

IMMUNOPATHOGENESIS OF HEPATITIS B

Carlo FERRARI, Gabriele MISSALE, Carolina BONI, Simona URBANI *Divisione Malattie Infettive ed Epatologia, Azienda Ospedaliera di Parma, Italy*



[Print this page](#)

SUMMARY

The type of cell-mediated responses expressed at the early stages of HBV infection can influence the subsequent outcome of hepatitis B. Indeed, recovery is associated with efficient activation of mechanisms of the innate immunity, which seem to be responsible for the early inhibition of viral replication. A subsequent activation of HBV-specific T cells is probably crucial to complement the effect of the innate immunity and to allow complete control of virus replication. Moreover, control of infection cannot be achieved without rapid and efficient development of anti-envelope neutralizing antibody responses that are needed for elimination of free viral particles and inhibition of cell to cell spread of the virus.

In patients with chronic hepatitis B, HBV-specific T cell responses are weak or undetectable in the peripheral blood and T cells are attracted into the infected liver where they are diluted among virus non-specific T and non-T cells that are the predominant cell population of the intrahepatic infiltrate. The high viral and antigen load may be the main responsible for the T cell hyporesponsiveness typical of chronic patients through exhaustion of T cell responses and expansion of T cells able to produce Th2 cytokines.

HBV is a typical non-cytopathic virus that can induce tissue damage of variable severity by stimulating a protective immune response that can simultaneously cause damage and protection, by curing intracellular virus through the destruction of virus infected cells. Therefore, immune elimination of infected cells can lead to the termination of infection when it is efficient, or to a persistent necroinflammatory disease when it is not.

Destruction of infected cells, however, is not the only mechanism implicated in the elimination of intracellular virus, as demonstrated by studies carried out in animal models of HBV infection and in human hepatitis B showing the importance of cytokine-mediated, noncytolytic mechanisms of anti-viral protection.

The first experimental evidence in favor of such mechanisms derives from studies performed in the transgenic mouse model (1). These studies showed that single-stranded and relaxed circular double stranded HBV-DNA replicative intermediates can be eliminated from the cytoplasm of HBV transgenic hepatocytes as a result of the anti-viral effect of IFN- α and TNF- γ released within the transgenic liver primarily by infiltrating HBV-specific CD8 $^{+}$ cells (2-4) but also by CD4 $^{+}$ T cells (5).

1. ACUTE HBV INFECTION

a. What is the sequence of early immune events after HBV infection? Because induction of the virus-specific immune response usually requires a number of days, the initial protection against HBV is believed to be afforded by nonspecific mechanisms that can be called into play in a very short time, ranging from minutes to hours (6). Among these mechanisms, killing of virus infected cells without HLA restriction or apparent specificity for viral antigens and secretion of antiviral cytokines by NK cells and by NKT cells is believed to play an important role in host defense against HBV (7-9). NKT cells have an extremely limited TCR repertoire with predominant TCR α/β expression (10,11). They depend on non-classical, MHC class I-like CD1 molecules for their development and they recognize mainly glycolipids presented by CD1. Their proliferation is supported by IL2, and stimulation of their CD3 complex can lead to production of IFN α and IL4. Interestingly, these cells have been described at high frequency in unchallenged livers suggesting a possible role for NKT cells in the pathogenesis of hepatic infections (10,11). Indeed, activation of NKT cells can cause liver injury in the transgenic mouse model of HBV infection, but can also contribute

to the noncytolytic clearance of HBV by secretion of large quantities of IFN- α (7-9)

Complete eradication and control of viral infections, however, cannot be accomplished by the natural immunity. Therefore, effector mechanisms capable of accurate recognition of specific viral structures are needed to deal successfully with HBV. Activation of naive T cells, which is essential for the initiation of adaptive immunity, requires two distinct signals: the first one provided by the recognition of the viral peptide-HLA complex through the T cell receptor, the second one derived from the interaction of costimulatory molecules on the surface of the APC with surface receptors on the T cells (12,13). Consequently, the type of cell that presents viral peptides to naive T cells can determine whether T cells are activated and proliferate, as occurs when both signals are delivered by professional APC, or inactivated and energized if antigen is presented in the absence of costimulatory signals, as can occur if they encounter antigen for the first time in tissues, like the liver, where the costimulatory molecules are not expressed (12,13). In particular, dendritic cells play a central role in the initiation of the specific immune response; they capture antigen at the site of entry into the body and then differentiate into professional APC during their migration to lymph nodes (21,13). Thus, induction of the primary immune response is most efficient within lymph nodes where initial priming of HBV-specific T cell responses is believed to occur. Indeed, lymph nodes contain all the cellular elements needed for T cell priming organized structurally into a unit that brings professional antigen presenting cells in close contact with naive T cells (12s,13,14).

