cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

Natural Killer Cells in Viral Hepatitis

Barbara Rehermann

Immunology Section, Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland

SUMMARY

This review focuses on the role of natural killer (NK) cells in acute and chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections and on cytokine-mediated mechanisms that contribute to alterations in NK cell phenotype and function.

Natural killer (NK) cells are traditionally regarded as first-line effectors of the innate immune response, but they also have a distinct role in chronic infection. Here, we review the role of NK cells against hepatitis C virus (HCV) and hepatitis B virus (HBV), two agents that cause acute and chronic hepatitis in humans. Interest in NK cells was initially sparked by genetic studies that demonstrated an association between NK cell–related genes and the outcome of HCV infection. Viral hepatitis also provides a model to study the NK cell response to both endogenous and exogenous type I interferon (IFN). Levels of IFN-stimulated genes increase in both acute and chronic HCV infection and pegylated IFNα has been the mainstay of HCV and HBV treatment for decades. In chronic viral hepatitis, NK cells display decreased production of antiviral cytokines. This phenotype is found in both HCV and HBV infection but is induced by different mechanisms. Potent antivirals now provide the opportunity to study the reversibility of the suppressed cytokine production of NK cells in comparison with the antigen-induced defect in IFNγ and tumor necrosis factor-α production of virus-specific T cells. This has implications for immune reconstitution in other conditions of chronic inflammation and immune exhaustion, such as human immunodeficiency virus infection and cancer. *(Cell Mol Gastroenterol Hepatol 2015;1:578–588; http://dx.doi.org/10.1016/j.jcmgh.2015.09.004)*

Keywords: HBV; HCV; Infection; Interferon; T Cell.

Natural killer (NK) cells were identified in 1975 as large granular lymphocytes with an ability to kill without priming target cells that do not express major histocompatibility complexes (MHCs).[1,2] With the recent discovery of innate helper-like cells, NK cells are now considered part of the family of innate lymphoid cells (ILCs), a classification of innate immune cells that mirrors that of CD8 and CD4 T cells in the adaptive immune system. NK cells represent the cytotoxic arm of the ILC family and share expression of the transcription factor eomesodermin with cytotoxic CD8 T cells. Three groups of eomesodermin-negative helper-like ILCs are the innate counterparts to adaptive CD4 T cells. ILC1 express the transcription factor Tbet, have a T_H1-like cytokine profile, and provides immunity against intracellular bacteria and parasites. ILC2s express the transcription factor GATA-3. Their cytokine profile of interleukin-4 (IL-4), IL-5, IL-9, and IL-13, and play a role in allergies and antihelminth responses that resembles that of T_H2 cells. ILC3 express RORγt and is the counterpart of T_H3 cells.[3–5] An additional classification differentiates between conventional NK cells and tissue-resident NK cells. Liver-resident NK cells use Tbet rather than eomesodermin and are only weakly cytotoxic. As strong tumor necrosis factor-α (TNFα) producers, they are closer to ILC1 than to conventional NK cells.[6,7] They were initially described in mice, but an equivalent population of liver-resident NK cells has recently also been reported in humans.[8]

In viral infections, NK cells exert rapid innate responses by exerting cytotoxicity against infected target cells and by releasing antiviral cytokines. By killing immature dendritic cells and secreting proinflammatory cytokines and chemokines, NK cells support the priming of T cells and orchestrate the recruitment of other immune cells to the site of infection.[9] These mechanisms enhance the adaptive arm of the immune response, which ultimately clears the infection and provides immune memory and protection upon reinfection. This classic division of labor between the innate and adaptive immune systems has recently been blurred, with some NK cells exhibiting features of adaptive cells, such as antigen-specific clonal expansion and contraction and development of long-lived memory. This feature was initially described for a subpopulation of liver-resident NK cells in mice that confers transferrable immunity against chemical

Abbreviations used in this paper: **CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; ILC, innate lymphoid cells; *ISG*, interferon-stimulated gene; KIR, killer-cell immunoglobulin-like receptor; LCMV, lymphocytic choriomeningitis virus; MHC, major histocompatibility complex; NCR, natural cytotoxicity receptor; NK, natural killer; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor β; TLR, Toll-like receptor; TNFα, tumor necrosis factor-α; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TRAIL-R2, TRAIL death receptor 2.**

Most current article

2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2015.09.004

haptens and viruses.[10,11] Further, mouse cytomegalovirus (CMV) is now known to induce memory NK cells via interaction of the viral glycoprotein with an NK cell receptor,[12] and human CMV has been shown to induce oligoclonal expansions and epigenetic modifications of human NK cells with memory-like functions.[13–15] A detailed review of the mechanisms of NK cell memory has been published elsewhere.[16]

NK cell responses are controlled by a large number of activating and inhibitory cell surface receptors. Activating receptors include natural cytotoxicity receptors (NKp30, NKp44, and NKp46), lectin-like receptors (NKG2C, NKG2D) that are expressed as dimers with CD94, and signaling lymphocyte activation molecule (SLAM) family receptors (2B4, CRACC, NTB-A), among others.[17] Inhibitory receptors include killer-cell immunoglobulin-like receptors (KIRs) and NKG2A/CD94. Receptors are expressed in a combinatorial manner, which creates an estimated 6000 to 30,000 phenotypically distinct NK cell subpopulations in the blood of each individual and an even larger diversity among individuals.[18]

In the absence of infection, inflammation, and other diseases, NK cells mostly receive inhibitory signals. Expression of inhibitory receptors is genetically determined.[18] It is thought that KIRs have coevolved with their ligands, the human leukocyte antigens (HLA), to ensure maintenance of self-tolerance. NK cells become activated when signals from inhibitory receptors decrease, such as when the expression of KIR-binding MHC molecules on virus-infected cells decreases, or when signals from activating receptors increase, such as when antibody-coated viral antigens and/or stress-induced ligands on infected cells are recognized. NK cells also respond to inflammatory cytokines such as type I interferons (IFNα and IFNβ), IL-2, IL-12, IL-15 and IL-18 that are commonly released in response to virus infections.[19] NK cell activation increases the expression level of activating receptors,[18] thereby allowing NK cells to acquire a more responsive state in the context of infection and inflammation.

This review discusses evidence for an antiviral role of NK cells in hepatitis C virus (HCV) infection, and then describes alterations of NK cell function in chronic HCV and hepatitis B virus (HBV) infection that are consistent with an increased regulatory role at the expense of antiviral function. The reversibility of this phenotype is discussed.

What Is the Role of Natural Killer Cells in Subclinical and Acute Hepatitis C Virus Infection?

