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Perspective

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Cytotoxic T Cells and Viral Hepatitis

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Overview

The hepatitis B (HBV)[1] and C (HCV) viruses are noncytopathic, hepatotropic viruses that cause acute and chronic hepatitis and hepatocellular carcinoma (1). The cellular immune response to HBV and HCV is thought to be responsible for viral clearance and disease pathogenesis during these infections. The T cell response to HBV is vigorous, polyclonal, and multispecific in acutely infected patients who successfully clear the virus, and it is relatively weak and narrowly focused in chronically infected patients. In contrast, the T cell response to HCV is relatively strong and multispecific in both acutely and chronically infected patients, suggesting that HCV may be less responsive to control by the T cell response than HBV. Both viruses, especially HCV, have a high mutation rate, creating the opportunity for selection of variant viral genomes to occur if a mutation confers a growth advantage or deletes a recognition site for the immune response.

Cytotoxic T lymphocytes (CTL) are generally thought to mediate viral clearance by killing infected cells. The number of HBV- and HCV-infected hepatocytes is so large relative to the number of virus-specific CTL, however, that clearance of these viruses may not be achievable by the relatively inefficient one-on-one process by which CTL kill their target cells. Recently, it has been shown that CTL can inhibit HBV gene expression and replication in the liver of transgenic mice noncytopathically by secreting antiviral cytokines that interrupt the HBV life cycle. Because this potentially "curative" process is much more efficient than killing, CTL-induced intracellular inactivation of HBV could be the principal mechanism of viral clearance during HBV infection. Whether HCV is susceptible to this kind of control is an open question at this point; however, the ability of HCV to persist despite a strong CTL response suggests that HCV may be either less visible to the CTL or less responsive to cytokine-mediated antiviral signals than HBV.

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1. *Abbreviations used in this paper:* CTL, cytotoxic T lymphocyte; HBV, hepatitis B virus; HCV, hepatitis C virus.

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The cytotoxic T cell response to HBV

The CTL response to HBV is vigorous, polyclonal, and multispecific in patients with acute hepatitis who ultimately clear the virus, and it is weak or barely detectable in patients with chronic hepatitis (2–5), except during acute exacerbations of chronic disease or after spontaneous or IFNα-induced viral clearance (6). Despite the vigor of the T cell response to HBV during acute viral hepatitis, very low levels of virus persist in the circulation for several decades after complete clinical and serological resolution of disease (7). Long-term persistence of trace amounts of viral DNA is associated with equally long-term persistence of HBV-specific CTL that display recent activation markers. This suggests that transcriptionally active virions can apparently maintain the CTL response indefinitely after recovery, perhaps for life (7). These new and unexpected results suggest that small quantities of HBV persist in an immunologically privileged reservoir after seroconversion and that spread of the infection is controlled by CTL, since several of these subjects lacked antibodies to hepatitis B surface antigen. The data also raise questions about the site of persistent infection, the basis for incomplete clearance, the chance of viral reactivation during immunosuppression, and the possibility that these individuals or their organs may be infectious for others. Clinical reports that occult HBV may be responsible for transmission of virus to liver transplant recipients (8) and after blood transfusions from HBV seronegative subjects (9) support the notion of incomplete viral clearance after recovery from acute viral hepatitis.

While the strong association between liver disease and the CTL response during acute HBV infection suggests an important role for CTL in the pathogenesis of acute viral hepatitis, proof of this hypothesis required the development of transgenic mice that express and replicate HBV in their hepatocytes and the demonstration that these animals develop an acute necroinflammatory liver disease after adoptive transfer of hepatitis B surface antigen–specific CTL lines and clones (10, 11). Importantly, the number of CTL injected into the mice, and the intrinsic cytopathic activity of these CTL can be easily manipulated such that the severity of the ensuing liver disease can be tightly controlled. Taking advantage of this opportunity, it has been shown recently that HBV gene expression and replication can be completely abolished in all of the hepatocytes in the liver by a noncytopathic antiviral process in which the viral nucleocapsids disappear from the cytoplasm and the viral RNAs are degraded in the nucleus of the hepatocytes under conditions in which $< 1\%$ of the hepatocytes is destroyed (12). As a result, all of the viral gene products and virions disappear from the liver and the serum in the absence of serum transaminase elevations or histological evidence of liver disease (12). Viral clearance in this model is completely blocked when antibodies to IFNγ and TNFα are injected be-

fore the CTL, indicating that these cytokines are responsible for the antiviral effect. Importantly, these results illustrate a new principle in viral immunology, i.e., CTL can activate HBV-infected cells to participate in the antiviral response by triggering them to produce cellular proteins that interrupt the viral life cycle.

