regenerative capacity in response to liver injury or other non-inflammatory stimuli (e.g., partial hepatectomy) (189–192). When mature hepatocytes are destroyed by the immune response, other mature hepatocytes are rapidly triggered to divide. Therefore, this process does not appreciably rely on hepatic progenitor cells (oval cells) or more undifferentiated stem cells (189–192). Oval cells (and perhaps stem cells) divide and differentiate into hepatocytes only when the replication capacity of mature hepatocytes has diminished, as may occur during late stage cirrhosis (see References 193 and 194 and also below). Along these lines, oval cell proliferation has been observed in experimental models of hepatocarcinogenesis (195), although their role in HCC is still uncertain (see below). Given that proliferating mature hepatocytes can readily serve as tumor precursors (196) and oval cells do not appear to generate tumors directly (195), it is likely that, if these cells play a role in hepatocyte transformation, they do it indirectly through the generation of hepatocytes.

Studies by Taub (192), Fausto (190, 191), and others (197) have defined the role of many immediate-early gene products in the initial phase of hepatocyte regeneration after partial hepatectomy. Likely, similar factors contribute to the early liver response to immunemediated injury. In particular, the entry of hepatocytes from a resting G0-phase into the cell cycle is induced by various cytokines, growth factors and components of the complement system. Examples of these factors include IL-6, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-α, hepatocyte growth factor (HGF), epidermal growth factor (EGF), insulin, C3a, and C5a (189, 190, 192, 198, 199). These ligands can activate transcription factors including nuclear factor (NF)-κB, signal transducer and activator of transcription 3 (STAT3), activator protein 1 (AP-1), and CCAAT/enhancer-binding protein (C/EBP) that then initiate the cascade of gene expression ultimately responsible for proliferation (200). Furthermore, a number of antiproliferative genes are also induced during the early phase of liver regeneration (197), in keeping with the concept of an autonomous control of cell-cycle entry by the hepatocyte and suggesting that tight regulation of liver cell proliferation originates very early after a regenerative stimulus.

The extent of hepatocyte turnover during viral hepatitis can be substantial. Indeed, a recent study using integrated viral DNA as a genetic marker for infected cells suggested that 100% of the hepatocytes may divide at least once during the resolution of self-limited WHV infection (201). A large amount of liver cell turnover has also been recently detected by the same technique in chronically infected woodchucks (202), and it has been inferred from a previous study that six to twelve complete liver turnovers per year may occur in this model of chronic infection (203). Although the degree of hepatocyte turnover in acute and chronic HBV or HCV infection in humans cannot be precisely measured, it is likely to be directly proportional to the relative severity and duration of liver disease. The fact that mature mouse hepatocytes can replicate at least 70 times in serial transplantation experiments...
(204) suggests that, in theory, mature human hepatocytes could cope easily with the recurrent regenerative stimuli that must occur during decades of chronic infection. If the relative regenerative capacity of hepatocytes diminishes over time (perhaps because of telomere shortening), this condition may facilitate the onset of cirrhosis (193, 194). It is important to note, however, that liver cell regeneration is likely to be directly linked to at least one possible mechanism of hepatocyte transformation. Indeed, liver cancer during HBV or HCV infection almost always occurs in the context of long-standing chronic hepatitis in which the coexistence of mitogenic (hepatocyte regeneration) and mutagenic (inflammation) events may favor random DNA damage that may lead to HCC. As we describe in greater details below, liver cancer can also be promoted by two additional mechanisms, both of which involve viral factors: insertional mutagenesis (for HBV) and expression of oncogenic viral proteins (for HBV and HCV) capable of deregulating liver cell growth or DNA repair.

Collectively, the results we summarize above suggest that liver cell regeneration during viral hepatitis contributes not only to the restoration of liver mass after the immune-mediated destruction of hepatocytes, but also to the long-term complications (cirrhosis and HCC) of chronic infections.