Hepatitis C virus (HCV) is an RNA virus of the Flaviviridae family that establishes persistent infection in the majority of infected patients. A potential role for NK cells in viral hepatitis was first suggested by genetic studies that described a higher odds ratio of spontaneous HCV clearance[20] and IFN-treatment-induced HCV clearance[21] in *KIR2DL3*$^+$ patients who are homozygous for *HLA-C1* alleles as compared with patients who are homozygous or heterozygous for *HLA-C2* alleles. *HLA-C1* and *HLA-C2* represent two groups of *HLA-C* alleles that differ in two amino acids in their respective HLA-Cw α1 domains. Because the interaction between KIRs on NK cells with HLA molecules on target cells plays a key role in NK cell inhibition, it has been suggested that the *KIR2DL3/HLA-C1* compound genotype results in a lower activation threshold of NK cells, thereby allowing faster NK cell activation compared with less favorable genotypes. This is supported by data in an in vitro influenza A virus infection model that demonstrate a larger HLA-C–regulated NK cell subset with more rapid NK cell IFN-γ secretion and cytotoxicity in *HLA-C1* than in *HLA-C2* homozygous patients.[22]

An increased prevalence of *KIR2DL3/HLA-C1* homozygosity is also observed in injection drug users who remain aviremic and antibody-negative despite high-risk behavior and frequent HCV exposure.[21] The apparent immune protection in such individuals is associated with KIR2DL3 expression on NK cells[23] and with an increased frequency of activated NK cells.[24,25] At the functional level, NK cells in the blood of exposed uninfected individuals display increased ex vivo IFNγ production[24] and increased in vitro cytotoxicity.[25] These results from cross-sectional cohorts are consistent with data from a prospective study of health care workers observed after an accidental needlestick.[26] Accidental exposure to minute amounts of HCV-containing blood resulted in a transient increase the frequency of activated NK cells in the blood and their effector functions (both cytotoxicity and IFNγ production). The magnitude of the NK cell response correlated with that of the subsequent HCV-specific T-cell response. This likely represents an early innate response to an abortive or rapidly contained and cleared infection, because neither viremia nor HCV-specific antibodies are detected.[26]

Collectively, these studies demonstrate that NK cells are sensitive biomarkers of subclinical HCV exposure. While it is possible that NK cells—along with other components of the innate immune system—contribute to viral containment in this setting, it is obvious that innate immune responses on their own cannot clear the infection once high-level HCV viremia is established. Data from prospectively studied humans and experimentally infected chimpanzees demonstrate that high-level HCV viremia persists for weeks despite induction of a large set of intrahepatic interferon-stimulated genes (*ISGs*).[27,28] This immune response is initiated in the cytoplasm and in endosomes of infected cells by the pattern recognition receptors protein kinase, retinoic acid inducible gene-I, and toll-like receptor 3 (TLR3).[29] Downstream signals, mediated by interferon regulatory factor 3 (IRF3) and nuclear factor-κB, result in the transcription of the IFNβ gene. IFNβ is released from infected cells, binds to the IFNα/β receptor (*IFNAR1* and *IFNAR2*) on neighboring cells, and induces a diverse *ISG* set that includes many antiviral and proinflammatory genes.[30] However, owing to HCV's elaborate strategies to escape from IFN responses,[29,31] there is no decrease in viremia, just a plateau. Patients are typically clinically asymptomatic during this period and do not seek medical attention.

The onset of clinically symptomatic acute hepatitis with increased alanine aminotransferase levels occurs 8 to 10 weeks after infection. Without treatment, two-thirds of

the infected patients develop chronic hepatitis C, which is associated with a 2–3 $\log_{10}$ reduction in viral titer. Because liver biopsies are clinically not indicated in the acute phase of hepatitis C, the intrahepatic effector responses responsible for the decrease in viremia have not been studied in patients. However, data from biopsy tissues of experimentally infected chimpanzees have clearly shown that the decrease in viremia coincides with an increase in intrahepatic IFNγ-mRNA levels.[27,28,32]

The relative contribution of T cells and NK cells to IFNγ production and antiviral response is not known at this time. Whereas the appearance and maintenance of HCV-specific T-cell responses in the blood, in particular CD4 T-cell proliferation and cytokine production, are the best predictors of viral clearance,[32–37] NK cells are also activated and display increased cytotoxicity and IFNγ production.[38–40] Pelletier et al[39] recently reported a correlation between the magnitude of T-cell response and the peripheral blood NK cell response in the acute phase of HCV infection, and Kokordelis et al[40] found that NK cells from patients who later cleared the infection have a greater antiviral effect in vitro than NK cells from patients who developed chronic HCV infection. This opens the interesting question of whether the increased NK cell activity in acute HCV infection is an independent event or is triggered by CD4 T-cell–derived IL-2. The latter would render NK cells amplifiers and even downstream effectors of the virus-specific T-cell response.

How Does Chronic Hepatitis C Virus infection Alter Natural Killer Cell Function?

NK cells are activated not just in acute but also in chronic HCV infection. They express increased levels of CD69 and HLA-DR, indicating recent and more distant stimulation, respectively, and increased levels of NKp30,[41] NKp44,[22] NKp46,[22,42,43] NKG2A, NKG2D,[44] and the IL-2 receptor β chain CD122.[22] Increased NKG2C expression has also been reported,[22,44] but has now been attributed to oligoclonal expansion of a highly differentiated NK cell subset during prior HCMV infection.[45] NK cell activation is influenced by location, as NK cells are generally more activated in liver than in blood, even in uninfected individuals.[41] NK cell activation is also influenced by additional factors; for example, NKp46 levels are strongly associated with female gender and Caucasian race.[46]

While exciting new information on liver-resident NK cells and NK cell memory is emerging in the general NK cell field, data on these topics are still sparse in HCV infection. This is mostly due to the limited number (about 100,000) of lymphocytes that can be isolated from subcutaneous liver biopsy tissues and the lack of liver tissue from uninfected controls. Only a single study performed a side-by-side comparison of intrahepatic NK cells from surgically resected liver tissue of patients with HCV infection and uninfected controls (who underwent cholecystectomy).[41] In that study, HCV infection was associated with an increased frequency (compared with uninfected livers) of intrahepatic NK cells that shared some phenotypic and functional features with the unique liver-resident NK cell population that has been proposed to represent the human equivalent to mouse memory NK cells in subsequent studies.[8,47] This raises the interesting, but as yet unstudied question of whether HCV infection affects NK cells with memory functions in the liver.

Chronic HCV infection leaves a distinct signature also on peripheral blood NK cells, which has now been confirmed by several independent studies. Most remarkable is a dichotomy in effector functions that is characterized by increased cytotoxicity (evidenced by increased degranulation and production of tumor necrosis factor-related apoptosis-inducing ligand [TRAIL]) and, upon in vitro stimulation with IL-12/IL-15 or IL-12/IL-18, decreased production of antiviral cytokines (IFNγ and TNFα).[22,44,48,49] This is unexpected because the size of the $CD56^{bright}$ subset, which constitutes about 10% of peripheral blood NK cells in uninfected individuals, doubles in chronic HCV infection. $CD56^{bright}$ NK cells are known to produce large amounts of IFNγ and TRAIL with little perforin/granzyme-mediated cytotoxicity.[50,51] Most of the remaining NK cells belong to the more mature $CD56^{dim}$ subset. These cells contain high levels of perforin and granzyme, but can also rapidly produce chemokines and cytokines.[50] The altered subset distribution and overall decrease in the number of intrahepatic and blood NK cells[44,48,52,53] should therefore not result in the selective decrease in cytokine production.

What is the mechanism underlying these divergent effector functions? Increasing signaling via NKp30, NKp44, and NKp46 may directly contribute to the increase in NK cell cytotoxicity because these molecules are natural cytotoxicity receptors (NCRs) and NCR^+ NK cells have been shown to release more perforin/granzyme containing granules and exert greater in vitro antiviral effect than NCR^- NK cells.[40,46] Increased expression of the activating receptor NKG2D and its ligands may also contribute to NK cell cytotoxicity,[54] as may decreased expression of the inhibitory receptor NKG2A.