One might predict from the foregoing observations that superinfection of the liver by other hepatotropic viruses might lead to the clearance of HBV if they induce the production of antiviral cytokines to which HBV is susceptible. Indeed, precisely these events have been shown to occur in the HBV transgenic mice during lymphocytic choriomeningitis virus infection (13) as well as during adenovirus- and cytomegalovirus-induced hepatitis (our unpublished observations). Intriguingly, isolated case reports have been published suggesting that superinfection by HAV is sometimes associated with clearance of HBV in chronically infected patients (14).

These results suggest that a strong intrahepatic CTL response to HBV during acute viral hepatitis can suppress HBV gene expression and replication and perhaps even "cure" infected hepatocytes of the virus in addition to killing them. Conversely, the data suggest that a weak immune response, such as that which occurs in chronically infected patients, could contribute to viral persistence and chronic liver disease by reducing the expression of viral antigens sufficiently for the infected cells to escape immune recognition but not enough for the virus to be eliminated. Therefore, the ability of CTL-derived cytokines to inhibit HBV replication could represent a survival strategy by the virus, contributing to persistence, or a tissue-sparing antiviral strategy by the host, contributing to viral elimination.

Mechanisms of HBV persistence

For a noncytopathic virus to persist, it must either not induce an effective antiviral immune response or it must overwhelm or evade it. Neonatal tolerance is probably responsible for both the lack of an antiviral immune response and for viral persistence after mother–infant transmission, which is the most common antecedent of persistent HBV infection worldwide (1). The immunological basis for viral persistence after adult onset infection is not well understood. Perhaps the simplest explanation is quantitative, possibly based on the relative kinetics of viral spread and to the induction of a CTL response during the early days of an infection. For example, viral persistence would be predicted if the size of the inoculum or the replication rate of an incoming virus exceeds the kinetics of the immune response, such that the effector-to-target cell ratio favors the virus even when the CTL response is fully in place.

Other factors must be involved as well, however, to be consistent with the repeated observation that the CTL response is much less vigorous in chronically infected patients than it is during acute infection (for review see reference 1). Reasonable candidates are the induction of peripheral tolerance or exhaustion of the T cell response by the high viral load that characterizes most persistently infected patients. Other candidate mechanisms that could contribute to viral persistence include early infection of immunologically privileged sites, viral inhibition of antigen presentation, selective immune suppression, downregulation of viral gene expression, and viral mutations that abrogate, anergize, or antagonize antigen recognition by virus-specific T cells (for review see reference 15). There is some evidence that privileged sites may play a role since HBV does infect extrahepatic tissues and HBV-specific CTL can recognize viral antigens in the liver but not in the kidney or brain of HBV transgenic mice (16).

Additionally, it has been suggested that infected cells that express Fas ligand can protect themselves against CTL-mediated injury by actively destroying the CTL via the same Fas ligand–Fas receptor pathway that CTL can use to kill their target cells, but in reverse (17). Importantly, it appears that hepatocytes can be induced to express Fas ligand during an inflammatory response (17). If this is correct, patients whose hepatocytes are induced to express Fas ligand could selectively delete their HBV-specific CTL and, therefore, become chronically infected. While this is a very appealing scenario to explain the apparent HBV-specific immunodeficiency that characterizes chronically infected patients, this notion is strictly speculative at present.

Finally, viral inhibition of antigen presentation, virus-induced suppression or neutralization of antiviral cytokines and virus-induced induction of selective immune suppression are survival pathways that are used by other animal viruses (for review see reference 18). While there is no direct proof that such processes are operative during HBV infection, recent evidence that the HBV X protein can interact with proteasome subunits in vitro is relevant and potentially important (19).

