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Conflicts of interest

The authors disclose no conflicts.

Reprint requests

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Direct-Acting Antivirals Cure Innate Immunity in Chronic Hepatitis C



See “Successful Interferon-free therapy of chronic hepatitis C virus infection normalizes natural killer cell function,” by Serti E, Chepa-Lotrea X, Kim YJ, et al, on page 190.

For a number of years, immunologists have strived to understand the mechanisms associated with treatment-induced eradication of hepatitis C virus (HCV). However, interferon (IFN)- α -based therapies did not allow studies examining the role of the virus itself on innate and adaptive immunity to HCV. The study by Serti et al¹ published in this issue of *Gastroenterology* provides a unique opportunity to assess the effect of virus on innate immunity, particularly natural killer (NK) cells, which were previously described by the same authors and other groups to be dysfunctional in this setting (reviewed by Golden-Mason and Rosen²).

HCV is characterized by a high propensity to persist owing to its remarkable skills to evade the host's innate and adaptive immune responses in complicity with permissive intrahepatic microenvironment, which panders to virus replication while limiting immunopathology.³ However, the available direct-acting antivirals (DAAs) have highlighted the vulnerability of HCV that, despite sophisticated countermeasures against host immune surveillance, can now be eradicated within weeks of treatment in most cases,⁴ whereas the chances of achieving success were significantly lower with the historical pegylated IFN- α /ribavirin combination therapy. The outcome of the latter was uncertain, even accounting for the strongest predictors such as IFN- λ 3 polymorphism and viral genotype. Therefore, several investigators sought possible pretreatment and treatment immunologic variables associated with success or failure. Importantly, those early studies clearly showed that T cells did not play a lead role in viral eradication, because no HCV-specific T-cell changes were observed in patients who did or did not achieve an early virologic response,⁵ and the overall vigor of the HCV-specific T-cell response decreased during treatment.⁶ The negative findings on T-cell responses prompted a proliferation of studies on NK cells in the context of hepatitis C treatment, particularly because early work on intrahepatic NK cells isolated from liver explants suggested that innate lymphoid cells were highly prevalent in the liver and constituted about one-half of intrahepatic

lymphocytes.⁷ Moreover, IFN- α is a potent activator of NK cells, thus providing a strong rationale for investigating their role in treatment responses. Two papers from the authors of the present study pointed to the importance of IFN- α -induced early NK cell activation⁸ and type I IFN signaling⁹ as a paradigm of response to therapy. Others added further variables associated with responses to IFN- α -based therapies while confirming NK activation as a key factor associated with sustained virologic response (SVR).¹⁰ In chronic HCV infection, NK cells are already activated before any exogenous IFN, and are polarized toward cytotoxicity with deficient IFN- γ secretion as a consequence of chronic exposure to endogenous IFN- α .^{11,12} This phenomenon is caused by type I IFN-induced phosphorylation of signal transducer and activation of transcription (STAT) 1, which displaces STAT4 at the IFN- α/β receptor resulting in decreased pSTAT4-dependent IFN- γ production and increased pSTAT1-dependent cytotoxicity (Figure 1).¹³ The net result is a “functional dichotomy” characterized by enhanced NK cytolytic activity and a simultaneous failure to produce adequate amounts of IFN- γ and tumor necrosis factor (TNF)- α , with consequent inability to eradicate HCV. It follows that IFN- α -based therapies can only accentuate the NK cell functional dichotomy as a result of massive exposure to exogenous IFN- α . Indeed, as a consequence of STAT1 activation, IFN- γ production decreases early in treatment and does not recover for several weeks after IFN- α therapy.⁸ Whether IFN- γ production is actually restored to normal values after SVR is not known, because the study did not have sufficient follow-up to answer this question.