Cytokine-dependent signals are thought to play a major role in the increase in NK cell cytotoxicity and decrease in NK cell cytokine production in chronic HCV infection (Figure 1). This is evidenced by increased ex vivo levels of signal transducer and activator of transcription 1 (STAT1), a key molecule of IFNα signaling in NK cells. Because STAT1 itself is an *ISG*, increased STAT1 levels are consistent with type I IFN-mediated signaling in chronic HCV infection. This is supported by increased ex vivo levels of phosphorylated STAT1 and decreased levels of phosphorylated STAT4 in NK cells of HCV-infected patients compared with NK cells of uninfected patients.[55,56] Chronic exposure to virus-induced type I IFN can explain the NK cell phenotype of increased cytotoxicity and decreased cytokine production because induction of cytotoxicity and production of IFNγ require differential STAT1/4 signaling.

Based on a model initially proposed by Miyagi et al[57] for lymphocytic choriomeningitis virus (LCMV) infection, NK cells produce IFNγ in the early phase of a virus infection because of their constitutively high STAT4 expression. Chronic exposure to type I IFN results in increased

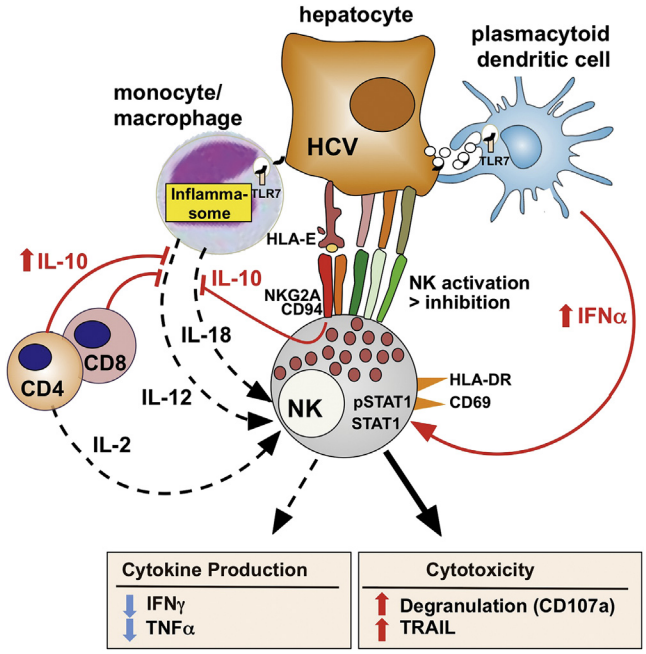

Figure 1. Altered natural killer (NK) cell function in chronic hepatitis C virus (HCV) infection. NK cells are activated in HCV infection with greater signaling from activating than inhibitory receptors (see text for details). Polarization of NK cell function toward increased (*solid line*) cytotoxicity and decreased (*dotted line*) production of antiviral cytokines[22,44,55] is driven by type I interferon (IFN), produced by Toll-like receptor 7 (TLR7)-stimulated plasmacytoid dendritic cells[62,63] and other nonparenchymal cells (not pictured). Mechanisms mediated by interleukin-10 (IL-10), which contribute to this NK cell phenotype in hepatitis B virus (HBV) infection, may also be operative in HCV infection because virus-specific T cells that secrete IL-10 have been demonstrated in the liver.[113] IL-10 is also directly induced in NK cells via interaction of the inhibitory NKG2A/CD94 receptor with human leukocyte antigen E (HLA-E) on HCV-infected hepatocytes.[74] HLA-E is thought to be stabilized by an endogenously processed HCVcore peptide and therefore expressed at increased levels.[75] Suboptimal production (*dotted line*) of IL-18 and IL-2 may further decrease IFNγ production by NK cells. Monocytes from patients with chronic HCV infection produce less IL-18 than monocytes from healthy controls upon coculture with HCV-replicating hepatocytes and NK cells.[70] HCV-specific CD4 T-cell responses and IL-2 production are reduced in HCV infection.[114]

expression of STAT1, and preferential STAT1 over STAT4 phosphorylation,[57] increased STAT1-dependent cytotoxicity (with TRAIL itself being an *ISG*), and decreased STAT4-dependent IFNγ production.[58] The observations of increased pSTAT1/pSTAT4 ratio and increased levels of the *ISG*s STAT1 and TRAIL in NK cells in chronic HCV infection therefore provide compelling evidence that type I IFN is produced locally in HCV infection even though it is detectable only rarely and at exceedingly low levels in the serum.[59–61]

Candidates for type I IFN production are nonparenchymal cells, such as liver-resident macrophages (Kupffer cells), plasmacytoid dendritic cells, and liver sinusoidal endothelial cells. Kupffer cells were identified as a local source of IFNα by immune-staining of liver sections.[61] Plasmacytoid dendritic cells were shown to respond to short-range transfer of HCV RNA-containing exosomes from hepatoma cells with TLR7-induced production of type I IFN in in vitro experiments.[62,63] Finally, primary human liver sinusoidal endothelial cells and immortalized liver endothelial cells were shown to produce type I IFN after internalization of HCV and stimulation of TLR7 and retinoic acid-inducible gene 1 (RIG-I).[64] The dichotomy of increased cytotoxicity and decreased cytokine production by NK cells is exacerbated when HCV-infected patients undergo IFN-based therapy.[55,65–67] This NK cell phenotype can be recapitulated when peripheral blood mononuclear cells of uninfected individuals are exposed to IFNα in vitro.[22,66]

In addition to the local increase in type I IFN, a relative decrease in IL-12, IL-18, and IL-2 production is thought to contribute to the suppressed cytokine production of NK cells in chronic HCV infection (see Figure 1). HCV is known to activate the inflammasome in monocytes and macrophages in an infection-independent process that requires recognition of viral RNA by endosomal TLR7. This can be achieved by clathrin-mediated uptake and uncoating of HCV particles and release of their RNA in endosomes.[68,69] It can also be achieved by cell-to-cell transfer of RNA as shown in cocultures of primary human monocytes with hepatoma cells that contain replicating subgenomic HCV RNA but do not release infectious HCV particles.[70] Inflammasome activation results in secretion of IL-18 and IL-1β in this coculture model,[70] and both cytokines are also detected at increased levels in the blood of patients with acute HCV infection.[71] IL-18 stimulates IFNγ production and down-regulation of HCV replication by NK cells in cocultures with HCV-replicon cells and monocytes.[70] Interestingly, monocytes from healthy IL-18 production and IFNγ-mediated antiviral activity of NK cells improve when monocytes from HCV-infected patients are replaced with monocytes from healthy blood donors in this system.[70] These data show that suboptimal monocyte activation and IL-18 production contribute to the defective cytokine production of NK cells in chronic HCV infection. A lack of IL-2 may further contribute to the decreased cytokine production of NK cells. This scenario in chronic HCV infection clearly contrasts with acute HCV infection where serum IL-18 levels peak,[71] and where HCV-specific CD4 T-cell responses[33,36] and IFNγ producing NK cell responses[40] are strong and predictive of HCV clearance.

