Natural killer cells and hepatitis C: action and reaction

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Natural killer cells and hepatitis C: action and reaction

Kuldeep Cheent, Salim I Khakoo

ABSTRACT
In 1989, hepatitis C virus (HCV) was first identified as the infectious agent responsible for human non-A, non-B hepatitis.1 Two decades later, HCV remains a global public health problem with a suboptimal response rate to treatment and the absence of a protective vaccine. Recent work has highlighted the influence of the innate immune system, and in particular natural killer cells, on the outcome and pathology of HCV infection. These cells are considerably more complex than was originally thought and their role in viral infections is currently being unravelled. This review summarises our emerging understanding of natural killer cells in HCV infection.

INTRODUCTION
Hepatitis C virus (HCV) is a small enveloped positive-stranded RNA virus belonging to the Hepacivirus genus of the Flaviviridae family.1 There are six major genotypes which vary by up to 50% in nucleotide sequence. The global importance of HCV is illustrated by the observation that worldwide there are currently 150–170 million people believed to be infected.2 The majority of individuals (50–85%) exposed to HCV develop chronic infection, with the associated risks of cirrhosis, liver failure and hepatocellular carcinoma.3 Therefore it is important to elucidate the mechanisms associated with both viral clearance and disease progression. Natural killer (NK) cells have been implicated in all stages of HCV infection in both genetic and functional studies. This role may be either direct, by targeting infected hepatocytes, or indirect by influencing other key immunocytes such as dendritic cells (DCs) or T cells.

NK CELL RECEPTORS AND FUNCTIONS
NK cells comprise 5–20% of peripheral blood mononuclear cells but make up a substantially greater proportion (50–50%) of lymphocytes in the liver.4 They were originally described based on their ability to directly kill major histocompatibility complex (MHC) class I-negative tumour targets, as distinct from classical cytotoxic T cells, which require the presence of MHC class I for target cell recognition.5 The majority of NK cells are defined phenotypically by the presence of the surface marker CD56 and the absence of CD3. Depending on the level of cell surface expression of CD56 they can be divided into two main subsets, CD56bright and CD56dim, each with distinct properties.6 CD56dim NK cells express a moderate level of CD56, represent a differentiated subset and form the majority (>90%) of the circulating NK cell pool. Compared to the CD56bright population, CD56dim NK cells express higher levels of killer cell immunoglobulin-like receptors (KIRs), CD16 and perforin and are regarded to have marked cytotoxic potential. The CD56bright subset, which expresses high levels of CD56, contribute up to 10% of the peripheral blood NK population and are regarded as a less mature subset with a potential to differentiate into CD56dim NK cells.7 CD56bright NK cells express a high level of the inhibitory receptor CD94:NKG2A and generally do not express KIRs or CD16. The primary function of CD56bright NK cells is cytokine production which enables them to have an immunoregulatory function at sites of inflammation where they may be expanded. The functional distinction between the CD56bright and CD56dim populations is not absolute, and CD56bright NK cells can express the degranulation marker CD107a while CD56 dim NK cells can produce cytokines. Indeed the total interferon γ (IFNγ) production from CD56dim NK cells can exceed that of the CD56bright fraction.8 Similarly, CD56bright NK cells can be cytotoxic through expression of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL).9

In contrast to T and B cells, NK cells do not require priming to recognise virus-infected cells, and can thus be rapidly activated by cytokine stimulation. They recognise potential target cells using a plethora of cell surface receptors that transduce activating or inhibitory signals. When the signals from the activating receptors exceed those from inhibitory receptors NK cell effector functions are initiated.10 These functions include secretion of the T-helper 1 (Th1) cytokines IFNγ and tumour necrosis factor α (TNFα), and direct cytotoxicity of target cells (figure 1). Target cell killing can be mediated by degranulation of cytotoxic granules, and by surface expression of ligands such as Fas ligand (FasL) and TRAIL, that activate death receptors on target cells. Additionally, the Th1 type cytokines can prime the adaptive immune response and IFNγ, in particular, can have a direct antiviral effect.