**Liver Repair in Response to Immune-Mediated Injury**

As we briefly mention above, liver repair in response to immune-mediated injury involves the rapid activation of a complex network of inflammatory and fibrogenic processes that resemble those of wound-healing and contribute to maintenance of the structural integrity of the organ (191, 205). Various cell types participate in these reparative mechanisms: Some, such as hepatic stellate cells (HCS), myofibroblasts, fibroblasts and Kupffer cells, reside within the liver, whereas others, such as monocytes, NK cells and T cells, reach the liver from the circulation (191, 205). Liver repair is characterized by deposition of components (e.g., collagen, laminin, fibronectin, and proteoglycans) of the extracellular matrix (ECM) by activated stellate cells, myofibroblasts and fibroblasts (191, 205). Activation of these cells is thought to be regulated by inflammatory mediators including cytokines (e.g., TGF-β, IL-6 and TNF-α), chemokines (e.g., CCL21), growth factors [e.g., platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF)-binding protein-1] and other stimuli (e.g., reactive oxygen intermediates, lipid peroxides and nitric oxide) that are produced by resident (e.g., fibroblasts and Kupffer cells) and non-resident (e.g., NK cells and T cells) liver cells (191, 205, 206). Although the regulation of this intricate pathway is beyond the scope of our review, it is important to emphasize that the initially beneficial reparative response can become deregulated, thereby facilitating excessive and qualitatively abnormal ECM deposition within the liver (205, 207). This deposition may occur during chronic HBV or HCV infection, when viral persistence and recurrent immune-mediated liver damage drive the pathogenesis of fibrosis and cirrhosis.

**Liver Fibrosis and Cirrhosis**

Liver fibrosis and cirrhosis are common consequences of chronic liver diseases of viral (e.g., HBV or HCV) and non-viral (e.g., ethanol, iron or copper accumulation, and biliary tract disorders) etiologies (205, 207). They are characterized by an imbalance between fibrogenesis and fibrolysis (205, 207), which results in the excessive intrahepatic deposition of matrix that is qualitatively different in its composition and organization from that of normal liver repair (205, 207). As a result of this process, a dense, reticulated ECM that is relatively resistant to enzymatic degradation is initially deposited around the portal areas of the liver (portal-based fibrosis) during...
chronic viral hepatitis. As a function of time, the fibrosis progressively expands into the lobules with the formation of septa that can eventually connect portal and central veins (portocentral bridging fibrosis) (205, 207). Liver cirrhosis represents the final phase of fibrosis in which fibrous septa surround nodules of regenerating hepatocytes causing profound architectural distortion of the liver, functional insufficiency, and diversion of venous blood containing intestinal toxins into the systemic circulation (205, 207). Liver fibrosis and cirrhosis are also responsible for the formation of a basal membrane separating hepatocytes from sinusoidal blood (205, 207). This event (often described as capillarization of the hepatic sinusoids) is thought to alter the normal exchange of soluble factors between blood and hepatocytes (205, 207). The rate by which liver fibrosis and cirrhosis (and cancer, see below) progress during chronic HBV or HCV infection is positively influenced by the severity and duration of liver disease. Thus, it is not surprising that factors known to be associated with more severe liver damage (e.g., older age at onset of infection, male gender, alcohol intake, obesity, diabetes, and HIV coinfection) are also implicated in faster progression of these complications (205, 207).

On the basis of the results summarized above, it is clear that the pathogenesis of fibrosis and cirrhosis during chronic viral hepatitis is multifactorial, involving recurrent immune-mediated liver injury, sustained inflammation, and activation of ECM-producing cells.