Lymph nodes represent the ideal environment also for initial priming of B cells that are generally dependent upon the helper function of CD4⁺ T cells for their optimal activation (15).

b. T cell-mediated immune response. Important information about the immune events occurring after HBV infection derives from the study of acutely infected chimpanzees (16) that can develop acute hepatitis and can mount immune responses to HBV proteins similar to those observed in humans. In this animal model, HBV DNA peaks in the liver and then starts to decline before biochemical and histological evidence of liver injury. Moreover, the detection of intrahepatic T cell mRNA is temporally associated with the evidence of liver disease, but CD8 mRNA is undetectable when HBV-DNA is disappearing from the liver. Thus, most viral DNA (at least 90% in the chimpanzee model) is cleared without the destruction of liver cells by noncytolytic mechanisms likely triggered by IFN- α and TNF- γ primarily produced by non-T cells. Indeed, macrophages, NK cells and NK-T cells represent the main cell populations in the infected liver before the onset of liver disease.

These conclusions are confirmed by immunological analysis of the early incubation phase of HBV infection in acutely infected humans (17). Also in human infection HBV replication is efficiently controlled before the onset of clinical symptoms when ALT levels are still normal. This confirms that HBV is not cytopathic and that virus control can be achieved without massive and biochemically detectable destruction of infected liver cells. Interestingly, HBV-specific CD8 T cells are detectable in the peripheral blood of acutely infected human subjects during the early incubation phase. Moreover, the peak frequency of circulating HBV-specific CTL in humans and intrahepatic CTL in chimpanzees coincides with clinical onset and ALT elevation.

Although immunological data are not available about early intrahepatic events in human infection, the scenario emerging from the available data is that IFN- α and IFN- β produced by infected hepatocytes are primarily responsible for the recruitment and the activation of cells of the innate immunity into the liver, e.g. macrophages, NKT cells and NK cells. Once activated, these cells can produce cytokines and monokines that upregulate HLA class I expression on hepatocytes that normally express HLA class I molecules only weakly. According to this interpretation, the quality and the magnitude of the innate immune response rather than HBV-specific CTL would start and influence the sequence of pathogenetic events operative at the early stages of infection. Therefore, initial viral control seems to be largely dependent upon non-cytolytic processes but the immune destruction of infected liver cells would represent a complementary mechanism needed to achieve a full and persistent control of the infection. These conclusions are also supported by the observation that duck and woodchuck hepatitis viruses can be efficiently cleared without massive hepatic necrosis even when all of the hepatocytes are infected (18), suggesting that noncytolytic clearance mechanisms may be operative in those models also. The observation that the maximal frequency of HBV-specific CD8 cells in natural HBV infection is detected in the circulation by tetramer staining concurrently with the peak of liver damage supports the view that this cell population plays an important role in the pathogenesis of liver damage (19).

A vigorous and Th1 oriented HLA class II restricted peripheral blood T cell response to HBcAg and HBeAg is detectable in virtually all patients during the symptomatic phase of acute self-limited hepatitis B and it appears to be directed against several epitopes, a few of which are strongly immunodominant and widely recognized by patients with different HLA backgrounds (19-23). The detection of a strong CD4-mediated response to nucleocapsid antigens during acute hepatitis B is temporally associated in most patients with the clearance of serum HBV envelope antigens (19,24). Release of cytokines by CD4 cells in response to HBV nucleocapsid antigens is likely required to supplement the antiviral activity of CTL-mediated events and to allow optimal activation of HBV-specific CTL to fully control the infection.

Surprisingly, HBV-specific helper and cytotoxic T cells are still present in an activated state several years after recovery from acute hepatitis (25,26). These responses seem to be maintained by continuous stimulation by minute amounts of persisting virus detectable only by PCR. Therefore, resolution of disease does not imply complete eradication of infection but more likely reflects the capacity of anti-viral CD4 and CD8 cells to keep HBV under tight and persistent control.

c. B cell function. Antibody production is critical for the neutralization of free HBV particles and for the interference with virus entry into the host cells. Therefore, protection by antibodies is most important before invasion of the host cells; afterwards, antibodies can contribute to limit cell to cell spread of viral particles but elimination of intracellular virus becomes the principal task of HLA class I restricted CTL and other effector cell types non-specifically recruited to the site of infection (24). Indeed, recognition of virally infected cells by antibodies and their killing through complement-mediated cytotoxicity or ADCC are less efficient than activation of HLA class I restricted CTL that require no more than 100 peptide/HLA complexes for activation. This high efficiency is in contrast with the millions of antibody molecules which are needed to destroy virally infected cells through a complement-mediated mechanism (27).

Anti-envelope antibodies are efficiently produced by patients who recover from acute infection and become positive by commercial assays (that detect only free antibodies) only after resolution of hepatitis and clearance of HBsAg. Actually, the antibody response starts much earlier after infection, but anti-envelope antibodies are not detectable because complexed with the excess of envelope antigens produced during virus replication (24).

While the neutralizing activity of anti-envelope antibodies is well documented, the pathogenetic role of the antibody response to the nucleocapsid antigens and to the HBV non-structural proteins still remains a debated issue. It is generally accepted that anti-HBc antibodies do not express virus-neutralizing activity, but protection of chimpanzees against HBV infection by passive immunization with anti-HBe antibodies has been observed, suggesting a possible but still undefined role for these antibodies in virus neutralization (28).