NK cell function is critically regulated by combinations of stimulatory, co-stimulatory and inhibitory receptors (table 1). The net balance of signals derived from these receptors determines whether the NK cell becomes activated. Activating receptors expressed on NK cells include the C-type lectin-like receptors NKG2D and CD94:NKG2C/E, natural cytotoxicity receptors NKp44, NKp30, NKp46, and CD16 (FC-γRIII), which is the low-
NK cells and the liver

The liver is relatively enriched in NK cells. This intrahepatic population is embedded in the endothelial lining of the liver sinusoids and was originally described as ‘pit’ cells. These cells are large granular lymphocytes that are capable of spontaneous cytotoxicity against an MHC deficient cell line without prior sensitisation, a defining NK cell characteristic. They contain characteristic granules and, uniquely, rod-cored vesicles. They can be sub-classified based on the density and size of their granules which are either low density and small granules; or high density and large granules, with NK cells that contain the latter resembling peripheral blood NK cells. It has been shown in rats that peripheral blood high-density large granular cells migrate to the liver and differentiate into liver-specific low-density small granular NK cells. Intrahepatic NK cells may behave differently to NK cells in other areas due to the ‘tolerogenic’ environment in the liver. Murine intrahepatic NK cells are hyporesponsive. They are less cytotoxic and have an altered cytokine profile producing lower levels of IFNγ and greater levels of immunoregulatory cytokines, such as interleukin-10 (IL-10), compared to peripheral blood and splenic NK cells. This hyporesponsive state has been described in the early stages of hepatitis B virus infection and may contribute to the establishment of chronic viral infection.

In addition to their role in protection against pathogens and tumour transformation, intrahepatic NK cells have been demonstrated to have anti-fibrotic functions via inhibition of hepatic stellate cells (HSCs). They are capable of directly inducing HSC apoptosis, and producing IFNγ which inhibits HSC activation. Interestingly, greater levels of peripheral blood NK cell cytotoxicity have been associated with less liver fibrosis in patients with chronic HCV consistent with the lysis of activated hepatic stellate cells.

NK cells and the outcome of HCV infection

Understanding the mechanisms by which HCV is successfully eradicated is especially important for therapeutic and vaccination strategies. NK cells were originally implicated in determining the outcome of HCV infection in an immunogenetic study of the KIR genes and their HLA-C ligands. The KIR genes are a highly polymorphic group of receptors, showing diversity at the levels of both the locus and allele. Different individuals have different combinations of KIR genes. Their MHC class I ligands are also highly polymorphic, therefore there is a substantial population diversity in this receptor-ligand system. This diversity makes the KIR genes ideal candidates for modulating disease outcomes. By comparing the presence or absence of KIR genes and their HLA ligands in over 1000 individuals exposed to HCV it was shown that the specific combination of the inhibitory receptor KIR2DL3 and its group 1 HLA-C ligand (HLA-C1) was protective against chronic HCV infection. The protective effect of this gene combination was limited to individuals infected by intravenous drug use or accidental needle-stick injury. No protective effect was observed in subjects infected by transfusion of blood products in whom the innate immune response was thought to be overwhelmed by the higher infecting inoculum. The association of KIR2DL3 and group 1 HLA-C was subsequently confirmed in a smaller study of intravenous drug users of Puerto-Rican origin, indicating that this protective effect is consistent across different populations. It has been postulated that this gene combination is protective because the KIR2DL3
Table 1: Key receptors for natural killer cells

<table>
<thead>
<tr>
<th>Function</th>
<th>Family</th>
<th>Receptor</th>
<th>Ligand</th>
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<tbody>
<tr>
<td>Activating C-type lectin</td>
<td></td>
<td>NKG2D</td>
<td>MIC-A/B, ULBPs, HLA-E</td>
</tr>
<tr>
<td>receptor</td>
<td></td>
<td>CD94-NKG2C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD94-NKG2E</td>
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<tr>
<td>Natural cytotoxicity</td>
<td></td>
<td>NKp30</td>
<td>B7-3, B7-H6, CMV pp65</td>
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<tr>
<td>receptor</td>
<td></td>
<td>NKp44</td>
<td>Viral haemagglutinin</td>
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<tr>
<td></td>
<td></td>
<td>NKp46</td>
<td>Viral haemagglutinin</td>
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<tr>
<td>Killer cell immunoglobulin</td>
<td></td>
<td>3DS1</td>
<td>HLA-Bw4</td>
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<tr>
<td>receptor</td>
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<td>CD16</td>
<td>IglA</td>
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<td></td>
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<td>Toll-like receptors</td>
<td>Pathogen-associated molecular patterns (PAMPs)</td>
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<tr>
<td>Inhibitory</td>
<td></td>
<td>2DL1</td>
<td>Group 2 HLA-C</td>
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<tr>
<td>Killer cell immunoglobulin</td>
<td></td>
<td>2DL2/3</td>
<td>Group 1 HLA-C</td>
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<td>receptor</td>
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<td>CD94-NKG2A</td>
<td>HLA-E</td>
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NK CELLS IN CHRONIC HCV INFECTION

In addition to genetic studies, NK cells have also been implicated in the acute phase of HCV infection. These include the NK cells associated cytokines IL-12, IL-18 and IFNγ. The type III interferon IL-28B (IFNλ3) has received much attention as a mediator of clearance of HCV. It is not known whether IL-28B has a similar or complementary functions to the type I interferon, IFNα, which is involved in NK cell activation. However, these cells share common signalling pathways there is likely to be at least some overlap in function.