Liver Cancer

As mentioned above, the pathogenetic mechanisms whereby HBV or HCV causes HCC involve both host and viral factors. As the interval between viral infection and HCC is typically several decades, these viruses are thought to be neither directly nor acutely oncogenic. Almost all cases of HCC take place after many years of chronic hepatitis (1, 208, 209). As described earlier, chronic hepatitis is characterized by sustained liver disease with associated hepatocellular regeneration (i.e., cellular DNA synthesis) and inflammation (i.e., the production of mutagens), which could precipitate random genetic and chromosomal damage and lead to the development of HCC. The pathogenetic relevance of immune-mediated hepatocyte damage in carcinogenesis during chronic HBV or HCV infection is reinforced by the fact that HCC in humans occurs in the context of necrosis, inflammation and regeneration in a variety of persistent liver diseases other than viral hepatitis, including primary biliary cirrhosis (210), glycogen storage disease (211), α1-antitrypsin deficiency (212), hemochromatosis (213), and alcoholism (214). Thus, it appears that chronic liver cell injury is a premalignant state that promotes cellular processes, like enhanced cellular DNA synthesis and production of inflammatory mutagens coupled with compromised cellular detoxification and repair functions, that could be oncogenic. Persistence of these events for a sufficiently long period of time may result in the multiple genetic and chromosomal alterations that are responsible for the development of HCC. In keeping with this hypothesis, HBV transgenic mice that do not spontaneously develop liver injury have been shown to develop HCC after many months of chronic hepatitis mediated by HBV-specific CTLs (215) (see also Figure 6). Importantly, the appearance of HCC in this setting occurs despite the absence of cofactors such as random viral integration, X gene expression or genotoxic agents that have been proposed to contribute to the development of HCC in humans (1, 208, 209).

Although the studies we describe suggest that persistent immune-mediated liver cell injury may be sufficient to trigger HCC, other viral factors may also contribute to hepatocarcinogenesis during chronic HBV or HCV infection. Indeed, HBV DNA integration has been reported to occur in the proximity of a variety of procarcinogenic genes...
Histopathological features of prolonged chronic immune-mediated hepatitis in hepatitis B virus (HBV) transgenic mice (adapted from Reference 215). Three weeks after adoptive transfer of HBV-immune spleen cells, HBV transgenic mice reconstituted with a syngeneic, nontransgenic immune system develop a chronic inflammatory liver disease characterized by a diffuse mononuclear and polymorphonuclear inflammatory infiltrate and focal hepatocellular necrosis and dropout (arrows; upper left panel). Three months later, the mice display multiple portal (and intralobular, not shown) inflammatory infiltrates indicative of chronic hepatitis (upper right panel). By eight months, the liver disease in these animals is more severe and preneoplastic foci [the edge of one of which is outlined (arrowheads)] are detected (lower left panel). Eventually, all animals sacrificed 17 months later display well-differentiated hepatocellular carcinoma characterized by trabecular cords composed of several layers of differentiated neoplastic hepatocytes (lower right panel). These hepatocytes contain typical abundant eosinophilic cytoplasm and prominent nucleoli and are covered by a thin endothelial lining (H&E stain, original magnification X 200).

(including oncogenes, growth factor receptor genes, and genes regulating telomerase activity or signal transduction pathways) and deregulate their expression. It must be noted, however, that HBV integration should not occur in resting hepatocytes and, therefore, if this process contributes to hepatocarcinogenesis, it is likely to be secondary to procarcinogenic events that trigger hepatocyte turnover (e.g., immune-mediated hepatitis). Moreover, the expression of HBV-derived polypeptides such as the X protein, a C-terminally truncated version of the M-HBs protein, and the full-length L-HBs protein has been linked to the process of malignant transformation of infected hepatocytes. These viral products may contribute to hepatocarcinogenesis by their capacity to activate a variety of cellular promoters (including AP-1 and NF-κB) and interact with several signal transduction pathways [including those related to Janus kinases 1 (Jak1), protein kinase C (PKC), phosphatidylinositol-3 (PI-3), and mitogen-activated protein...
The HBV X protein has also been shown to interact and interfere with numerous transcription factors including Cal(2+)/cAMP-response element binding protein (CREB), activating transcription factor 3 (ATF), nuclear factor interleukin-6 (NFIL6), early growth response-1 (Egr1), Ets-1, octamer-binding protein (Oct1), and retinoid x receptor (RXR), tumor suppressor genes (including p53), and proteins involved in DNA repair functions (including p53 and UVDD1) (reviewed in Reference 208).