The antibody production by B cells is generally a T cell dependent phenomenon that requires the helper effect of CD4+ T cells through both cognate interaction and release of cytokines needed for differentiation of B lymphocytes into antibody producing cells (15). In contrast to the antibody production against HBV envelope antigens and HBeAg that is strictly T cell dependent, the antibody response to HBcAg has been suggested to be also T cell-independent based on the finding that antibody production can be induced by HBcAg in nude athymic mice (29). This property is explained by the structure of HBcAg (30,31). Clustering of individual HBcAg subunits gives rise to spikes distributed over the surface of the HBcAg shell that project out of it. The orientation of these spikes, on the top of which the dominant B cell epitopes are located (30), may be optimal for cross-linking the B cell membrane receptor and can explain the exceptional B cell activation capacity of the HBV core molecule. These features can also explain the clinical evidence that anti-HBc antibodies are produced by virtually all HBV infected patients, regardless of their clinical status, and that their level is particularly high during chronic HBV infection as a likely result of a continuous B cell stimulation by HBcAg (24).

HBcAg binding to B cell receptors by repeated epitopes carried by appropriately spaced protein spikes can induce co-stimulatory molecules on naïve HBcAg-specific B cells and can greatly enhance the naïve B cell antigen presenting function compared to non-B antigen presenting cells (32). At the molecular level, HBcAg can be bound by a linear motif expressed by some heavy and light chains of the B cell receptor (33), thereby providing a basis for HBcAg binding to a high fraction of naïve B cells.

2. CHRONIC HBV INFECTION

a. Cell-mediated and humoral responses. HBV-specific helper and cytotoxic T cells are generally undetectable in the blood of patients with chronic hepatitis, who are unable to control the virus and display high degree of liver damage and high levels of HBV replication (20,21,34-37). This low level of responses has been proposed to be due to deletion by exhaustion, to anergy, to cytokine imbalance (24), but also minor populations of HBV-specific CD8 cells has been described in this setting that are only “partially tolerant” being unable to bind specific tetramers, to produce IFN- γ , to lyse and to expand following stimulation with the specific peptide (38). Therefore, these cells would be able to escape peripheral deletion and to persist in the face of a high concentration of viral antigens, ignoring the infecting virus.

Suppression of T cell responses is more profound in highly viremic patients (39) and can be at least partially overcome in the reactivation phases of infection that has been reported to be associated with enhanced levels of peripheral blood CD4-mediated T cell reactivity to HBV nucleocapsid antigens (40,41). It has been suggested that these responses are triggered by the increasing concentrations of serum HBeAg and intracellular HBcAg (42). High antigen concentrations would be required for induction of the T cell response, because of the low avidity of HBcAg and HBeAg-specific T cells in chronic patients (43). Hepatitis flares can also be followed by a significant rise in IL-12 levels that can precede or occur simultaneously with HBeAg seroconversion (41). The observation that a significant increase in IL12 and Th1 cytokine production always follow the ALT flares and is detected only in patients who are able to control HBV replication may support the concept of a non-cytopathic control of virus replication also in chronic hepatitis B (41).

HBeAg and HBcAg-specific CD4 and CD8 cells have been reported to be cross-reactive (21,34-45) because HBcAg and HBeAg share most of their amino acid sequences, with the exception of 10 amino terminal residues, deriving from the intracellular processing of the pre-core gene product, that are present on serum HBeAg but not on particulate HBcAg. However, T cells able to recognize HBeAg but not HBcAg have been described (46). Since these cells can cause liver damage in vivo when transferred into HBeAg transgenic mice (43) and are cytolytic in vitro against endogenously synthesized HBeAg (46), it has been proposed that they can play a role in the selection of precore-negative mutants of HBV. Longitudinal studies in patients infected by mixed populations of wild type and e-minus variants of HBV indicate that reactivation of chronic liver disease is generally preceded by the reappearance of serum HBeAg resulting from enhanced replication of wild type HBV capable of producing HBeAg (47). When HBeAg concentration is high enough, activation of low avidity HBeAg-specific CD4 cells would occur, mediating liver injury and the preferential elimination of liver cells expressing endogenously synthesized HBeAg. Negative selection of HBeAg negative mutants will ensue, HBeAg will disappear from serum and the HBeAg reactive T cells will return to a quiescent condition. Although these interpretations are appealing, additional studies are needed to confirm this sequence of events.

In patients with chronic hepatitis, HBV-specific T cells are more easily detectable within the liver, where their frequency is however low because they are diluted among a large number of virus non-specific T cells, that may play an important role in the pathogenesis of liver cell damage (39,48-51). At this stage of infection most liver infiltrating T cells are Th0 and are able to secrete large amounts of Th2 cytokines (52) which may contribute to the T cell hyporesponsiveness typical of this condition.

A different situation is present in chronic asymptomatic HBV carriers without signs of liver damage. They have generally been considered to lack an active CTL response against HBV. However, recent studies carried out by tetramer staining and functional analysis of circulating and intrahepatic CTL show that functionally active HBV-specific CD8⁺ T cells are present in the circulation and the liver of these patients, despite the absence of liver damage (39). HBV-specific CTL present in the circulation of these patients express the phenotype of antigen-primed resting T cells, expand efficiently, display cytolytic activity and secrete anti-viral cytokines in vitro upon stimulation with viral antigen (39). Moreover, a high fraction of liver infiltrating CD8⁺ T cells are HBV-specific in these patients (39).