After SVR, one would expect a complete reconstitution of immune cell function. Instead, there is evidence that T-cell function remains profoundly altered in most cases after successful IFN- α /ribavirin therapy with T-cell hyporesponsiveness to exogenous stimulation persisting for a long time after virus control, probably as a result of prolonged exposure to viral antigens and consequent exhaustion.¹⁴ A hierarchy of residual HCV-specific T-cell dysfunction was demonstrated with cytotoxicity and IL-2 production being mostly affected. Whether such T-cell functional defects are relevant clinically is unclear presently, although they have the potential to impinge on the efficiency of protection upon reexposure to HCV. NK cell dysfunction is probably not as pervasive,¹⁵ but NK cell efficiency would be highly desirable, being an essential component of the innate/adaptive immunity cross-talk.³

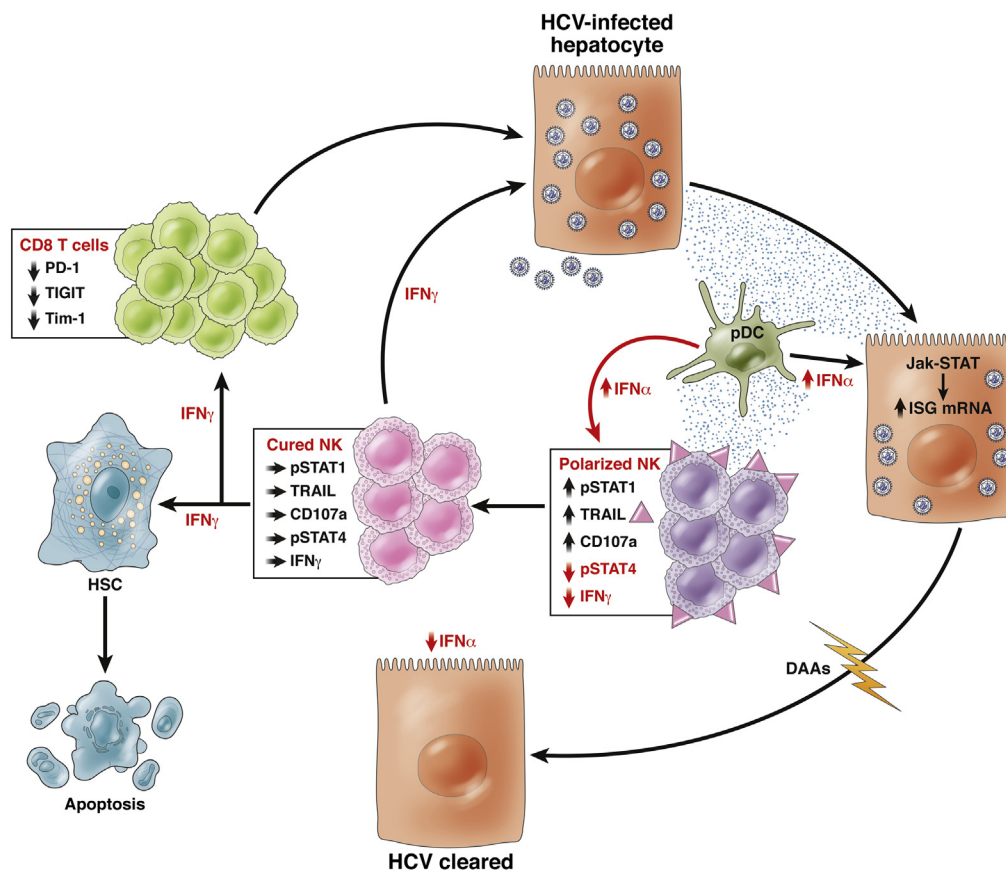


Figure 1. Continuous exposure to interferon (IFN)- α increases signal transducer and activation of transcription (STAT)1 expression in natural killer (NK) cells and results in increased pSTAT1-dependent cytotoxicity (CD107a expression and tumor necrosis factor-related apoptosis-inducing ligand [TRAIL] expression) and decreased pSTAT4-dependent IFN- γ production, polarizing NK cells toward cytotoxicity. Plasmacytoid dendritic cells (pDC) contribute to IFN- α secretion. Eradication of hepatitis C virus (HCV) by direct-acting antivirals (DAAs) results in normalization of IFN- α concentration in the micro-environment with increased IFN- γ production and reduction of cytotoxicity, whereas this was not possible with IFN- α -based therapies. Increased IFN- γ production by NK cells may protect from reinfection, improve the development of adaptive immunity via reduction of expression of exhaustion markers such as PD-1,²⁰ T-cell immunoreceptor with immunoglobulin (Ig) and ITIM domains (TIGIT) and T-cell immunoglobulin and mucin domain 1 (TIM-1; unpublished data—Burchill MA, Golden-Mason L, Rosen HR. Reversal of T-cell exhaustion in chronic HCV-infected patients by direct-acting antivirals. *Hepatology* 2014;60:311A. Abstract 224). IFN- γ can also promote apoptosis of hepatic stellate cells (HSC), which are key players in liver fibrogenesis. Cured NK and CD8 T cells can contribute with DAAs to noncytolytic eradication of HCV.