In contrast to HBV, HCV is an RNA virus that does not integrate into the host genome; yet HCV causes chronic hepatitis (i.e., inflammation, liver cell destruction and regeneration) and HCC. It is noteworthy that several proteins encoded by the HCV genome (e.g., the core protein, NS3, and NS5A) have been reported to regulate cell proliferation and viability. The HCV core protein has been shown to activate several major cellular transduction pathways including those involving extracellular signal-regulated kinases (ERK)/JNK/MAP kinases, NF-κB and STAT-1 to bind proteins involved in cell growth and survival including tumor necrosis factor-alpha receptor (TNF-αR), lymphotoxin-beta receptor (LT-βR), retinoid X receptor-alpha (RXR-α), and apolipoprotein (apo) A2 and to affect apoptosis. Furthermore, transgenic mice expressing this protein develop HCC (31). NS3 and NS5A can induce a transformed phenotype in fibroblasts by regulating various pathways, including those that involve p53, p21, and Cdk1/2-cyclin complexes (reviewed in Reference 209).

It must be noted that most of the studies pertaining to the potential transforming capacity of HBV or HCV proteins have been done either with transformed cell lines or in transgenic mice that overexpress a specific viral product (reviewed in Reference 209). Although further studies need to be performed in order to understand the relevance of these observations in the context of chronic viral hepatitis and HCC development in humans, it is reasonable to assume that host and viral factors may indeed cooperate in hepatocarcinogenesis (see also Figure 7), thereby explaining why the incidence of HCC in chronic HBV or HCV infection is so high, and so much higher than that observed in chronic liver diseases other than viral hepatitis (1, 208, 209).

Liver Steatosis
Liver steatosis is a metabolic disorder characterized by hepatocellular fat accumulation (216), and it is a common histopathological finding in chronic HCV (but not HBV) infection (217). As with HCC, both host and viral factors are thought to predispose and/or contribute to its development. In fact, steatosis in chronically infected patients has been associated with obesity and diabetes as well as with specific genotypes of HCV (217). It is also worth mentioning that recent studies in cell lines and transgenic mice have shown that the core and NS5 proteins of HCV can intracellularly colocalize with lipid droplets and interact with lipid-binding protein such as apoA1 and apoA2 (26, 33, 218, 219). It is believed that such interactions may induce varying degrees of lipid metabolism deregulation, thereby facilitating the occurrence of steatosis (220). Finally, it has also been suggested that the steatotic process may be facilitated by HCV core protein accumulation within mitochondria; this accumulation may subsequently induce oxidative stress and perturb lipid metabolism (30, 33).

EXTRAHEPATIC DISEASES ASSOCIATED WITH HBV OR HCV INFECTION
As we mention above, acute HBV and chronic HBV or HCV infection are also associated with extrahepatic manifestations that affect a variety of organs or tissues, including kidney, blood vessels, skin, and joints. It is
Figure 7

Hepatocarcinogenesis in viral hepatitis. Although a vigorous (+++ ) immune response to hepatitis B virus (HBV) or hepatitis C virus (HCV) may lead to viral clearance and an absent (−) immune response may lead to the healthy carrier state, an intermediate (+) immune response produces chronic hepatitis. This indolent necroinflammatory and potentially mutagenic liver disease is characterized by chronic liver cell necrosis that stimulates a sustained regenerative response. The collaboration of these mutagenic and mitogenic stimuli has the potential to cause cellular and viral DNA damage, chromosomal abnormalities and genetic mutations that deregulate cellular growth control in a multistep process that leads eventually to hepatocellular carcinoma (HCC).

generally assumed that the main pathogenetic mechanism behind most of these complications involves transient or persistent antigenic stimulation and tissue deposition of circulating antigen-antibody immune complexes. Although a detailed description of all extrahepatic diseases associated with HBV or HCV infection is beyond the scope of this review, a brief summary of some of the most common disorders is provided below.