NK cells are also inhibited by the heterodimeric receptor CD94:NKG2A, which has the oligomeric MHC class I molecule HLA-E as its ligand. In a genetic study homozygosity for the HLA-E allele has been shown to be protective against chronic infection with HCV genotypes 2 and 3. This protective effect was thought to be due to an effect on HLA-E restricted T cells, although the HLA-E allele may have a lower affinity for peptides and hence be expressed at lower levels. This could therefore lead to less inhibition of NK cells via the CD94-NKG2A receptor.

Similarly, a number of cytokines involved in NK activation or function have been implicated in the outcome of HCV infection. These include the NK cells associated cytokines IFNα and IL-15. Associated with this, an attenuation of NK cell IFNγ secretion has been demonstrated, although this has not been universally reported. A full description of the innate immune response in HBV infection is beyond the scope of this article, and has recently been comprehensively reviewed. However, summarising, HBV and HCV appear to have distinct effects on NK cell activation in the acute phase of infection, underpinning key differences in the pathobiology of these hepatotrophic viruses.

NK CELLS IN ACUTE HCV INFECTION

In addition to genetic studies, NK cells have also been implicated in the acute phase of HCV infection (figure 2). Nowadays, these patients rarely come to the attention of clinicians, as they are often asymptomatic and therefore do not seek medical assistance. Amadei et al recently reported an increase in CD56bright NK cells (with an associated reduction in the CD56dim subset) in acute HCV patients compared to healthy individuals.37 Individuals who spontaneously cleared the virus showed a decline in the CD56bright population, with levels comparable to healthy control individuals after 1–3 months, indicating a return to baseline which was not observed in those that went on to have a chronic infection. Expression of the activating receptor NKG2D was also increased in the acute phase of infection. Functional experiments showed augmented IFNγ production and cytotoxicity in these patients and a trend for more NK cell degranulation in individuals expressing HLA-C1 specific KIR receptors, which was maximal in those with self-limiting infection. Thus in the acute phase of HCV infection there is activation of NK cells indicating their role in the immune response at this stage. Pelletier et al have also studied individuals in the acute phase of HCV infection. They also found increased activity of NK cells as determined by a degranulation assay, but found that the NK cells from intravenous drug users had generally lower levels of IFNγ secretion as compared to healthy controls, and suggest that this may be related to opioid use.

These findings contrast with acute HBV infection in which it was recently shown that there may be relatively little secretion of the innate cytokines IL-12, IFNα1 and IL-15. Associated with this, an attenuation of NK cell IFNγ secretion has been demonstrated, although this has not been universally reported. A full description of the innate immune response in HBV infection is beyond the scope of this article, and has recently been comprehensively reviewed. However, summarising, HBV and HCV appear to have distinct effects on NK cell activation in the acute phase of infection, underpinning key differences in the pathobiology of these hepatotrophic viruses.

NK CELLS IN CHRONIC HCV INFECTION

More amenable to study are NK cells from individuals with chronic HCV infection where comparisons with healthy donors have shown perturbations in NK cell frequency, phenotype and function (figure 3). Peripheral blood NK cell frequencies, both absolute number and percentage of total lymphocyte population, are reduced in chronic HCV compared to healthy individuals.21 The reduction in NK cell frequency may be a consequence of HCV infection, or a predisposing factor to chronic HCV infection, and both explanations have some support. In individuals with chronic HCV infection, NK cell frequency increases following successful antiviral therapy while a reduction in peripheral blood NK cell frequency in individuals with chronic HCV as compared to spontaneous resolvers has also been noted. IL-15, a pivotal cytokine for NK cell development, proliferation and function, may be relevant to this observation. Meier et al showed a significant reduction in IL-15 levels in HCV...
patients as compared to healthy controls and demonstrated that exogenous IL-15 rescued HCV NK cells from apoptosis, increasing ex vivo proliferation and function. Furthermore, DCs are an important source of IL-15 and have been shown to cross-talk with NK cells. In chronic HCV infection IL-15 production by IFNα-stimulated DCs is deficient. Thus a downstream consequence of this dendritic cell dysfunction could be inadequate production or proliferation of NK cells.