In addition to monocytes, monocyte-derived dendritic cells have a reduced cytokine response in HCV infection. Specifically, they produce less IL-15 than those of healthy controls, which results in reduced expression of MHC class I-related chain A and B (MICA/B) and in reduced NK cell stimulation via the MICA/B ligand NKG2D.[72,73] Conversely, NK cells from HCV patients do not activate dendritic cells as much as NK cells from healthy donors.[74] This is thought to be due to stabilization of HLA-E on HCV-infected hepatocytes by an endogenously processed HCVcore peptide.[74,75] HLA-E binds to the inhibitory NKG2A/CD94 receptor on NK cells and induces IL-10 and TGF-β production.[74]

What are the consequences of an NK cell phenotype that is biased toward an increase in cytotoxicity and a decrease

in cytokine production? NK cells have been shown to exert antiviral effects both via perforin/granzyme-mediated cytotoxicity and via IFNγ-mediated down-regulation of HCV replication in in vitro models.[66,76,77] As initially proposed by Guidotti and Chisari,[78] a cytokine-mediated antiviral effect may be more efficient than cytotoxicity in the infected liver because secreted cytokines can reach many infected hepatocytes whereas cytotoxicity requires a 1:1 interaction between cells.[78] The altered functional phenotype of NK cells in chronic HCV infection may therefore facilitate chronic inflammation via killing of infected cells but not allow viral clearance due to impaired IFNγ production. Because suppressed cytokine secretion is also a key characteristic of the T-cell response in chronic HCV infection,[79] this may be a general mechanism to dampen the immune response of the host and to ameliorate disease pathogenesis.

Does the Altered Natural Killer Cell Phenotype Extend to Other Forms of Chronic Hepatitis?

To answer the question whether the altered NK cell phenotype represents a specific mechanism of HCV persistence or a general host response to decrease immunopathology, it is interesting to study NK cells in other viral infections. HBV is a good example because it belongs to a different family of viruses and employs different mechanisms to establish persistence.[31] Most HCV infections occur in immune-competent adults whereas most HBV infections occur during the neonatal period and in early childhood when immune responses are less mature and the inflammatory response is lower than in adulthood. One of the most striking differences to HCV is the absence of detectable *ISG* responses, even in the acute phase of hepatitis.[80,81] This suggests that type I IFN—the main factor that drives the increase in NK cell cytotoxicity and the decrease in IFNγ production in HCV infection—does not play a major role in HBV infection.

However, despite the differential type I IFN response, alterations in the NK cell phenotype and function in HBV infection are strikingly similar to those in HCV infection. The overall percentage of NK cells in the peripheral blood mononuclear cell population is decreased,[44,82,83] with a relative and absolute increase of the CD56^{bright} NK cell subset.[84] Likewise, NK cells display increased expression of activating receptors such as NKp30, NKp46, and NKG2C[43] with decreased expression of the inhibitory marker NKG2A.[43,52]

As in HCV infection, NK cells of chronic HBV patients have a reduced capacity to produce IFNγ compared with healthy controls.[44,84,85] Conversely, NK cell cytotoxicity is maintained, and the percentage of TRAIL-expressing CD56^{bright} NK cells is increased. This altered NK cell phenotype is already apparent in pediatric patients after perinatal acquisition of HBV infection from their mothers.[86]

Although NK cell phenotype and function are similar in HCV and HBV infection, the underlying causative mechanisms are different (Figure 2). In HBV infection, alterations in NK cell function appear to be induced by IL-10 and TGFβ rather than IFNα.[84] Accordingly, the cytokine-induced dichotomy in NK cell effector functions increases during phases of increased disease activity with increased IL-10 serum levels.[84] Some of the IL-10 may be derived from regulatory B cells, which have been demonstrated in HBV infection.[87] This NK cell phenotype can be recapitulated by in vitro exposure to IL-10, which suppresses IFNγ production without altering cytotoxicity and IL-10 production. Conversely, blockade of IL-10 and/or TGFβ restores IFNγ production of both CD56^{bright} and CD56^{dim} NK cells in vitro.[84]

Thus, the common theme of preserved NK cell cytotoxicity and impaired IFNγ production is found in both chronic HBV and HCV infections. Like HCV, HBV is sensitive to the antiviral effects of IFNγ. IFNγ from adoptively transferred HBV-specific CD8^{+} T cells[88] and from therapeutically activated NK cells has been shown to down-regulate HBV replication in transgenic mouse models.[89] The lack of IFNγ production by NK cells in chronic HBV infection may therefore result in reduced viral control and inability to clear HBV, and the preserved cytotoxicity appears to contribute to

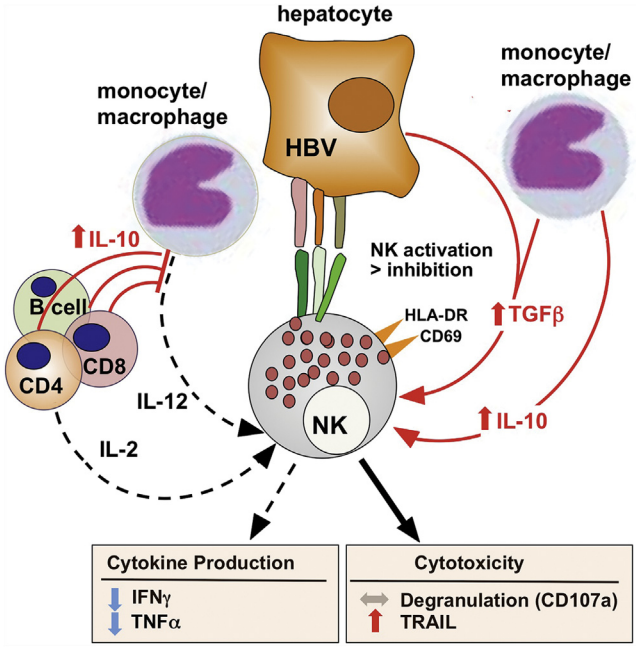

Figure 2. Altered natural killer (NK) cell function in chronic hepatitis B virus (HBV) infection. Activated NK cells display a similar functional phenotype of increased cytotoxicity (increased tumor necrosis factor-related apoptosis-inducing ligand [TRAIL] production, conserved cytotoxicity) and decreased cytokine production in HBV infection as seen in chronic hepatitis C virus (HCV) infection,[44] but it appears to be induced by transforming growth factor β (TGFβ) and interleukin-10 (IL-10) rather than by type I interferon (IFN). Neutralization of TGFβ and IL-10 normalizes NK cell function in vitro.[84] IL-10–producing regulatory B cells have been described in HBV infection and may contribute to the phenotype.[87] In contrast to HCV infection, increased interferon-stimulated gene (*ISG*) levels are not a typical feature of chronic HBV infection.[81]

liver inflammation. Along this line, polymorphisms in the IL-10 promoter are associated with increased IL-10 production, and with increased disease severity and progression in chronic HBV infection.[90,91] Furthermore, liver inflammation correlates with TRAIL expression on NK cells and TRAIL-expressing NK cells are enriched in the liver.[92]

What could be the reason for the preserved NK cell cytotoxicity in chronic viral hepatitis? NK cell cytotoxicity may serve a regulatory role, aimed at controlling virus-specific T-cell responses. HBV-specific T cells up-regulate TRAIL death receptor 2 (TRAIL-R2), which renders them susceptible to apoptosis by TRAIL-expressing NK cells. Increased TRAIL-R2 expression is specifically observed on intrahepatic HBV-specific $CD8^+$ T cells in close contact with TRAIL-expressing NK cells, and it correlates with the HBV titer.[93] In contrast, the majority of T cells of other specificity do not up-regulate TRAIL-R2, which indicates that T-cell receptor stimulation by cognate antigen rather than bystander activation is the underlying mechanism. Indeed, in vitro depletion of NK cells from peripheral blood mononuclear cells of HBV-infected patients increases HBV-specific but not CMV-specific $CD8^+$ T-cell responses.[93] A similar regulatory role of NK cells is also found in the mouse model of chronic LCMV-induced hepatitis.[94] NK cells specifically kill LCMV-specific CD4 T cells in a perforin-dependent manner, and a reduced CD4 T-cell response is associated with a secondary reduction of the LCMV-specific CD8 T-cell response.[94] In addition, activated CD8 T cells become targets of $NKG2D^+$ NK cells because they up-regulate NKG2D ligands in LCMV infection.[95] Consequently, depletion of NK cells restores CD4 T-cell help and thereby enhances the LCMV-specific CD8 T-cell response,[94] and blocking of the activating receptor NKG2D on NK cells directly increases the frequency of functioning, antigen-specific CD8 T cells.[95] It has therefore been proposed that NK cells function as "rheostats" for virus-specific T-cell responses and that therapeutic targeting of NK cells may have differential effects based on the degree of virus-driven T-cell exhaustion.[96]

Are Altered Immune Functions Reversible by Antiviral Therapy?