**Extrahepatic Manifestations of Acute Viral Hepatitis**

Although acute HCV infection is usually asymptomatic, acute HBV infection is often
associated with prodrome of extrahepatic manifestations that are secondary to tissue deposition of immune complexes and resemble serum sickness (reviewed in References 147, 148, and 221). The symptoms may include urticaria, maculopapular rash, and purpura as well as mild, localized or generalized arthritis, frequently involving the small joints of the hands. Skin and joint symptoms usually precede the onset of hepatitis and tend to disappear by the time the patients becomes jaundiced (147, 148, 221). Another fairly common extrahepatic manifestation of acute HBV infection is polyarteritis nodosa (PAN), which also occurs (albeit less frequently) during chronic HBV or HCV infection (147, 148, 221). PAN is a systemic inflammatory disease characterized by vasculitis of small- and medium-sized small arteries caused by immune-complex deposition. PAN can manifest itself as arthritis, glomerulonephritis, neuritis, and skin rashes. The symptoms may include hypertension, renal insufficiency, peripheral neuropathy, myopathy, joint pains, and ischemic myalgias (147, 148, 221). Even though multisystem involvement is typical of PAN, occasionally only one organ or system may be involved (147, 148, 221).

**Renal Diseases in Chronic Viral Hepatitis**

Epidemiological, clinical and immunological observations indicate that various forms of immune-complex glomerulonephritis are common extrahepatic complications of persistent HBV and HCV infection (reviewed in References 221 and 222). The nature of the viral antigens implicated in these diseases may vary according to the histopathological lesions. For example, HBV-induced membranous nephropathy is mainly characterized by deposits consisting of HBeAg and IgG, although HBsAg and the HCV core protein as well as IgG and C3 are the most abundant components of the immune complexes observed in HBV- or HCV-induced membra-noproliferative glomerulonephritis, respectively (221, 222).

**Mixed Cryoglobulinemia and B Cell Non-Hodgkin’s Lymphoma in Chronic Viral Hepatitis**

Mixed cryoglobulinemia (MC) is a benign B cell proliferative disorder characterized by polyclonal B cell activation and antibody production that can lead to local or systemic vasculitis secondary to the deposition of circulating immune complexes in small vessels (146). MC is the most common extrahepatic manifestation associated with chronic HCV infection (146, 149, 222) and has also been described in patients chronically infected with HBV (221). MC can be either asymptomatic or evident as relatively mild cutaneous vasculitis or severe vasculitis involving the kidneys and central nervous system. The MC deposits often consist of IgM rheumatoid factors (RF), complement components, polyclonal IgG anti-HCV or anti-HBV antibodies and HCV- or HBV-related antigens and viral nucleic acids (146, 149, 221, 222). Importantly, there is an unusually high prevalence of B cell non-Hodgkin’s lymphoma (NHL) in patients chronically infected with HCV, and recent studies demonstrating lymphoma regression following successful antiviral therapy and clearance of HCV support a causative role for HCV in this malignant B cell proliferative disorder (223). The causative mechanism(s) are not well-defined, although persistent antigenic stimulation and polyclonal B cell activation by circulating viral proteins are likely candidates. In keeping with this, binding of the HCV E2 glycoprotein to CD81 on B cells has been shown to induce proliferation and activation of these cells in vitro (224), and low level HCV replication has been reported in primary or transformed B cell lines (including some obtained from HCV-infected NHL) (225). Further studies aimed at understanding how HCV promates B cell proliferative disorders are certainly warranted.
**Porphyria Cutanea Tarda and Lichen Planus in Chronic Viral Hepatitis**

Porphyria cutanea tarda (PCT) is an interesting dermatological complication of chronic HBV and HCV infections (146, 149, 221, 222). PCT belongs to a group of disorders (porphyrias) of the heme biosynthesis pathway resulting from decreased activity of uroporphyrinogen decarboxylase (UROD) in the liver. PCT can be either inherited or acquired, and it has been proposed that, in addition to hepatic iron overload, HBV and HCV may be additional factors contributing to PCT disease progression (226). Lichen planus (LP) is a chronic inflammatory disease that affects skin and mucous membranes of squamous cell origin. Various reports suggest that HCV infection is relatively common in patients with LP and that HCV may cause LP, especially the oral form of the disease. A constant feature of LP patients with chronic HCV infection is the presence of polyclonal hypergammaglobulinemia, and immune complexes have been implicated in the pathogenesis of HCV-related LP (227). In addition, evidence for HCV infection of LP lesions (227) and secondary infiltration of virus-specific T cells (228) has been recently provided, suggesting that T cell-mediated immunopathology may also participate in this disorder.