**Skewing of subset distribution**

A number of studies have documented a relative increase in circulating CD56bright, but not CD56dim NK cells, in chronic HCV compared to healthy individuals and spontaneous resolvers. Bonorino et al demonstrated that the relative proportions of CD56dim and CD56bright NK cells in the liver are altered in chronic HCV infection. They found that 80.5% of intrahepatic NK cells were CD56dim as compared to 94% in peripheral blood and 19.5% intrahepatic NK cells were CD56bright as compared to 6.0% in peripheral blood. This implies that the decreased frequency of CD56dim NK cells in the periphery is not related to their sequestration in the liver, although there is no clear data on the relative proportions of CD56dim and CD56bright NK cells in the healthy liver.

CD56–CD16+ NK cells appear to be more terminally differentiated NK cells and there is an expansion of this subset in chronic HCV infection. These cells have reduced perforin expression as compared with CD56dim NK cells and have been shown to be hypofunctional, particularly in their interactions with dendritic cells. In HCV, chemokine production by this subset was skewed towards MIP-1β, and there was also a reduction in IFNγ and TNFα secretion compared to the CD56+ NK population. Thus, overall, there is a skewing of NK cells away from the CD56dim CD16+ subset, which is thought to be the main cytotoxic subset of NK cells. This may be an effect of IFNα, as there is a strong IFNα response to HCV infection, and therapy with pegylated IFNα and ribavirin leads to an increase in CD56bright and a decline in CD56dim NK cells.

**Alterations in phenotype**

Changes in phenotype may reflect changes in subset distribution and also the effect of cytokines on specific subsets of NK cells. CD56bright NK cells are

![Figure 2](naturalkiller.png) Natural killer (NK) cells in acute hepatitis C virus (HCV) infection. NK cells are activated in the acute phase of HCV infection. Downregulation of major histocompatibility complex (MHC) class I on virus-infected hepatocytes may reduce the inhibitory signal to NK cells, shifting the balance towards NK cell activation. Dendritic cells (DCs) engage with NK cells via the Nkp30 receptor, and produce cytokines which boost NK cell proliferation towards an ‘NK1’ phenotype. Activated NK1 cells produce cytokines such as interferon γ (IFNγ) and tumour necrosis factor α (TNFα) which suppress HCV replication, reciprocally activate DCs, and prime naive CD4 T cells inducing a T-helper (Th)-1 response. IL, interleukin.
KIR-negative and NKG2A-positive, and the most consistent finding has been an increase in NKG2A expression in chronic HCV infection. This occurs on both intrahepatic and peripheral blood NK populations, with some studies reporting a decrease in KIR-expressing NK cells. Overall, there is also an increase in activating receptor expression. Such receptors include NKG2C, NKp44, NKp46 and NKp30. Initial reports of decreased expression of NKp46 have not been subsequently confirmed. Similarly there is conflicting evidence with respect to NKG2D expression which has been reported as being upregulated, downregulated and also unchanged.

Such contrasting data may relate to genuine differences in NK cell phenotype and function, or technical issues such as sample preparation, cytokine stimulation or freezing. There is also substantial population diversity in both NK cell phenotype and function which may account for these conflicting findings, especially if the study populations are small. Thus consistent with the findings from longitudinal studies of individuals with acute HCV infection, NK cells from those with chronic HCV infection appear to be chronically activated.

Altered function

Initial reports suggested diminished natural cytotoxicity in chronic HCV that was restored by successful HCV clearance with IFNα and ribavirin therapy. However, the number of cytotoxic CD56dim NK cells in the peripheral blood is depressed and hence, recent studies which take into account cytotoxicity per NK cell have demonstrated normal or increased NK cell cytotoxicity in chronic HCV. There is greater expression of activation markers such as CD122 (a subunit of IL-2 receptor which is crucial for IL-2 and IL-15 signalling), CD69, and NKp44. Upregulation of TRAIL on NK cells may also be an important mechanism underlying the anti-HCV effect of NK cells. Liver NK cells expressing TRAIL kill autologous hepatocytes in mice, and may therefore contribute to liver injury. NK cell TRAIL expression is increased in chronic HCV, and these cells have a phenotype consistent with IFNα stimulation.