The behavior of the CTL response in chronic asymptomatic carriers of HBV supports the notion that control of HBV replication may be exerted by HBV-specific CTL without causing cell destruction. Indeed, the presence of functionally active CTL in the liver and circulation of these patients is associated with inhibition of virus replication in the absence of liver damage.

Neutralizing anti-envelope antibodies are generally undetectable in patients with chronic HBV infection and their defective production has been widely regarded as an important factor contributing to HBV persistence. However, studies with sensitive immunoassays (which can detect antibodies also in the presence of excess serum antigen) reveal that anti-envelope antibodies are produced in the majority of patients with chronic hepatitis B but they are complexed with surface antigens present at high concentrations in the serum of these patients (42, 53). Thus, the failure to detect anti-envelope antibodies in chronic patients by commercial immunoassays may simply reflect the enormous synthetic capacity of the liver to produce large amounts of envelope antigen that the immune system cannot neutralize, rather than defective antibody production per se (24).

b. Mechanisms of chronic viral persistence. For a noncytopathic virus like HBV to persist it must either overwhelm or not induce an effective antiviral immune response or it must be able to evade it (54). Data from transgenic mice indicate that neonatal tolerance to HBeAg is a crucial mechanism responsible for the lack of an antiviral immune response following mother to infant transmission (55). In this context, a cytokine imbalance oriented towards preferential Th2 responses, as a result of the capacity of Th2 cells to evade tolerance more efficiently than Th1 cells, has been suggested to favor chronic evolution of HBV infected neonates (56).

The immunological basis for viral persistence during adult onset infection is not well understood. It is well known that the T cell response is much less vigorous in chronically infected patients than it is during acute infection (24). However, this T cell hyporesponsiveness is more likely to be the consequence of the active viral replication in persisting chronic infections rather than the primary cause of viral persistence. Peripheral tolerance or exhaustion of the T cell response by the high viral load that characterizes most persistently infected patients can be its cause (24). In support of this possibility, recovery of HBV-specific CD4 and CD8-mediated responses has been observed in viremic HBeAg⁺ patients following the decline of viremia caused by lamivudine treatment (57,58).

Several observations indicate also a role for soluble HBeAg in the down-regulation of HLA class II restricted T cell responses. Immunization of mice with HBeAg can favor the production of Th2 cytokines (59) which in turn can cause suppression of Th1 production. In the transgenic mouse model, circulating HBeAg can also delete Th1 cells by Fas-FasL-mediated mechanisms, leading to predominance of Th2 cells (60). Given the role of Th1 cytokines in the non-cytolytic control of virus replication, a T cell response skewed towards a prevalent Th2 profile may contribute to viral persistence. These findings raise the question of whether and how a Th1/Th2 imbalance actually occurs at the early stages of HBV infection when the most relevant pathogenetic events determining the outcome of HBV infection are believed to take place.

Although definitive demonstrations are still lacking, several candidate mechanisms have been proposed to contribute to HBV persistence. First, there is some evidence that privileged sites may play a role since HBV can infect extrahepatic tissues (24). Moreover, in HBV transgenic mice that express the virus in the liver and the kidney, circulating HBV specific CTL can cause hepatitis, but not nephritis, due to the limited access of the CTL to antigen positive cells present on the other side of microvascular barriers that exist in the kidney but are not present in the liver sinusoid (61).

Second, hepatocytes can be induced to express Fas ligand during an inflammatory response (62) and HBV itself might be able to induce Fas ligand expression by the hepatocyte. Since 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 use to kill their target cells (63), individuals with the highest hepatocyte expression of Fas ligand could delete their HBV-specific CTL more efficiently and, therefore, could have a higher probability to become chronically infected. All of these theories are testable, but they are strictly speculative at present.

Third, virus-specific CTL that might otherwise become activated by antigen recognition in the immunostimulatory context of secondary lymphoid organs might be inactivated if antigen is presented in the absence of costimulatory signals in the liver. In this context, presentation of soluble antigens by liver endothelial cells has been shown to induce specific T cell tolerance (64).

Forth, HBV envelope antigens represent an exception to the rule that CTL activation is selectively induced by recognition of endogenously synthesized antigen, because also exogenous forms of HBV envelope proteins can gain access to the class I pathway of antigen processing and presentation (65-67). CTL activation by this mechanism has

been suggested to cause selective killing of HBV envelope-specific B cells acting as antigen presenting cells. Suppression of the anti-envelope antibody response might ensue, facilitating chronic evolution of HBV infection (65).

Additionally, viral mutations that abrogate or antagonize antigen recognition by virus specific T cells have been reported in the context of strong and narrowly focused CTL responses in patients with chronic HBV infection (68,69). In view of the multispecificity of the CTL response in the acute phase of HBV infection, current data favor the notion that selection of CTL escape mutants is most likely to occur after a persistent infection is already established. In this setting, viral mutations could solidify the chronicity of the infection and perhaps even make it irreversible. Whether such mutations can also serve as the primary cause of viral persistence in the context of a multispecific T cell response remains to be proven.