The observed down-regulation of IFNγ production by both NK cells and T cells in HCV and HBV infections raises the interesting question of whether their impaired function can be restored by antiviral therapy. As described in the previous sections, the suppression of NK cell IFNγ production is cytokine-driven—that is, driven by an excess of IFNα, IL-10, and transforming growth factor β (TGFβ) and by a relative lack of IL-12, IL-18, and IL-2. In contrast, impaired cytokine production of T cells is attributed to chronic T-cell receptor stimulation by viral antigens.[97] Chronic T-cell receptor signaling induces inhibitory molecules such as programmed death 1 (PD-1), T-cell immunoglobulin domain and mucin domain-3 (Tim-3), and cytotoxic T lymphocyte antigen 4 (CTLA-4),[28,98–101] and promotes an "exhausted" T-cell phenotype that is also observed in diseases such as HIV infection and cancer.[102]

Antiviral drugs that rapidly clear HCV infection and significantly reduce HBV viremia provide an excellent clinical model to answer the question of whether the described changes in NK cell and T-cell function are reversible. IFN-based treatment regimens are not suitable to answer these questions because IFN exerts not only antiviral, but also immune-modulatory function.[103] Specifically, IFN-based therapies exacerbate the functional dichotomy of NK cells toward increased cytotoxic effector functions and reduced IFNγ production,[65,67] suppress proliferation of T-cell and IFNγ production of T cells,[104,105] and do not fully restore T-cell effector function even if they achieve viral clearance.[106]

In contrast, effective removal of HCV by an IFN-free regimen of direct-acting antivirals normalizes both phenotype and function of NK cells, as now shown in two independent studies on combination therapy with the HCV NS5A inhibitor daclatasvir and the NS3 protease inhibitor asunaprevir.[49,107] Successful treatment of IFN nonresponders with this IFN-free regimen is associated with a rapid decrease of NK cell activation within the first 24 hours of treatment initiation and with normalization of NK cell phenotype and function by week 8 of the 24-week regimen.[49] Both NK cell cytotoxicity and IFNγ production reach levels that are indistinguishable from those of healthy controls.[49] The concomitant decrease in the frequency of pSTAT1-expressing NK cells confirms the role of virus-induced type I IFN for the altered function of NK cell function in chronic HCV infection.[49] This is consistent with a decrease in the expression level of the *ISGs RSAD2, ISG15, OAS3,* and *IFIT1* in the total blood leukocyte population.[107]

The effect of treatment-induced HCV clearance on the T-cell response is quite different from its effect on NK cells. Spaan et al[107] studied HCV-specific T-cell responses in parallel to NK cell responses in five HLA-$A2^+$ patients treated with daclatasvir and asunaprevir. They used dextramers of HCV-peptide loaded HLA-A2 molecules to detect HCV-specific T cells ex vivo irrespective of their function, and they report an increase in the frequency of HCV-specific T cells in the blood at the treatment time point of week 12. This is consistent with an earlier report on an increased ex vivo frequency and improved in vitro proliferation of HCV-specific CD8 T cells in 51 HCV patients who were treated with the protease inhibitor faldaprevir, the HCV NS5B polymerase inhibitor deleobuvir with or without ribavirin.[108] CD8 T-cell populations that target the sequence of the infecting virus showed a greater improvement in in vitro proliferation than those that target sequences in which the virus had escaped, thus excluding any bias for preexisting memory T cells. Notably, however, an improved ex vivo T-cell function has not yet been demonstrated in any study. In our own study of asunaprevir/daclatasvir-treated patients,[49] we did not observe any recovery of the IFNγ production of HCV-specific T cells when ex vivo Elispot assays with sets of overlapping HCV peptides or minimal optimal T-cell epitopes were performed (B. Rehermann, unpublished results). Combined, these early results suggest that successful IFN-free therapy normalizes phenotype and function of NK cells, and that it restores the proliferative

capacity but not necessarily the ex vivo effector function of HCV-specific T cells.

Treatment of HBV infection differs from treatment of HCV infection in the sense that IFN-free HBV-specific antiviral regimens significantly decrease viremia but do not induce complete viral clearance.[109] This is because the covalently closed circular form of the HBV DNA persists in infected cells and serves as its transcriptional template. Consequently, only very few patients clear HBsAg with long-term nucleos(t)ide analogue treatment.[110] Consistent with suppression but not complete elimination of HBV, antiviral therapy with nucleoside analogues corrects some but not all of the abnormal NK cell parameters. The treatment-induced decrease in HBV titer is paralleled by normalization of the percentage of NK cells in peripheral blood mononuclear cells, the size of the $CD56^{bright}$ NK cell subset, and TRAIL expression on NK cells. This is associated with reduced liver inflammation. However, consistent with low-level HBV persistence, there is no or only partial[85] restoration of the NK cells' capacity to produce IFNγ.

As in HCV infection, restoration of T cell responses in HBV infection is mainly detectable with T cell assays that require more than a week of in vitro stimulation with viral antigens. Restoration of T-cell proliferation is greater in patients who respond to long-term antiviral treatment with suppression of viremia and loss of HBsAg than in those who show a decrease in viremia but remain HBsAg positive.[111] While the expanded T-cell lines also displayed detectable IL-2, TNFα, and IFNγ responses upon restimulation with their cognate antigen, T cells remain dysfunctional in ex vivo assays. Furthermore, even in vitro responses are not maintained when treatment is discontinued.[112] The T-cell responses of successfully treated patients therefore remain inferior to those of spontaneously recovered patients.

Collectively, these results suggest, that cytokine-induced alterations in NK cell function do normalize when the virus is cleared whereas antigen-induced alterations in T-cell function are not fully reversible. The observation that complete normalization of the NK cell phenotype and function can be achieved in HCV-infected but not in HBV-infected patients fits the observation that HCV can be completely cleared with antiviral treatment whereas HBV persists at low levels.

Conclusions and Implications

Research over the past 10 years has expanded our view on NK cells as both an antiviral and a regulatory immune cell population in viral hepatitis. Chronic HCV and HBV infection impose similar alterations on NK cell phenotype and function. Impairment of IFNγ production is observed for both NK cells and T cells, but induced by different mechanisms. With new direct-acting antiviral treatment regimens now available as standard of care for HCV infection, research into the reversibility of these immune alterations has just begun. An expansion of this new area of research may provide useful information and strategies for immunotherapy of infections such as HBV that cannot be cleared with antiviral drugs alone and require an active immune response. Further research in the emerging field of ILC subtypes, liver-resident NK cells, and NK-cell-mediated memory functions is important to increase our knowledge of the immune surveillance in the liver and may lead to novel strategies for immunotherapy of hepatocellular carcinoma.