**Sjögren’s Syndrome and Other Diseases in Chronic Hepatitis C Viral Infection**

Sjögren’s syndrome (SS) is an autoimmune disease that primarily affects salivary and lachrymal glands and is associated with chronic HCV but not HBV infection (146, 149, 222). Indeed, histological changes typical of SS have been detected in the salivary glands of patients chronically infected with HCV (229). Like LP, it has been proposed that the pathogenesis of SS may involve both tissue deposition of immune complexes as well as T cell-mediated immunopathology (146, 149, 222). In keeping with this, HCV replication has been detected in epithelial cells from salivary glands of patients with SS (230), and a lymphocytic exocrinopathy resembling SS has been described in the salivary and lacrimal glands of transgenic mice that express HCV E1 and E2 (24). Finally, HCV has also been linked to additional diseases such as type II diabetes and autoimmune thyroiditis, although these associations remain a matter of controversy (229). The interesting observation that HCV core transgenic mice are insulin resistant (231) suggests that HCV may contribute to the development of type II diabetes in humans.

**CONCLUSION**

Thanks to technological advances and model systems that have made it possible to study many of the host-virus interactions that influence these infections, our understanding of the immunobiology and pathogenesis of HBV and HCV infections has greatly improved in recent years. The basic principles of HBV and HCV replication and gene expression have been uncovered, infectious viral genomes have been cloned and sequenced, and all viral gene products have been essentially characterized (9, 232); a variety of animal models have been developed for HBV (233–235), and several cell-culture-adapted HCV replicons have been established (21) that now make it possible to dissect the mysteries of HCV replication and to test the antiviral activity of cellular gene products and candidate drugs.

It is quite clear that both viruses replicate noncytopathically in the hepatocyte, and that most of the clinical syndromes associated with these infections reflect the immune response. It is also apparent that the innate immune response does not contribute significantly to the pathogenesis of liver disease or viral clearance in either infection, whereas the adaptive immune response, especially the virus-specific CTL response, contributes to both. Recent observations reveal, however, that antigen-nonspecific inflammatory cells can enhance
CTL-induced immunopathology in the liver, and that platelets facilitate the intrahepatic accumulation of CTLs. These observations suggest that the host response to these infections is a highly complex but coordinated process. On the basis of these results, it is evident that CTL effector functions play a prominent role in the resolution of HBV and HCV infection, and that viral persistence reflects the failure to induce or maintain these CTL-dependent events. Moreover, according to this scenario, an inefficient T cell response that fails to clear the infections creates a smoldering chronic necroinflammatory process, the end result of which is hepatic fibrosis, cirrhosis and HCC. In addition, these infections are associated with a variety of extrahepatic disorders, which are largely immunological in origin.

In spite of these accomplishments, however, many issues pertaining to the biology, immunobiology and pathogenesis of HBV and HCV infections remain unresolved, some of which are highlighted in the Unresolved Issues/Future Directions section of this review. Future studies aimed at addressing these issues will not only improve our understanding of the host-virus interactions that determine the pathogenesis and outcome of infection, but may also lead to the discovery of new approaches for the prevention and treatment of HBV and HCV infections and their life-threatening complications.

SUMMARY POINTS
1. HBV and HCV replicate noncytopathically in the hepatocyte.
2. The innate immune response does not contribute significantly to the pathogenesis of liver disease or viral clearance in either infection.
3. The adaptive immune response, especially the virus-specific CTL response, plays a major role in both liver disease pathogenesis and viral clearance during HBV or HCV infection.
4. Antigen-nonspecific inflammatory cells enhance CTL-induced immunopathology in the liver.
5. Platelets may facilitate CTL homing within the liver.
6. Viral persistence reflects the failure to induce or maintain CTL-effector functions and/or viral evasion of the immune response.
7. Chronic inflammation contributes to the development of the complications (fibrosis, cirrhosis, and HCC) of persistent HBV or HCV infection.
8. HBV and HCV infections are associated with a variety of extrahepatic disorders, which are mainly immunological in origin.