A change in the cytokine profile of NK cells in chronic HCV may be relevant to the persistence of HCV infection. Failure of NK cell production of IFNγ in chronic HCV has been reported.
IFNγ has potent direct anti-HCV properties, blocking HCV replication in a dose-dependent manner.⁶⁸⁻⁷⁰ IFNγ also indirectly suppresses HCV activity by polarising T cell differentiation towards a virus specific Th1 phenotype. Additionally, increased HCV-NK cell production of Th2 cytokines such as IL-10 and transforming growth factor β (TGFβ), and the chemokine IL-8, may skew the cytokine profile towards an environment which is more permissive for HCV.⁴⁴ ⁴⁹ ⁵⁵ ⁶⁹ ⁷¹ This phenotype, which is infrequently found in healthy individuals, is similar to the NK2 phenotype as described by Peritt et al in which NK cells secrete the Th2 cytokines IL-5 and IL-13.⁷² NK cells can be polarised towards this phenotype under the influence of IL-4. Thus the Th2 environment found in chronic HCV infection may affect the differentiation and maturation of NK cells, towards this NK2 phenotype which further contributes to the Th2 environment in a positive feedback loop.

The cytokine microenvironment may affect NK cell phenotype and also function. In chronic HCV infection the dominant effect on NK cells appears to be of IFNα, but the roles of IL-4, IL-10 and IL-13 warrant further investigation, as the profile of NK cells in chronic HCV is consistent with a reduced maturation of CD56bright NK cells, and also enhanced differentiation of CD56dim NK cells towards a CD56− CD16+ phenotype (figure 4).

NK cells may also be modulated by direct cellular interactions, especially with DCs. All mature NK cells express the activating receptor NKG2D, the ligands for which are MHC class I chain-related (MIC) proteins. In HCV infection there is an impairment of MIC-A/B expression which results in lower levels of NK cell activation.⁴⁸ NK cell activation of DCs may be reciprocally perturbed. When co-cultured with human hepatic cells, NK cells enhance maturation and activation of DCs to promote a Th1-polarised CD4 T-cell response.⁵⁵ NK cells from HCV-infected individuals have a reduced capacity to activate DCs, due to NK cell inhibition by the CD94:NKG2A receptor and a consequent increase in NK expression of the immunoregulatory cytokines IL-10 and TGFβ, which promote Th2 type differentiation. Interestingly, inhibition of NKG2A restored the ability of HCV-NK cells to activate DCs, and also the production of the Th1 cytokines IFNγ and TNFα. This may be important as HCV can upregulate HLA-E, the ligand for NKG2A, in vivo and so represents a mechanism by which HCV may modulate the NK cell response.⁵⁶

The activation status of NK cells correlates with liver inflammation. Increased expression of NKG2A, CD69 and CD107a (a marker of NK cell degranulation) on peripheral blood NK cells have all been linked with disease activity, and in the study of Oliveiro et al an inverse correlation of peripheral NK cell NKG2D expression with alanine amino transferase (ALT) was found.⁹ ⁴¹ ⁴⁴ ⁶⁰ Furthermore, NK cells have been localised to necrotic areas in liver biopsy specimens in chronic HCV, but not chronic HBV.⁴¹ These intrahepatic NK cells express higher levels of TRAIL and the activating receptor NKp46.⁶⁰ Bonorino et al also found an inverse correlation between NKG2A-positive NK cells and viral load.⁴¹ However, although correlations with disease and RNA levels have significant p values, they have relatively low

### Figure 4

A model of abnormal natural killer (NK) cell differentiation in chronic hepatitis C virus (HCV) infection. The CD56bright CD16− NK cell population is expanded in chronic HCV as a consequence of a Th2 type cytokine microenvironment, either in the periphery or within the liver, leading to a failure of their maturation to the CD56dim CD16+ subset. Chronic stimulation by activating receptors and cytokines within the liver may then lead to accelerated differentiation of CD56dim CD16+ subset towards the functionally defective CD56− CD16+ subset, which is also expanded. These processes lead to a reduced frequency of the functionally mature, cytotoxic CD56bright CD16+ subset and the observed relative increase in CD56bright CD16− NK cells.
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values for the correlation coefficient \( r \), indicating that much of the variability in inflammation and RNA levels is accounted for by factors other than those specifically studied.