References

1. Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells: specificity and distribution according to genotype. Eur J Immunol 1975;5:112–117.
2. Kiessling R, Klein E, Pross H, et al. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur J Immunol 1975;5:117–121.
3. Eberl G, Colonna M, Di Santo JP, et al. Innate lymphoid cells. Innate lymphoid cells: a new paradigm in immunology. Science 2015;348:aaa6566.
4. Artis D, Spits H. The biology of innate lymphoid cells. Nature 2015;517:293–301.
5. Diefenbach A. Profiling the diversity of innate lymphoid cells. Nat Immunol 2015;16:222–224.
6. Gordon SM, Chaix J, Rupp LJ, et al. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. Immunity 2012;36:55–67.
7. Klose CS, Flach M, Mohle L, et al. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. Cell 2014;157:340–356.
8. Marquardt N, Beziat V, Nystrom S, et al. Cutting edge: identification and characterization of human intrahepatic CD49a+ NK cells. J Immunol 2015;194:2467–2471.
9. Vivier E, Raulet DH, Moretta A, et al. Innate or adaptive immunity? The example of natural killer cells. Science 2011;331:44–49.
10. Paust S, Gill HS, Wang BZ, et al. Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. Nat Immunol 2010;11:1127–1135.
11. Peng H, Jiang X, Chen Y, et al. Liver-resident NK cells confer adaptive immunity in skin-contact inflammation. J Clin Invest 2013;123:1444–1456.
12. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. Nature 2009;457:557–561.
13. Lee J, Zhang T, Hwang I, et al. Epigenetic modification and antibody-dependent expansion of memory-like NK cells in human cytomegalovirus-infected individuals. Immunity 2015;42:431–442.
14. Luetke-Eversloh M, Hammer Q, Durek P, et al. Human cytomegalovirus drives epigenetic imprinting of the IFNG locus in NKG2Chi natural killer cells. PLoS Pathog 2014;10:e1004441.
15. Schlums H, Cichocki F, Tesi B, et al. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. Immunity 2015;42:443–456.
16. Beaulieu AM, Madera S, Sun JC. Molecular programming of immunological memory in natural killer cells. Adv Exp Med Biol 2015;850:81–91.

17. Bryceson YT, March ME, Ljunggren HG, et al. Activation, coactivation, and costimulation of resting human natural killer cells. Immunol Rev 2006;214:73–91.
18. Horowitz A, Strauss-Albee DM, Leipold M, et al. Genetic and environmental determinants of human NK cell diversity revealed by mass cytometry. Sci Transl Med 2013;5:208ra145.
19. Biron CA, Nguyen KB, Pien GC, et al. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol 1999;17:189–220.
20. Khakoo SI, Thio CL, Martin MP, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science 2004;305:872–874.
21. Knapp S, Warshow U, Ho KM, et al. A polymorphism in IL28B distinguishes exposed, uninfected individuals from spontaneous resolvers of HCV infection. Gastroenterology 2011;141:320–325.
22. Ahlenstiel G, Titerence RH, Koh C, et al. Natural killer cells are polarized toward cytotoxicity in chronic hepatitis C in an interferon-alfa-dependent manner. Gastroenterology 2010;138:325–335.
23. Thoens C, Berger C, Trippler M, et al. KIR2DL3^{+}NKG2A^{-} natural killer cells are associated with protection from productive hepatitis C virus infection in people who inject drugs. J Hepatol 2014;61:475–481.
24. Sugden PB, Cameron B, Mina M, et al. Protection against hepatitis C infection via NK cells in highly-exposed uninfected injecting drug users. J Hepatol 2014;61:738–745.
25. Golden-Mason L, Cox AL, Randall JA, et al. Increased natural killer cell cytotoxicity and NKp30 expression protects against hepatitis C virus infection in high-risk individuals and inhibits replication in vitro. Hepatology 2010;52:1581–1589.
26. Werner JM, Heller T, Gordon AM, et al. Innate immune responses in hepatitis C virus-exposed healthcare workers who do not develop acute infection. Hepatology 2013;58:1621–1631.
27. Bigger CB, Brasky KM, Lanford RE. DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection. J Virol 2001;75:7059–7066.
28. Su AI, Pezacki JP, Wodicka L, et al. Genomic analysis of the host response to hepatitis C virus infection. Proc Natl Acad Sci USA 2002;99:15669–15674.
29. Horner SM, Gale M Jr. Regulation of hepatic innate immunity by hepatitis C virus. Nat Med 2013;19:879–888.
30. Schoggins JW, MacDuff DA, Imanaka N, et al. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. Nature 2014;505:691–695.
31. Park SH, Rehermann B. Immune responses to HCV and other hepatitis viruses. Immunity 2014;40:13–24.
32. Thimme R, Bukh J, Spangenberg HC, et al. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. Proc Natl Acad Sci USA 2002;99:15661–15668.
33. Diepolder HM, Zachoval R, Hoffmann RM, et al. Possible mechanism involving T-lymphocyte response to non-structural protein 3 in viral clearance in acute hepatitis C virus infection. Lancet 1995;346:1006–1007.
34. Missale G, Bertoni R, Lamonaca V, et al. Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. J Clin Invest 1996;98:706–714.
35. Urbani S, Amadei B, Fisicaro P, et al. Outcome of acute hepatitis C is related to virus-specific CD4 function and maturation of antiviral memory CD8 responses. Hepatology 2006;44:126–139.
36. Kaplan DE, Sugimoto K, Newton K, et al. Discordant role of CD4 T-cell response relative to neutralizing antibody and CD8 T-cell responses in acute hepatitis C. Gastroenterology 2007;132:654–666.
37. Thimme R, Oldach D, Chang KM, et al. Determinants of viral clearance and persistence during acute hepatitis C virus infection. J Exp Med 2001;194:1395–1406.
38. Amadei B, Urbani S, Cazaly A, et al. Activation of natural killer cells during acute infection with hepatitis C virus. Gastroenterology 2010;138:1536–1545.
39. Pelletier S, Drouin C, Bedard N, et al. Increased degranulation of natural killer cells during acute HCV correlates with the magnitude of virus-specific T cell responses. J Hepatol 2010;53:805–816.
40. Kokordelis P, Kramer B, Korner C, et al. An effective interferon-gamma-mediated inhibition of hepatitis C virus replication by natural killer cells is associated with spontaneous clearance of acute hepatitis C in human immunodeficiency virus-positive patients. Hepatology 2014;59:814–827.
41. Varchetta S, Mele D, Mantovani S, et al. Impaired intrahepatic natural killer cell cytotoxic function in chronic hepatitis C virus infection. Hepatology 2012;56:841–849.
42. De Maria A, Fogli M, Mazza S, et al. Increased natural cytotoxicity receptor expression and relevant IL-10 production in NK cells from chronically infected viremic HCV patients. Eur J Immunol 2007;37:445–455.
43. Nattermann J, Feldmann G, Ahlenstiel G, et al. Surface expression and cytolytic function of natural killer cell receptors is altered in chronic hepatitis C. Gut 2006;55:869–877.
44. Oliviero B, Varchetta S, Paudice E, et al. Natural killer cell functional dichotomy in chronic hepatitis B and chronic hepatitis C virus infections. Gastroenterology 2009;137:1151–1156.
45. Beziat V, Dalgard O, Asselah T, et al. CMV drives clonal expansion of NKG2C+ NK cells expressing self-specific KIRs in chronic hepatitis patients. Eur J Immunol 2012;42:447–457.
46. Golden-Mason L, Stone AE, Bambha KM, et al. Race- and gender-related variation in NKp46 expression associated with differential anti-HCV immunity. Hepatology 2012;56:1214–1222.
47. Hudspeth K, Donadon M, Cimino M, et al. Human liver-resident CD56/CD16 NK cells are retained within hepatic sinusoids via the engagement of CCR5 and CXCR6 pathways. J Autoimmun 2015, Published online. http://dx.doi.org/10.1016/j.jaut.2015.08.011.