CTL escape mutations

The role of viral escape mutations in well-defined CTL epitopes has attracted considerable interest as a cause of viral persistence in recent years (20–25). Many conditions must be fulfilled, however, for a mutant virus to be selected by CTL-mediated immune pressure (for review see reference 1). Perhaps the most important condition is the occurrence of a strong, functionally monoclonal CTL response that is focused on a single viral epitope. This scenario would favor the outgrowth of variant viruses that do not express the epitope because they would be invisible to the immune system. This type of CTL response is extremely unusual, however, during HBV infection since the CTL response is typically vigorous and multispecific during acute hepatitis and weak or undetectable during chronic hepatitis. Accordingly, immune selection of viruses containing mutant CTL epitopes is very uncommon during chronic HBV infection (26).

Nonetheless, strong, narrowly focused CTL responses are seen occasionally in these patients, and in this setting viral escape mutations can occur (20). Vigorous oligoclonal expansions of T cells have been described in other persistent viral infections, such as HIV (21, 25), HTLV-1 (24), and EBV(23). Even in these infections, however, viral mutations that affect recognition of an epitope by some CTL clones, even antagonizing the CTL response to the wild-type epitope (22), do not automatically affect all CTL clones specific for the same epitope since different T cell clones can bind different amino acid residues in the same epitope. While CTL escape can certainly confer a strong survival advantage to a virus, it is important to emphasize that selection of escape variants in all of these infections occurs in the setting of a preexisting persistent infection; that is, viral persistence probably favors the selection of escape variants, not the reverse. This is an important concept that is much too often ignored or underemphasized in the literature.

The common occurrence of a G-A mutation at nucleotide 1896 of the HBV genome creating a translational stop codon

that precludes precore protein synthesis and causes seroconversion from hepatitis B e antigen positivity to hepatitis B e antigen negativity in patients with chronic hepatitis is often cited as evidence of immune selection (27). This notion is probably incorrect since it implies that the precore specific immune response is critical for viral clearance. Unfortunately, there is no direct evidence to support this notion. On the contrary, because the core and precore protein sequences overlap extensively, T cell recognition of shared epitopes remains intact in the absence of the precore protein. Indeed, the ability of core or precore specific CTL to recognize and efficiently kill target cells that express both of these proteins has been demonstrated experimentally (3). Finally, no human helper or cytotoxic T cell responses that are unique to the precore protein have been demonstrated to date. Therefore, the immune selection hypothesis is insupportable at present as an explanation for the outgrowth of precore mutants, and should be viewed very cautiously until direct evidence for this notion is provided. On the contrary, there is considerable evidence that viral genomes containing the precore mutation enjoy a growth advantage over wild-type viruses, independent of the immune response. Indeed, the classical precore mutation has been reported to increase the conformational stability of the encapsidation signal in the viral RNA which plays a critical role in viral replication, thereby enhancing the HBV replication rate (28, 29). Additionally, precore protein expression has been shown recently to downregulate HBV replication efficiency in vivo (30). All of this evidence suggests a positive selection mechanism whereby the putative precore "escape" mutants actually have a replication advantage over wild-type viruses and are not likely to be selected by the immune response.

The CTL response to HCV

The mechanisms whereby HCV causes acute hepatocellular injury and initiates the sequence of events leading to chronic liver disease and ultimately hepatocellular carcinoma are not nearly as well understood as they are for HBV. Although the immune response almost certainly plays an important, perhaps a central, role in HCV pathogenesis, chimpanzees can be repetitively infected when they are exposed to an infectious inoculum of HCV after recovery from a previous infection with the same inoculum and the development of what would otherwise appear to be a perfectly competent immune response (31). While this may be related to the mutability of the virus and the presence of a diverse viral quasi-species in any given inoculum, the apparent absence of a protective neutralizing humoral or cellular response to common conserved determinants in the various viral proteins is puzzling. However, there is ample precedent for this pattern in influenza virus infection which is so highly mutable that recurrent pandemics reflect the inadequacy of the global immune response to this virus year after year.