UNRESOLVED ISSUES/FUTURE DIRECTIONS
These issues have been addressed recently in an “Action Plan for Liver Disease Research,” prepared by the NIH with strong input from the scientific community (see the following Web site: http://www.niddk.nih.gov/fund/divisions/ddn/ldrbr/ldrbr_action_plan.htm). The highest priority issues, in our opinion, are as follows:
1. Tissue culture and small-animal models of HBV and HCV infections need to be developed.
2. The biology of viral entry, replication, egress, and spread of HBV and HCV needs to be elucidated.
3. The host factors that control the induction and maintenance of an effective antiviral immune response against both viruses need to be identified.
4. An effective vaccine needs to be developed for the prevention of HCV, and improved antiviral strategies need to be produced for the treatment of chronic HBV and HCV infections.

RELATED RESOURCES

ACKNOWLEDGMENTS
This work was supported by grants ai20001, ca76403, ca54560, ca108304, ca40489 (to F.V.C.), and ai40696 (to L.G.G.) from the National Institutes of Health. This is manuscript number 17742-mem from the Scripps Research Institute.

LITERATURE CITED


monokine induced by IFN-gamma activity in vivo reduces the pathogenetic but not the antiviral potential of hepatitis B virus-specific cytotoxic T lymphocytes. J. Exp. Med. 194:1755–66


58  Guidotti • Chisari


A significant amount of hepatocyte turnover and clonal expansion of hepatocytes may occur during chronic hepadnaviral infection.


Contents

Frontispiece

Morris J. Karnovsky ........................................................... xii

A Pathologist’s Odyssey

Morris J. Karnovsky ........................................................... 1

Immunobiology and Pathogenesis of Viral Hepatitis

Luca G. Guidotti and Francis V. Chisari ..................................... 23

The Pathogenesis of Helicobacter pylori–Induced Gastro-Duodenal Diseases

John C. Atherton ................................................................ 63

Molecular Pathology of Malignant Gliomas

David N. Louis ................................................................ 97

Tumor Stroma and Regulation of Cancer Development

Thea D. Tlsty and Lisa M. Coussens ......................................... 119

Neurodegenerative Diseases: New Concepts of Pathogenesis and Their Therapeutic Implications

Daniel M. Skovronsky, Virginia M.-Y. Lee, and John Q. Trojanowski ........................................ 151

The Endothelium as a Target for Infections

Gustavo Valbuena and David H. Walker ..................................... 171

Genetic Regulation of Cardiogenesis and Congenital Heart Disease

Deepak Srivastava ................................................................ 199

Regulation of Lung Inflammation in the Model of IgG Immune-Complex Injury

Hongwei Gao, Thomas Neff, and Peter A. Ward .............................. 215

Integrative Biology of Prostate Cancer Progression

Scott A. Tomlins, Mark A. Rubin, and Arul M. Chinnaiyan .................. 243

KSHV Infection and the Pathogenesis of Kaposi’s Sarcoma

Don Ganem ........................................................................ 273
Inflammation and Atherosclerosis
  Göran K. Hansson, Anna-Karin L. Robertson, and Cecilia Söderberg-Nauclér ....... 297
Lung Cancer Preneoplasia
  Ignacio I. Wistuba and Adi F. Gazdar .................................................. 331
Pathogenesis of Nonimmune Glomerulopathies
  Christopher Kwok, M. Brendan Shannon, Jeffrey H. Miner, and Andrey Shaw ....... 349
Spectrum of Epstein-Barr Virus–Associated Diseases
  J.L. Kutok and F. Wang ........................................................................... 375
Calcium in Cell Injury and Death
  Zheng Dong, Pothana Saikumar, Joel M. Weinberg, and Manjeri A. Venkatachalam .................................................. 405
Genetics of Soft Tissue Tumors
  Matt van de Rijn and Jonathan A. Fletcher .............................................. 435
Severe Sepsis and Septic Shock: The Role of Gram-Negative Bacteremia
  Robert S. Munford ............................................................................. 467
Proteases in Parasitic Diseases
  James H. McKerrow, Conor Caffrey, Ben Kelly, P'ng Loke, and Mohammed Sajid .... 497
INDEX
Subject Index .................................................................................................. 537