48. Morishima C, Paschal DM, Wang CC, et al. Decreased NK cell frequency in chronic hepatitis C does not affect ex vivo cytolytic killing. Hepatology 2006;43:573–580.
49. Serti E, Chepa-Lotrea X, Kim YJ, et al. Successful interferon-free therapy of chronic hepatitis C virus infection normalizes natural killer cell function. Gastroenterology 2015;149:190–200.
50. Fauriat C, Long EO, Ljunggren HG, et al. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. Blood 2010;115:2167–2176.
51. De Maria A, Bozzano F, Cantoni C, et al. Revisiting human natural killer cell subset function revealed cytolytic $CD56^{dim}CD16^{+}$ NK cells as rapid producers of abundant IFN-gamma on activation. Proc Natl Acad Sci USA 2011; 108:728–732.
52. Bonorino P, Ramzan M, Camous X, et al. Fine characterization of intrahepatic NK cells expressing natural killer receptors in chronic hepatitis B and C. J Hepatol 2009; 51:458–467.
53. Dessouki O, Kamiya Y, Nagahama H, et al. Chronic hepatitis C viral infection reduces NK cell frequency and suppresses cytokine secretion: reversion by anti-viral treatment. Biochem Biophys Res Comm 2010; 393:331–337.
54. Sene D, Levasseur F, Abel M, et al. Hepatitis C virus (HCV) evades NKG2D-dependent NK cell responses through NS5A-mediated imbalance of inflammatory cytokines. PLoS Pathog 2010;6:e1001184.
55. Edlich B, Ahlenstiel G, Azpiroz AZ, et al. Early changes in interferon signaling define natural killer cell response and refractoriness to interferon-based therapy of hepatitis C patients. Hepatology 2012;55:39–48.
56. Miyagi T, Takehara T, Nishio K, et al. Altered interferon-alpha-signaling in natural killer cells from patients with chronic hepatitis C virus infection. J Hepatol 2010; 53:424–430.
57. Miyagi T, Gil MP, Wang X, et al. High basal STAT4 balanced by STAT1 induction to control type 1 interferon effects in natural killer cells. J Exp Med 2007; 204:2383–2396.
58. Nguyen KB, Cousens LP, Doughty LA, et al. Interferon alpha/beta-mediated inhibition and promotion of interferon gamma: STAT1 resolves a paradox. Nat Immunol 2000;1:70–76.
59. Meissner EG, Wu D, Osinusi A, et al. Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome. J Clin Invest 2014;124:3352–3363.
60. Shin EC, Seifert U, Kato T, et al. Virus-induced type I IFN stimulates generation of immunoproteasomes at the site of infection. J Clin Invest 2006;116:3006–3014.
61. Lau DT, Negash A, Chen J, et al. Innate immune tolerance and the role of Kupffer cells in differential responses to interferon therapy among patients with HCV genotype 1 infection. Gastroenterology 2013;144:402–413.
62. Dreux M, Garaigorta U, Boyd B, et al. Short-range exosomal transfer of viral RNA from infected cells to plasmacytoid dendritic cells triggers innate immunity. Cell Host Microbe 2012;12:558–570.
63. Takahashi K, Asabe S, Wieland S, et al. Plasmacytoid dendritic cells sense hepatitis C virus-infected cells, produce interferon, and inhibit infection. Proc Natl Acad Sci USA 2010;107:7431–7436.
64. Giugliano S, Kriss M, Golden-Mason L, et al. Hepatitis C virus infection induces autocrine interferon signaling by human liver endothelial cells and release of exosomes, which inhibits viral replication. Gastroenterology 2015; 148:392–402.e13.
65. Ahlenstiel G, Edlich B, Hogdal LJ, et al. Early changes in natural killer cell function indicate virologic response to interferon therapy for hepatitis C. Gastroenterology 2011; 141:1231–1239.
66. Stegmann KA, Bjorkstrom NK, Veber H, et al. Interferon-alpha-induced TRAIL on natural killer cells is associated with control of hepatitis C virus infection. Gastroenterology 2010;138:1885–1897.
67. Oliviero B, Mele D, Degasperi E, et al. Natural killer cell dynamic profile is associated with treatment outcome in patients with chronic HCV infection. J Hepatol 2013; 59:38–44.
68. Chattergoon MA, Latanich R, Quinn J, et al. HIV and HCV activate the inflammasome in monocytes and macrophages via endosomal Toll-like receptors without induction of type 1 interferon. PLoS Pathog 2014; 10:e1004082.
69. Negash AA, Ramos HJ, Crochet N, et al. IL-1beta production through the NLRP3 inflammasome by hepatic macrophages links hepatitis C virus infection with liver inflammation and disease. PLoS Pathog 2013; 9:e1003330.
70. Serti E, Werner JM, Chattergoon M, et al. Monocytes activate natural killer cells via inflammasome-induced interleukin 18 in response to hepatitis C virus replication. Gastroenterology 2014;147:209–220.
71. Chattergoon MA, Levine JS, Latanich R, et al. High plasma interleukin-18 levels mark the acute phase of hepatitis C virus infection. J Infect Dis 2011; 204:1730–1740.
72. Jinushi M, Takehara T, Kanto T, et al. Critical role of MHC class I-related chain A and B expression on IFN-alpha-stimulated dendritic cells in NK cell activation: impairment in chronic hepatitis C virus infection. J Immunol 2003;170:1249–1256.
73. Jinushi M, Takehara T, Tatsumi T, et al. Autocrine/paracrine IL-15 that is required for type I IFN-mediated dendritic cell expression of MHC class I-related chain A and B is impaired in hepatitis C virus infection. J Immunol 2003;171:5423–5429.
74. Jinushi M, Takehara T, Tatsumi T, et al. Negative regulation of NK cell activities by inhibitory receptor CD94/NKG2A leads to altered NK cell-induced modulation of dendritic cell functions in chronic hepatitis C virus infection. J Immunol 2004;173:6072–6081.
75. Nattermann J, Nischalke HD, Hofmeister V, et al. The HLA-A2 restricted T cell epitope HCV core 35–44 stabilizes HLA-E expression and inhibits cytolysis mediated by natural killer cells. Am J Pathol 2005;166:443–453.
76. Kramer B, Korner C, Kebschull M, et al. NKp46(High) expression defines a NK cell subset that is potentially involved in control of HCV replication and modulation of liver fibrosis. Hepatology 2012;56:1201–1213.