In the absence of efficient in vitro systems to support and measure HCV replication, the neutralizing potential and specificity of the different anti-HCV antibodies remain largely undefined. Nonetheless, HCV specific neutralizing antibodies have been demonstrated recently in the plasma of chronically infected patients by in vitro neutralization of the capacity of HCV inocula to infect continuous T cell lines (32) and chimpanzees (33). While these antibodies can protect against infection by HCV strains previously present in the patients from which they were derived, they fail to neutralize viral strains prevalent in the patient at the time the antibodies are detected. Since the neutralizing antibody response appears to be directed against epitopes located within the highly variable HCV envelope proteins, it is likely that the humoral immune response contributes to viral heterogeneity by selecting for mutant viruses that lack the corresponding epitopes.

The role of the CTL response in HCV clearance is obscured by the fact that, in contrast to the relatively weak antiviral T cell response in patients with chronic hepatitis B, the CTL response to HCV is fairly vigorous in patients with chronic hepatitis C. Indeed, HLA class I–restricted HCV-specific $CD8^+$ CTL are detectable in the peripheral blood and the intrahepatic lymphocytic infiltrate in patients with chronic hepatitis C (34–39) and in the liver of infected chimpanzees (40), suggesting that the virus can persist in the presence of these CTL. The CTL identified thus far in infected patients are able to recognize both conserved and variable regions of all of the HCV proteins in the context of several different HLA molecules (34–39). Moreover, the response is often multispecific. CTL clones have been isolated from the intrahepatic compartment using only antigen nonspecific stimuli, implying that these cells are present at relatively high frequency within the intrahepatic infiltrate. In contrast, it is necessary to stimulate peripheral blood mononuclear cells with HCV-derived peptides to demonstrate a CTL response to HCV. Nonetheless, the HCV-specific CTL response is stronger than the response to HBV during chronic infection displaying CTL precursor frequencies of roughly 1 per 50,000 PBMC (41), which is 20 times higher than that seen in patients chronically infected by HBV (6).

Thus, HCV appears to be more immunogenic than HBV in chronically infected patients, but less responsive to immunological control. This is very surprising since the expression of HLA and intercellular adhesion molecules (42) and Fas antigen (43) are upregulated in the liver during HCV infection, which should facilitate antigen recognition, T cell–hepatocyte binding affinity, and hepatocellular apoptosis. Additionally, it is noteworthy that several related flaviviruses have been shown recently to upregulate MHC class I expression in infected cells (44), raising the possibility that HCV might do the same. Nonetheless, HCV is rarely eliminated despite an apparently vigorous immune response. These observations are compatible with the notion that it may not be possible to eradicate HCV infection simply by killing the infected cells, for the quantitative reasons discussed earlier in the context of HBV. If so, the common occurrence of persistent HCV infection may suggest that HCV is resistant to control by T cell–derived antiviral cytokines, unlike HBV. Alternatively, HCV might induce a T cell response that does not produce the particular antiviral cytokines to which HCV might be susceptible. It is also possible that the T cell response elicited by this relatively low titer virus is simply not vigorous enough to control it. Finally, since the HCV mutation rate is at least 10-fold higher than HBV, escape mutants may play a greater role in the primary establishment of HCV persistence than is likely for HBV. Importantly, CTL escape has been observed in a chronically HCV-infected chimpanzee (45), but the extent to which the mutation contributed to or was a consequence of persistent HCV infection in this case, however, remains to be determined.

Summary and conclusions

Thanks to the intense scrutiny of the immune response to HBV and HCV over the past several years, a general picture is

emerging that may explain the immunopathogenesis of these two infections. The T cell response to HBV is strong and broadly specific in acutely infected patients (1), and these T cells typically secrete type 1 antiviral cytokines such as IFNγ and TNFα upon antigen stimulation (46, 47). In contrast, the T cell response to HBV is weak and narrowly focused in chronically infected patients (1), except during exacerbations of liver disease or after viral clearance, and the cytokine profile of the intrahepatic HBV-specific T cells is variable (48, 49). Finally, the T cell response to HCV is relatively strong in patients with acute and chronic hepatitis who fail to clear the virus. Importantly, most of the peripheral blood and intrahepatic HCV-specific T cell clones that have been analyzed in these chronically infected patients produce primarily type 1 cytokines (35), and the overall intrahepatic cytokine profile in the livers of these patients is also primarily type 1 (50). These observations suggest that HCV may not be sensitive to control by type 1 cytokines. If this is correct, clearance of HCV would depend entirely on the cytopathic activity of the CTL response which, as we have discussed above, may simply be quantitatively insufficient to reach, recognize, and kill all of the HCV-infected hepatocytes in view of the fact that the infected hepatocytes can outnumber the HCV-specific CTL by 1,000-fold or more.