**NK CELLS AND INTERFERON TREATMENT**

IFN\( \alpha \) is a potent NK cell activating cytokine that is the cornerstone of treatment for HCV infection. NK cells have been implicated as determining the outcome of treatment for HCV in genetic, phenotypic and functional analyses. Genetic studies have indicated that a similar combination of KIR and HLA-C genes (KIR2DL3 and group 1 HLA-C) are associated with a beneficial response to treatment with IFN\( \alpha \) based regimens, as has been observed for spontaneously resolving HCV infection.\(^75\)\(^76\) This demonstrates a consistency in the protective effects of these genes in HCV infection, which has also been extended to multiply exposed intravenous drug users.\(^75\)

Pre-treatment peripheral blood NK cell phenotype may also predict the response to pegylated interferon and ribavirin therapy. An 80% sustained virological response (SVR) rate was observed in individuals in whom the frequency of CD56–CD16+ NK cells was in the normal range, compared to only 25% SVR in individuals where CD56–CD16+ frequency was significantly above the normal range.\(^52\) Additionally, in a small study, individuals with an SVR were found to have more KIR-negative NK2A-positive CD56\(^\text{dim}\) NK cells than non-responders.\(^59\) Thus both KIR-positive and KIR-negative NK cells may have a role to play in the outcome of HCV treatment.

During IFN\( \alpha \) therapy there is an increase in NK cell cytotoxicity,\(^4\)\(^46\)\(^63\)\(^64\) which may be due to augmented degranulation and also the capacity to induce apoptosis. TRAIL induces apoptosis of cells expressing the death receptors DR4 and DR5,\(^77\) and has emerged as an important marker associated with the response to IFN\( \alpha \).\(^9\) In vitro, HCV infection sensitises hepatocytes to TRAIL-mediated apoptosis and TRAIL on NK cells has been associated with liver inflammation in HBV infection.\(^78\) Baseline levels of TRAIL on NK cells in chronic HCV may be normal or elevated.\(^9\)\(^69\) However, upon IFN\( \alpha \) stimulation NK cells express higher levels of TRAIL, and upregulation of TRAIL on CD56\(^\text{bright}\) NK cells is observed in individuals undergoing anti-viral therapy. Additionally, analysis of paired liver biopsy samples has shown that SVR is associated with an increase in the frequency and total number of intrahepatic NK cells following IFN\( \alpha \) and ribavirin treatment.\(^79\)

**POSSIBLE HCV TO ESCAPE NK CELL SURVEILLANCE**

HCV has a number of strategies to evade the host immune response and these may specifically affect NK cells. The virus has multiple mechanisms to interfere with secretion of type I interferons, which may attenuate anti-viral NK cell activity.\(^60\) HCV core protein has been reported to substantially upregulate the expression of MHC class I on Hep\( \beta \)B cells with an associated downregulation of NK cytotoxicity.\(^81\) Similarly, a peptide derived from HCV core (HCV\(_{35\text{–}44}\)) is capable of upregulating HLA-E, leading to CD94:NKG2A mediated inhibition of NK cells.\(^56\) This peptide is also presented by HLA-A2 to cytotoxic T lymphocytes (CTL), and the presence of CTL specific to this peptide in individuals with chronic HCV implies that it can be endogenously processed and presented.\(^82\) Thus in vivo it could also be loaded onto HLA-E and so represents a tenable mechanism by which HCV may escape NK cell activity. Conversely, the NS4A/B protein of HCV has been shown to downregulate MHC class I as a consequence of an inhibition of its trafficking through the ER and this could lead to NK cell activation.\(^83\)

Thus the effects of HCV on MHC class I are complex and the net effect that these changes may have on NK cells in vivo are not clear at present.

HCV-E2 has been shown to reduce NK cell IFN\( \gamma \) secretion by binding to CD81 on NK cells.\(^69\) The reduction in NK cytotoxicity was partly attributed to a direct effect of the HCV envelope protein E2 cross-linking tetraspanin CD81 receptors on NK cells, a theory supported by in vitro evidence from two groups.\(^65\)\(^84\) Both of these studies used a high concentration of truncated, plate bound HCV-E2 protein, rather than complete infectious virions, which have been developed relatively recently. These earlier studies have now been challenged. Although anti-CD81 antibody inhibited NK cells, HCV-E2 protein when part of complete soluble infectious particles is unable to do so.\(^66\)\(^69\) Interestingly, Crotta et al found that immobilised HCV virions inhibited IFN\( \gamma \) production by IL-12-activated NK cells and this effect was due to engagement of cellular CD81 by HCV-E2. However, given the observations of Yoon et al and also that NK cells appear to be activated in acute HCV infection, it appears that the direct effects of HCV-E2 on NK cells are unlikely to be significant in vivo.\(^57\)\(^58\)\(^66\)