MAIN CONCEPTS IN HBV IMMUNOPATHOGENESIS (summary)

1. Humoral immune response to HBV

a. Accepted concepts. The antibody response to HBV envelope antigens is a T cell dependent process that plays a critical role in viral clearance by complexing with free viral particles, preventing the infection of susceptible cells.

b. Unsolved issues. The role of antibodies to the capsid and nonstructural proteins in HBV immunobiology is less clear, although passively administered anti-HBe antibodies have been reported to protect chimpanzees against HBV infection

2. Cell-mediated immune response to HBV

a. Accepted concepts.

- ◆ Elimination of intracellular HBV is caused not only by the cytolytic activity of HBV-specific CTL but also by the suppression of viral gene expression and replication by soluble factors, including IFN α and TNF γ , released by liver infiltrating T and non-T cells. This is supported by the observation that in both human infection and animal models, control of viral replication and elimination of most HBV-DNA molecules occur before the onset of liver damage and clinical symptoms.
- ◆ In acute HBV infection, peripheral blood CD4- and CD8-mediated responses to HBV antigens are easily detectable in vitro and are much stronger than in viremic patients with chronic hepatitis B; while the HBV nucleoprotein is the most powerful immunogen for CD4 cells, both structural and non-structural HBV antigens can be efficient stimulators for CD8 cells.
- ◆ In patients with chronic HBV infection and active viral replication, peripheral blood CD4 and CD8 cells are hyporesponsive or totally non-responsive to HBV antigen stimulation in vitro as a likely result of their functional depression in vivo. In these patients HBV-specific CTL are more easily detectable within the liver, where their frequency is low because they are diluted among a large number of virus non-specific T cells, that are believed to play an important role in the pathogenesis of liver cell damage.
- ◆ Soluble HBeAg has been shown to play an important role in the pathogenesis of neonatal T cell tolerance to HBV.
- ◆ Chronic asymptomatic carriers of HBV show efficient CTL responses to HBV antigens detectable in the circulation and liver. These findings challenge the previous view that the absence of signs of liver damage was due to the lack of active CTL responses against HBV.
- ◆ HBV-specific CD4- and CD8-mediated responses are detectable even decades after clinical resolution of acute hepatitis B and are sustained by residual minute amounts of virus detectable by PCR in the serum of subjects recovered from acute hepatitis; this implies that resolution of acute hepatitis does not mean eradication of infection but rather control of residual virus by an efficient and persistent immune response.

b. Unsolved issues.

- ◆ The role of the innate immunity in the evolution of HBV infection must be further clarified.
- ◆ The cause of the T cell hyporesponsiveness in chronic hepatitis B is still largely undefined. A role for the high viral load has been suggested by studies of the cell-mediated immune response in patients treated with

lamivudine; also soluble HBeAg has been suggested to contribute to the T cell hyporesponsive state.

- ◆ Information about the features of the immune response in different subgroups of patients with chronic HBV infection and different behavior of viremia and disease activity (i.e. HBeAg+ vs anti-HBe+; persistently elevated vs fluctuating ALT) are still incomplete. Peaks of disease activity that can precede improvement of liver inflammation have been reported to be associated with recovery of T cell responsiveness to HBV nucleocapsid antigens. Since this temporal association is never stringent, the contribution of these phenomena to liver cell damage and virus control must be further investigated by longitudinal studies carried out with more sensitive technologies.

3. General unsolved issues

- ◆ The primary causes of viral persistence are still unknown. Candidate mechanisms include infection of immunologically privileged sites, deletion of HBV-specific CTL by FAS ligand expressing hepatocytes, anergy induced by the effect of the liver environment, suppression of the anti-envelope antibody response by killing of envelope specific B cells, downregulation of viral gene expression or emergence of viral mutations that abrogate, anergize or antagonize antigen recognition by virus specific T cells.

REFERENCES

1. Guidotti GG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu. Rev. Immunol.* 2001;19:65-91.
2. Guidotti LG, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity.* 1996;4:25-36.
3. Guidotti LG, Borrow P, Hobbs MV, Matzke B, Gresser I, Oldstone MB, Chisari FV. Viral cross talk: intracellular inactivation of the hepatitis B virus during an unrelated viral infection of the liver. *Proc. Natl. Acad. Sci. U S A.* 1996;93:4589-94.
4. Heise T, Guidotti LG, Cavanaugh VJ, Chisari FV. Hepatitis B virus RNA-binding proteins associated with cytokine-induced clearance of viral RNA from the liver of transgenic mice. *J Virol.* 1999;73:474-81.
5. Franco A, Guidotti LG, Hobbs MV, Paschetto V, Chisari FV. Pathogenetic effector function of CD4-positive T helper 1 cells in hepatitis B virus transgenic mice. *J Immunol.* 1997;159:2001-8.
6. Marrack P, Kappler J. Subversion of the immune system by pathogens. *Cell* 1994; 76: 323-332.
7. Kakimi K, Guidotti LG, Koezuka Y, Chisari FV. Natural killer T cell activation inhibits hepatitis B virus replication in vivo. *J.Exp.Med.* 2000; 192: 921-930.
8. Kakimi K, Lane TE, Chisari FV, Guidotti LG. Inhibition of hepatitis BB virus replication by activation of NKT cells does not require inflammatory cell recruitment to the liver. *J Immunol.* 2001; 167: 6701-6705.
9. Baron JL, Gardiner L, Nishimura S, Shinkai K, Locksley R, Ganem D. Activation of a nonclassical NKT cell subset in a transgenic mouse model of hepatitis B virus infection. *Immunity* 2002; 16: 583-594.
10. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense. *Annu.Rev.Immunol.*, 1999; 17: 189-220.
11. Bendelac A, Bonneville M, Kearney JF. Autoreactivity by design: innate B and T lymphocytes. *Nature Reviews.* 2001; 1:177-186.
12. von Adrian UH, Mackay CR. T cell function and migration. Two sides of the same coin. *N.Engl.J.Med.* 2000;343:1020-1034.
13. Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: intermediates, effectors and memory cells. *Science* 2000; 290:92-97.
14. Doherty PC, Allan W, Eichelberger M, Carding SR. Roles of T cell subsets in viral immunity. *Annu.Rev.Immunol.* 1992; 10: 123-151.
15. Parker DC. T cell-dependent B cell activation. *Annu.Rev.Immunol.* 1993; 11: 331-360.

16. Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. *Science*, 1999; 284: 825-829.
17. Webster GJ, Reignat S, Maini MK, Whalley SA, Ogg GS, King A, Brown D, Amlot PL, Williams R, Vergani D, Dusheiko GM, Bertoletti A. Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. *Hepatology* 2000; 32: 1117-1124.
18. Jilbert AR, Wu TT, England JM, Hall PM, Carp Nz, O'Connell AP, Mason WS. Rapid resolution of duck hepatitis B virus infections occurs after massive hepatocellular involvement. *J.Virol.* 1992; 66: 1377-1388.
19. Maini MK, Boni C, Ogg GS, King AS, Reignat S, Lee CK, Larrubia JR, Webster GJ, McMichael AJ, Ferrari C, Williams R, Vergani D, Bertoletti A. Direct ex vivo analysis of hepatitis B virus-specific CD8+ T cells associated with the control of infection. *Gastroenterology*, 1999; 117: 1-13.
20. Ferrari C, Penna A, Bertoletti A, Valli A, Degli Antoni A, Giuberti T, Cavalli A, Petit M-A, Fiaccadori F. Cellular immune response to hepatitis B virus-encoded antigens in acute and chronic hepatitis B virus infection. *J.Immunol.* 1990; 145: 3442-3449.
21. Jung MC, Spengler U, Schraut W, Hoffmann R, Zachoval R, Eisenburg J, Eichenlaub D, Riethmuller G, Paumgartner G, Ziegler-Heitbrock HW. Hepatitis B virus antigen-specific T-cell activation in patients with acute and chronic hepatitis B. *J.Hepatol.* 1991; 13: 310-317.
22. Ferrari C, Bertoletti A, Penna A, Cavalli A, Valli A, Missale G, Pilli M, Giuberti T, Fowler P, Chisari FV, Fiaccadori F. Identification of immunodominant T cell epitopes of the hepatitis B virus nucleocapsid antigen. *J Clin Invest*, 1991;88:214-222.
23. Penna A, Del Prete G, Cavalli A, Bertoletti A, D'Elios MM, Sorrentino R, D'Amato M, Boni C, Pilli M, Fiaccadori F, Ferrari C. Predominant T helper 1 cytokine profile of hepatitis B virus nucleocapsid-specific T cells in acute self-limited hepatitis B. *Hepatology*. 1997; 25:1022-1027.
24. Chisari, F. V. and C. Ferrari. Hepatitis B virus immunopathogenesis. *Ann. Rev. Immunol.*, 1995; 13:29-60.
25. Penna A, Artini M, Cavalli A, Levrero M, Bertoletti A, Pilli M, Chisari FV, Rehmann B, Del Prete G, Fiaccadori F, Ferrari C. Long-lasting memory T cell responses following self-limited acute hepatitis B. *J. Clin. Invest.* 1996;98:1185-94.
26. Rehmann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nature Med.* 1996;2:1104-1108.
27. Oldstone MD. Molecular anatomy of viral persistence. *J.Virol.* 1991; 65: 6381-6386.
28. Stephan W, Prince AM, Brotman B. Modulation of hepatitis B infection by intravenous application of an immunoglobulin preparation that contains antibodies to hepatitis B e and core antigens but not to hepatitis B surface antigen. *J.Virol.* 1984; 51: 420-424.
29. Milich DR, McLachlan A. The nucleocapsid of hepatitis B virus is both a T-cell-independent and a T-cell-dependent antigen. *Science* 1986; 234: 1398-1401.
30. Botcher B, Wynne SA, Crowther RA. *Nature* 1997; 386:88-91.
31. Conway JF, Cheng N, Zlotnich A, Wingfield PT, Stahl SJ, Steven A. *Nature* 1997; 386:91-94.
32. Milich DR, Chen M, Schodel F, Peterson DL, Jones JE, Hughes JL. Role of B cells in antigen presentation of the hepatitis B core. *Proc.Natl.Acad.Sci.USA.* 1997;94:14648-14653.
33. Lazdina U, Cao T, Steinbergs J, Alheim M, Pumbens P, Peterson DL, Milich DR, Leroux-Roel G, Sallberg M. Molecular basis for the interaction of the hepatitis B virus core antigen with the surface immunoglobulin receptor of naïve B cells. *J.Virol.* 2001; 75:6367-6374.
34. Penna A, Chisari FV, Bertoletti A, Giuberti T, Fowler P, Missale G, Fiaccadori F, Ferrari C. Cytotoxic T lymphocytes recognize an HLA A2 restricted epitope within the hepatitis B virus nucleocapsid antigen. *J Exp Med*, 1991; 174: 1565-1570.
35. Missale G, Redeker A, Person J, Fowler P, Guilhot S, Schlicht HJ, Ferrari C, Chisari FV. HLA-A31 and HLA-Aw68 restricted cytotoxic T cell responses to a single hepatitis B virus nucleocapsid epitope during acute viral hepatitis. *J Exp Med*, 1993; 177(3):751-62
36. Nayersina R, Fowler P, Guilhot S, Missale G, Cerny A, Schlicht HJ, Vitiello A, Chesnut R, Person JL, Redeker AG. HLA-A2-restricted