77. Larkin J, Bost A, Glass JI, et al. Cytokine-activated natural killer cells exert direct killing of hepatoma cells harboring hepatitis C virus replicons. J Infect Dis 2006; 26:854–865.
78. Guidotti LG, Chisari FV. To kill or to cure: options in host defense against viral infection. Curr Opin Immunol 1996; 8:478–483.
79. Wedemeyer H, He XS, Nascimbeni M, et al. Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. J Immunol 2002;169:3447–3458.
80. Dunn C, Peppa D, Khanna P, et al. Temporal analysis of early immune responses in patients with acute hepatitis B virus infection. Gastroenterology 2009;137: 1289–1300.
81. Wieland S, Thimme R, Purcell RH, et al. Genomic analysis of the host response to hepatitis B virus infection. Proc Natl Acad Sci USA 2004;101:6669–6674.
82. Duan XZ, Wang M, Li HW, et al. Decreased frequency and function of circulating plasmocytoid dendritic cells (pDC) in hepatitis B virus infected humans. J Clin Immunol 2004;24:637–646.
83. Zou Z, Xu D, Li B, et al. Compartmentalization and its implication for peripheral immunologically-competent cells to the liver in patients with HBV-related acute-on-chronic liver failure. Hepatol Res 2009;39:1198–1207.
84. Peppa D, Micco L, Javaid A, et al. Blockade of immunosuppressive cytokines restores NK cell antiviral function in chronic hepatitis B virus infection. PLoS Pathogens 2010;6:e1001227.
85. Tjwa ET, van Oord GW, Hegmans JP, et al. Viral load reduction improves activation and function of natural killer cells in patients with chronic hepatitis B. J Hepatol 2011;54:209–218.
86. Heiberg IL, Pallett LJ, Winther TN, et al. Defective natural killer cell anti-viral capacity in paediatric HBV infection. Clin Exp Immunol 2015;179:466–476.
87. Das A, Ellis G, Pallant C, et al. IL-10-producing regulatory B cells in the pathogenesis of chronic hepatitis B virus infection. J Immunol 2012;189:3925–3935.
88. Ando K, Moriyama T, Guidotti LG, et al. Mechanisms of class I restricted immunopathology. A transgenic mouse model of fulminant hepatitis. J Exp Med 1993; 178:1541–1554.
89. Cavanaugh VJ, Guidotti LG, Chisari FV. Interleukin-12 inhibits hepatitis B virus replication in transgenic mice. J Virol 1997;71:3236–3243.
90. Cheong JY, Cho SW, Hwang IL, et al. Association between chronic hepatitis B virus infection and interleukin-10, tumor necrosis factor-alpha gene promoter polymorphisms. J Gastroenterol Hepatol 2006; 21:1163–1169.
91. Miyazoe S, Hamasaki K, Nakata K, et al. Influence of interleukin-10 gene promoter polymorphisms on disease progression in patients chronically infected with hepatitis B virus. Am J Gastroenterol 2002;97:2086–2092.
92. Dunn C, Brunetto M, Reynolds G, et al. Cytokines induced during chronic hepatitis B virus infection promote a pathway for NK cell-mediated liver damage. J Exp Med 2007;204:667–680.
93. Peppa D, Gill US, Reynolds G, et al. Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion. J Exp Med 2013;210:99–114.
94. Waggoner SN, Cornberg M, Selin LK, et al. Natural killer cells act as rheostats modulating antiviral T cells. Nature 2012;481:394–398.
95. Lang PA, Lang KS, Xu HC, et al. Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting CD8+ T-cell immunity. Proc Natl Acad Sci USA 2012;109:1210–1215.
96. Welsh RM, Waggoner SN. NK cells controlling virus-specific T cells: Rheostats for acute vs. persistent infections. Virology 2013;435:37–45.
97. Rehermann B. Pathogenesis of chronic viral hepatitis. Differential roles of T cells and NK cells. Nat Med 2013; 19:859–868.
98. Raziorrouh B, Schraut W, Gerlach T, et al. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8+ T-cell function. Hepatology 2010;52:1934–1947.
99. Bengsch B, Seigel B, Ruhl M, et al. Coexpression of PD-1, 2B4, CD160 and KLRG1 on exhausted HCV-specific CD8+ T cells is linked to antigen recognition and T cell differentiation. PLoS Pathog 2010;6:e1000947.
100. Nakamoto N, Cho H, Shaked A, et al. Synergistic reversal of intrahepatic HCV-specific CD8 T cell exhaustion by combined PD-1/CTLA-4 blockade. PLoS Pathog 2009; 5:e1000313.
101. Golden-Mason L, Palmer BE, Kassam N, et al. Negative immune regulator Tim-3 is overexpressed on T cells in hepatitis C virus infection and its blockade rescues dysfunctional CD4+ and CD8+ T cells. J Virol 2009; 83:9122–9130.
102. Wherry EJ. T cell exhaustion. Nat Immunol 2011; 12:492–499.
103. Rehermann B, Bertoletti A. Immunological aspects of antiviral therapy of chronic hepatitis B virus and hepatitis C virus infections. Hepatology 2015;61:712–721.
104. Rahman F, Heller T, Sobao Y, et al. Effects of antiviral therapy on the cellular immune response in acute hepatitis C. Hepatology 2004;40:87–97.
105. Lauer GM, Lucas M, Timm J, et al. Full-breadth analysis of CD8+ T-cell responses in acute hepatitis C virus infection and early therapy. J Virol 2005;79:12979–12988.
106. Missale G, Pilli M, Zerbini A, et al. Lack of full CD8 functional restoration after antiviral treatment for acute and chronic hepatitis C virus infection. Gut 2012; 61:1076–1084.
107. Spaan M, van Oord G, Kreefft K, et al. Immunological analysis during interferon-free therapy for chronic hepatitis C virus infection reveals modulation of the natural killer cell compartment. J Infect Dis 2015, Published online. http://dx.doi.org/10.1093/infdis/jiv391.
108. Martin B, Hennecke N, Lohmann V, et al. Restoration of HCV-specific CD8+ T cell function by interferon-free therapy. J Hepatol 2014;61:1219–1219.
109. Kwon H, Lok AS. Hepatitis B therapy. Nat Rev Gastroenterol Hepatol 2011;8:275–284.
110. Wursthorn K, Jung M, Riva A, et al. Kinetics of hepatitis B surface antigen decline during 3 years of telbivudine

treatment in hepatitis B e antigen-positive patients. Hepatology 2010;52:1611–1620.
111. Boni C, Laccabue D, Lampertico P, et al. Restored function of HBV-specific T cells after long-term effective therapy with nucleos(t)ide analogues. Gastroenterology 2012;143:963–973.
112. Boni C, Penna A, Bertoletti A, et al. Transient restoration of anti-viral T cell responses induced by lamivudine therapy in chronic hepatitis B. J Hepatol 2003;39:595–605.
113. Accapezzato D, Francavilla V, Paroli M, et al. Hepatic expansion of a virus-specific regulatory $CD8^+$ T cell population in chronic hepatitis C virus infection. J Clin Invest 2004;113:963–972.
114. Semmo N, Krashias G, Willberg C, et al. Analysis of the relationship between cytokine secretion and proliferative capacity in hepatitis C virus infection. J Viral Hepat 2007; 14:492–502.

Received July 13, 2015. Accepted September 10, 2015.

Correspondence
Address correspondence to: Barbara Rehermann, MD, Immunology Section, Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, DHHS, Bethesda, Maryland 20892. e-mail: rehermann@nih.gov; fax: 301-402-0491.

Conflicts of interest
The author discloses no conflicts.

Funding
This study was funded by the intramural research program of NIDDK, National Institutes of Health.