**NK CELLS IN HCV-ASSOCIATED CIRRHOSIS AND HEPATOCELLULAR CARCINOMA**

Approximately 20% of patients with chronic HCV infection will develop cirrhosis after 20 years of infection. NK cells may impact on this both by controlling infection and by directly affecting the fibrogenic process. This is because NK cells can cause apoptosis of the fibrogenic stellate cells by NK2D and TRAIL mediated signalling, and also because the pro-inflammatory cytokine IFN\( \gamma \) is anti-fibrogenic.\(^85\)\(^86\) Reduction in intrahepatic NK frequency also correlates with the progression of HCV and the development of cirrhosis.\(^87\) Hepatocellular carcinoma (HCC) is a common complication of cirrhosis, and reductions in peripheral and intrahepatic NK cell frequency, cytotoxicity and IFN\( \gamma \) production have been reported in individuals with HCC as compared to healthy control subjects.\(^88\)\(^89\) Depressed NK cell activity may be mediated by regulatory T cells, which suppress the anti-tumour functions of NK cells.\(^90\) An anti-
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The tumour effect of NK cells is supported by an immunogenetic study in which the combination of the activating NK cell receptor KIR3DS1 and its HLA-Bw4٢٠ ligand was under-represented in HCV-positive patients who developed HCC, as compared to those who remained tumour-free.٢٣ TRAIL has been implicated as one important molecule in this anti-tumour function of NK cells,٢٤ with another being NGK2D. NGK2D recognises the stress ligands MICA/B, which have a restricted expression on healthy cells but are upregulated by viral infection and tumour transformation thereby providing a strong signal for NK cell activation via NGK2D. Shedding of MICA/B into the circulation leads to downregulation of NGK2D on T and NK cells and provides a mechanism of tumour escape.٢٥٢٦ This may happen in HCC and the anti-HCC drug sorafenib has been shown to inhibit this proteolytic cleavage of MICA/B from HCC cells, thus enhancing NK cell mediated cytolyis of tumour cells.٢٧

NK CELLS AND LIVER TRANSPLANTATION

HCV-related liver disease is a frequent indication for orthotopic liver transplantation (OLT) in the Western world. As NK cells express inhibitory receptors for polymorphic HLA class I molecules and liver transplants are not HLA matched, there is a strong potential for alloreactivity of recipient NK cells against the donor liver, especially if the recipient does not have a ligand for the donor inhibitory receptors. This model is supported by two retrospective analyses of patients who underwent OLT for a variety of causes.٢٨٢٩ In the latter study of 416 UK patients, individuals who received donor allografts expressing one group 2 HLA-C allele had less graft loss and a 13.6% improvement in survival at 10 years compared to those receiving group 1 HLA-C homozygous allografts. The outcome figures were even better for those receiving a group 2 HLA-C homozygous allograft with 26.5% reduction in graft loss and lower frequencies of both chronic rejection and recurrent cirrhosis. This is consistent with the group 2 HLA-C:KIR interaction being stronger than that of group 1 HLA-C:KIR and thus resulting in greater NK cell inhibition.٢٩ The authors suggest that group 2 homozygous allografts may be better allocated to high risk recipients (eg, second transplants) to maximise the chances of long-term graft function. Unfortunately, these findings were not replicated in a large European study.٣٠ Population diversity may play a role in these differences as individual HLA alleles within the group 1 and group 2 HLA-C ‘supertypes’ may have different interactions with their KIR ligands.٣١

Recurrence of HCV post-transplantation is universal and often has an accelerated course with allograft cirrhosis in up to 50% of individuals at 5 years post-transplant.٣٢ Predicting which individuals are more likely to develop aggressive HCV recurrence post-transplant may be a useful tool given the shortage of donor organs. In OLT recipients expressing KIR2DL3, the mismatching of HLA-KIR ligands correlated with the progression to liver fibrosis.٣٣ Therefore KIR2DL3 recipients may be better suited to HLA-KIR matched allografts.

A prospective study looking at phenotypic changes of peripheral NK cells pre- and post-transplant has also been reported.٣٤ A reduction in peripheral blood NK frequency was noted in the first week after transplant returning to pre-transplant levels after 1 month. This was attributed to homing of NK cells to the liver, possibly due to graft re-population or de novo graft infection. A progressive increase in NGK2C expression was also noted post-OLT and appeared to be related to HCV recurrence. Increased NK cell natural cytotoxicity receptor expression correlated with ALT levels supporting the theory that NK cells contribute to liver inflammation. Thus the effects of NK cells in post-transplant HCV are complex, with weaker inhibitory signals leading to a more pronounced anti-viral effect and carrying an increased risk of rejection.