- cytotoxic T lymphocyte responses to multiple hepatitis B surface antigen epitopes during hepatitis B virus infection. *J Immunol*, 1993;150:4659-4671.
37. Rehermann B, Fowler P, Sidney J, Person J, Redeker A, Brown M, Moss B, Sette A, Chisari FV. Department of Molecular and Experimental Medicine, Scripps Research Institut. The cytotoxic T lymphocyte response to multiple hepatitis B polymerase epitopes during and after acute viral infection. *J Exp Med*, 1995;181:1047-1058
38. Reignat S, Webster GJM, Brown D, Ogg GS, King A, Seneviratne SL, Dusheiko G, Williams R, Maini MK, Bertoletti A. Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection. *J. Exp. Med.* 2002; 195: 1089-1101.
39. Maini MK, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, King AS, Herberg J, Gilson R, Alisa A, Williams R, Vergani D, Naoumov NV, Ferrari C, Bertoletti A. The role of virus-specific CD8+ cells in liver damage and viral control during persistent hepatitis B virus infection. *J.Exp.Med.*, 2000; 191: 1269-1280.
40. Tsai S L, Chen P J, Lai MY, Yang P M, Sung J L, Huang J H, Hwang L H, Chang T H, Chen D S. Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for hepatitis B e antigen seroconversion. *J. Clin. Invest.*, 1992; 89:87-96.
41. Rossol S, Marinos G, Carucci P, Singer MV, Williams R, Naoumov NV. Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. *J.Clin. Invest.* 1997; 99: 3025-3033.
42. Maruyama T, McLachlan A, Iino S, Koike K, Kurokawa K, Milich DR. The serology of chronic hepatitis B infection revisited. *J.Clin.Invest.* 1993; 91: 2586-2595.
43. Sallberg MC, Thung SN, Hughes J, Jones J, Milich DR. Nondeletional T-cell receptor transgenic mice: model for the CD4+ T-cell repertoire in chronic hepatitis B virus infection. *J. Virol.* 2000; 74: 7587-7599.
44. Bertoletti A, Ferrari C, Penna A, Fiaccadori F, Margolskee R, Schlicht HJ, Fowler P, Guilhot S, Chisari FV. HLA class I restricted cytotoxic T cells in acute viral hepatitis type B. *Proc. Natl. Acad. Sci. USA* 1991;88: 10445.
45. Jung MC, Diepolder HM, Spengler U, Wierenga EA, Zachoval R, Hoffmann RM, Eichenlaub D, Frosner G, Will H, Pape GR. Activation of a heterogeneous hepatitis B core and e antigen-specific CD4+ T cell-population during seroconversion to anti-HBe and anti-HBs in hepatitis B virus infection. *J.Virol.* 1995; 69: 3358-3368.
46. Diepolder HM, Ries G, Jung MC, Schlicht HJ, Gerlach JT, Gruner N, Caselmann WH, Pape GR. Differential antigen-processing pathways of the hepatitis B virus e and core proteins. *Gastroenterology.* 1999; 116:650-657.
47. Brunetto MR, Monti Gorin J, Citico G, Oliveri F., Colombatto P, Capalbo M, Saracco G, Barbera C, verme G and Bonino F. Pre-core/core gene mutants of hepatitis B virus:pathogenetic implications. In: Rizzetto M, Purcel R, Gerin J, Verme G, editors. *Viral Hepatitis and Liver Diseases.* Turin: Edizioni Minerva Medica. 1997. p. 127-137
48. Ferrari C, Mondelli MU, Penna A, Fiaccadori F, Chisari FV. Functional characterization of cloned intrahepatic, hepatitis B virus nucleoprotein-specific helper T cell lines. *J.Immunol.* 1987; 139: 539-544.
49. Ferrari C, Penna A, Giuberti T, Tong MJ, Ribera E, Fiaccadori F, Chisari FV. Intrahepatic, nucleocapsid antigen-specific T cells in chronic active hepatitis B. *J.Immunol.* 1987; 139: 2050-2058.
50. Barnaba V, Franco A, Alberti A, Balsano C, Benvenuto R, Balsano F. Recognition of hepatitis B envelope proteins by liver-infiltrating T lymphocytes in chronic HBV infection. *J.Immunol.* 1989; 143: 2650-2655.
51. Barnaba V, Franco A, Paroli M, Benvenuto R, De Petrillo G, Burgio VL, Santilio I, Balsano C, Bonavita MS, Cappelli G. Selective expansion of cytotoxic T lymphocytes with a CD4+CD56+ surface phenotype and a T helper type 1 profile of cytokine secretion in the liver of patients chronically infected with Hepatitis B virus. *J Immunol.* 1994; 152:3074-87.
52. Bertoletti A, D'Elios MM, Boni C, De Carli M, Zignego AL, Durazzo M, Missale G, Penna A, Fiaccadori F, Del Prete G, Ferrari C. Different cytokine profiles of intrahepatic T cells in chronic hepatitis B and hepatitis C virus infections. *Gastroenterology.* 1997;112: 193-199.
53. Maruyama T, Iino S, Koike K, Yasuda K, Milich DR. Serology of acute exacerbation in chronic hepatitis B. *Gastroenterology* 1993; 105: 1141-1151.
54. Ferrari C, Chisari FV. Immune mechanisms of viral clearance and disease pathogenesis during viral hepatitis. In: Arias IM, Boyer JL,

