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REVIEW

# Natural Killer Cells in Viral Hepatitis

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**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)***

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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.**

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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 (IFN$\alpha$ and IFN$\beta$), 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 $\alpha$1 domains. Because the interaction between KIRs on NK cells with HLA molecules on target cells plays a key role in NK cell inhibition, it has been suggested that the *KIR2DL3/HLA-C1* compound genotype results in a lower activation threshold of NK cells, thereby allowing faster NK cell activation compared with less favorable genotypes. This is supported by data in an in vitro influenza A virus infection model that demonstrate a larger HLA-C–regulated NK cell subset with more rapid NK cell IFN-$\gamma$ 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 IFN$\gamma$ 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 IFN$\gamma$ 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-$\kappa$B, result in the transcription of the IFN$\beta$ gene. IFN$\beta$ is released from infected cells, binds to the IFN$\alpha/\beta$ 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