As illustrated in Fig. 1, the foregoing observations suggest a scenario in which viral clearance from organs like the liver that contain very large numbers of infected cells depends on the development of a vigorous CTL response, the destruction of some of the infected cells, the production of antiviral cytokines, and the susceptibility of the infecting virus to cytokine-mediated control. If the CTL response is strong and rapid, the number of infected cells is low, and the virus is susceptible to cytokine-mediated control, viral clearance should occur while only a fraction of the infected cells are actually killed, resulting in a self-limited inflammatory liver disease. This is compatible with the course of events during acute hepatitis B.

On the other hand, if the T cell response is quantitatively suboptimal, the virus will persist even if the appropriate antiviral cytokines are present since they will be produced in limited quantities that are likely to suppress viral gene expression without fully clearing the virus, thus causing it to be less visible to the immune system and leading to persistent infection. This may be the case in patients with chronic hepatitis B. Finally, even a strong CTL response may not be able to clear a massive viral infection unless the cytokine-mediated curative limb of the response illustrated in Fig. 1 is called into play since the cytopathic function of the immune response may simply not be able to destroy all of the infected cells, thus leading to persistent infection and chronic liver disease. This may occur either if the CTL fail to produce the appropriate antiviral cytokines, or if the virus is not susceptible to cytokine-mediated control, as may be true for HCV. If the foregoing hypothesis is correct, strategies designed to boost the CTL response (e.g., virus-specific immunotherapy) or to enhance or mimic the regulatory functions of the CTL response in the liver (e.g., intrahepatic cytokine induction therapy) could help to terminate chronic HBV and HCV infection.

The insight the foregoing studies have provided into the immunobiology and pathogenesis of HBV and HCV infections suggest new therapeutic approaches designed to focus the antiviral power of the CTL response at the site of viral replication in chronically infected patients so that the combined curative and destructive functions of the CTL response can eliminate these viruses from the liver. It would appear that these objectives are within reach for HBV. A clearer understanding of the immunopathogenesis of HCV is needed, however, before the antigen-specific immunotherapy of chronic HCV infection will be possible.

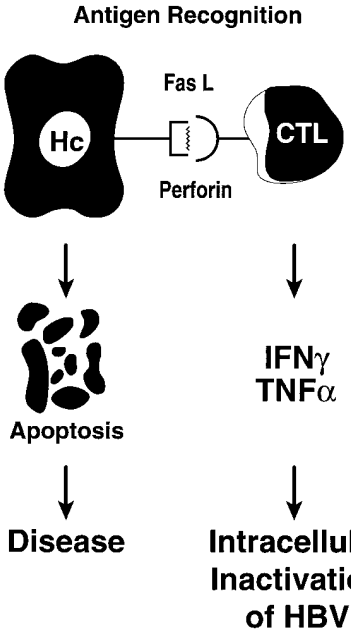

Figure 1. Noncytolytic clearance of HBV from the hepatocyte by CTL-derived cytokines. Upon antigen activation, CTL deliver an apoptotic signal to their target cells, killing them. They also secrete IFNγ and TNFα, cytokines that have been shown to abolish HBV gene expression and viral replication in vivo, curing them. The curative effect of the CTL response is several orders of magnitude more efficient than its destructive effect. The outcome of an infection may depend on the relative balance of these two effects with a predominantly curative response leading to viral clearance, and a predominantly destructive response leading to viral persistence and chronic liver disease. Importantly, if the curative process abolishes viral gene expression and replication but does not eliminate the viral cccDNA from the hepatocyte, it could paradoxically lead to viral persistence by rendering the virus immunologically invisible without removing its transcriptional template.

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