THERAPEUTIC PROSPECTS AND FUTURE DIRECTIONS

There are several potential mechanisms of modifying NK cell function to improve their anti-viral and anti-tumour properties. These would include manipulating NK cell receptor expression (eg, blocking NK inhibitory receptors), cytokine therapy (eg, IL-2, IL-12, IL-15), and infusion of ex vivo expanded autologous NK cell populations. NK cells can be successfully expanded ex vivo to upregulate TRAIL, FasL and NGK2D expression with a consequent increase in anti-tumour activity.٣٥ A phase I clinical trial based on these findings using adoptive infusion of ex vivo expanded autologous NK cells in patients with metastatic tumours is currently in progress. Furthermore, adoptive transfer of TRAIL-expressing NK cells may represent a potential therapy for reducing recurrence of HCC following partial hepatectomy and liver transplantation.٣٦٣٧ Ishiyama et al reported that IL-2-stimulated NK cells extracted from donor liver graft perfusate have marked anti-tumour activity (via increased TRAIL expression) against a hepatocellular carcinoma cell line.٣٨

While adoptive immunotherapy may have a role in HCC, targeted NK therapies have not been used

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Key points 1: Natural killer cells in hepatitis C virus infection

- NK cells are effector cells of the innate immune system that can directly lyse infected cells and modulate adaptive immune responses.
- In acute HCV infection NK cells are activated, displaying increased cytotoxicity and increased IFNγ secretion.
- In chronic HCV infection, NK cell frequency is reduced, with changes in phenotype towards NK2 type cytokines (IL-10 and TGFβ).
- NK cell abnormalities in chronic HCV infection are distinct from those in chronic HBV infection.
- HCV core encoded peptides may upregulate MHC-class I and inhibit NK cells.
- In vitro NK cells are capable of inducing apoptosis of HCV infected cells.
Key points 2: KIR and MHC genetics in hepatitis C virus infection

- NK cells are regulated by polymorphic inhibitory receptors (KIR) with polymorphic MHC class I ligands.
- These receptors are crucial for both maturation and regulation of NK cell activity.
- The combination of the inhibitory receptor KIR2DL3 and its group 1 HLA-C ligand is beneficial in spontaneous and treatment-induced resolution of HCV infection.
- Recipient–donor KIR/HLA interactions may influence the outcome of liver transplantation. Associations have been made with graft and patient survival and HCV recurrence post-transplant.
- Allelic diversity of KIR and MHC genes may account for differences in findings from genetic association studies.

for the treatment of HCV; although it is notable that IFNα2 is a potent NK activating cytokine. In order to target NK cells, deeper insights into their role in HCV need to be gained. It will be important to translate genetic insights into functional ones; in particular, why KIR2DL3 and group 1 HLA-C alleles are protective. The recruitment of NK cells to the liver and their role in the acute phase of infection has received little attention to date due to difficulties in obtaining patient derived material. However these cohorts will be key to understanding mechanisms by which NK cells are involved in the eradication of HCV. The potential for NK cells to interface with dendritic cells, T cells and also IL-28B in a synergistic response to acute HCV infection requires further exploration. Bringing together genetic and functional observations to understand mechanisms by which the KIR–HLA interactions can alter the cross-talk between these cells may be particularly important to understanding eradication of HCV. Whilst there is now a substantial body of information on NK cells in chronic HCV infection, relatively little progress has been made as to how they may be manipulated in order to generate a successful anti-viral immune response. Finally, our understanding of NK cell biology continues to evolve and this will continue to generate novel ideas related to viral eradication and disease progression.

CONCLUSION

Our emerging understanding of NK cells is giving us new insights into their role in HCV infection. Critically, HCV is the prototypic infection for which inhibitory KIR determine outcome against chronicity. In chronic HCV infection NK cell function is attenuated under the influence of cytokines, and probably inefficient receptor-mediated stimulation. A weak NK cell response is one factor in the failure to generate an adequate adaptive immune response. Addressing both the innate and adaptive immune systems will hold key to the development of successful vaccination strategies for HCV.

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REFERENCES

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63. Par G, Rukavina D, Podack ER, et al. Decrease in CD3-negative, CD8dim (+) and Vdelta2/Vgamma9 T+ peripheral blood lymphocyte counts, low perforin expression and the impairment of natural killer cell activity is associated with chronic hepatitis C virus (HCV) infection. J Hepatol 2009;50:508–16.