- Chisari FV, Fausto N, Schachter D, Shafritz DA, editors. *The liver. Biology and pathobiology*. Philadelphia: Lippincott Williams & Wilkins. 2001. p. 763-782.
55. Milich DR, Jones JE, Hughes JL, Price J, Raney AK, McLachlan A. Is a function of the secreted hepatitis B e antigen to induce immunologic tolerance in utero? *Proc. Natl. Acad. Sci. USA* 1990; 87:6599-6604.
56. Milich DR, Schodel F, Peterson DL, Jones JE, Hughes JL. Characterization of self-reactive T cells that evade tolerance in hepatitis B e antigen transgenic mice. *Eur. J. Immunol.* 1995; 25: 1663-1672.
57. Boni C, Bertoletti A, Penna A, Cavalli A, Pilli M, Urbani S, Scognamiglio P, Boehme R, Panebianco R, Fiaccadori F, Ferrari C. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. *J. Clin. Invest.* 1998;102:968-75.
58. Boni C, Penna A, Ogg GS, Bertoletti A, Pilli M, Cavalo C, Cavalli A, Urbani S, Boehme R, Panebianco R, Fiaccadori F, Ferrari C. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. *Hepatology* 2001;33: 963-971.
59. Milich DR, Schodel F, Hughes JL, Jones JE, Peterson D. The hepatitis B virus core and e antigens elicit different Th cell subsets: antigen structure can affect Th cell phenotype. *J. Virol.* 1997; 71: 2192-2201.
60. Milich DR, Chen MK, Hughes JL, Jones JE. The secreted hepatitis B precore antigen can modulate the immune response to the nucleocapsid: a mechanism for persistence. *J. Immunol.* 1998; 160: 2013-2021.
61. Ando K, Guidotti LG, Cerny A, Ishikawa T, Chisari FV. CTL access to tissue antigen is restricted in vivo. *J Immunol.* 1994 Jul 15;153 (2):482-8.
62. Galle PR, Hofmann WJ, Walczak H, Schaller H, Otto G, Stremmel W, Krammer PH, Runkel L. Involvement of the CD95 (APO-1/Fas) receptor and ligand in liver damage. *J. Exp. Med.*, 1995;182: 1223-1230
63. Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA. Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science*, 1995;270: 1189-1192.
64. Limmer A, Ohl J, Kurts C, Ljunggren H-G, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle P. Efficient presentation of exogenous antigen by liver endothelial cells to CD8⁺ T cells results in antigen-specific T cell tolerance. *Nat. Med.* 2000; 6: 1348-1354.
65. Barnaba V, Franco A, Alberti A, Benvenuto R, Balsano F. Selective killing of hepatitis B envelope antigen-specific B cells by class I-restricted, exogenous antigen-specific T lymphocytes. *Nature* 1990; 315: 258-260.
66. Schrimbeck R, Melber K, Kuhrober A, Janowicz ZA, Reimann J. Immunization with soluble hepatitis B virus surface protein elicits murine H-2 class I-restricted CD8⁺ cytotoxic T lymphocyte responses in vivo. *J. Immunol.* 1994; 152: 1110-1119.
67. Schrimbeck R, Melber K, Mertens T, Reimann J. Antibody and cytotoxic T-cell responses to soluble hepatitis B virus (HBV) S antigen in mice: implication for the pathogenesis of HBV-induced hepatitis. *J. Virol.* 1994; 68: 1418-1425.
68. Bertoletti A, Costanzo A, Chisari FV, Levrero M, Artini M, Sette A, Penna A, Giuberti T, Fiaccadori F, Ferrari C. Cytotoxic T lymphocyte response to a wild type hepatitis B virus epitope in patients chronically infected by variant viruses carrying substitutions within the epitope. *J Exp Med*, 1994;180: 933-943.
69. Bertoletti A, Sette, Chisari FV, Penna A, Levrero M, De Carli M, Fiaccadori F, Ferrari C. Natural variants of cytotoxic epitopes are T cell receptor antagonists for anti-viral cytotoxic T cells. *Nature*, 1994;369: 407-410.