With this in mind, Serti et al¹ designed a flawless prospective study to ask whether a purely antiviral regimen could restore NK cell phenotype and function. To achieve this, they were able to obtain frozen peripheral blood mononuclear cells from patients with chronic HCV infection receiving a combination of a first wave NS3 protease inhibitor, asunaprevir, and daclatasvir, a first-in-class NS5A inhibitor. The data showed that the expression of several NK cell-activating receptors decreased to normal levels within hours of the commencement of treatment. Remarkably, IFN- γ production normalized by week 2 of therapy, whereas markers of cytotoxicity such as expression of the degranulation marker CD107a and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) lagged behind, to normalize by treatment week 8, suggesting a clear hierarchy of restoration of NK cell function. Interestingly, inhibition of HCV replication decreased concentrations of CXCR3 ligands CXCL10 and

CXCL11, which are products of IFN-stimulated genes. More important, normalization of NK function was maintained through the end of treatment, indicating that viral cure is equivalent to an immunologic cure, eliminating the deleterious effects of chronic type I IFN, which constitutes the basis of the NK functional dichotomy illustrated (Figure 1). Indeed, type I IFNs, beside eliciting direct antiviral effects during acute infection, may concurrently boost immunoregulatory responses that prevent robust adaptive immune responses. The recent demonstration that blockade of IFN-I signaling during persistent infection redirects the immune environment to enable control of infection and is associated with enhanced IFN- γ production^{16,17} is in keeping with this assumption.

The hitherto unrecognized findings reported by Serti et al¹ are instrumental to our understanding of the pathogenesis of hepatitis C and have several relevant translational implications. The fundamental importance of IFN- γ in the

control of viral infections has been shown in several studies, including its powerful noncytolytic mechanism of viral clearance from infected hepatocytes.¹⁸ Those studies performed *in vitro* and in animal models were also applicable, for instance, to NK cells from a cohort of patients who developed acute HCV infection in the context of human immunodeficiency virus-1 co-infection.¹⁹ Thus, NK cells from patients who spontaneously cleared HCV displayed a greater IFN- γ secretion than those from patients developing chronic infection, and could inhibit HCV replication in the replicon system *in vitro*. DAA therapy has also been shown to rescue T-cell function from the state of exhaustion induced by co-inhibitory receptors like PD-1.²⁰ Interestingly, expression of this molecule gradually decreased under IFN-free treatment, providing direct evidence for a role of chronic exposure to viral antigens in the pathogenesis of T-cell exhaustion (Figure 1). Reconstitution of adaptive immunity to HCV, aided by fully functional NK cells, may also have important clinical implications. Indeed, in contrast with impaired HCV-specific CD8⁺ T cells after pegylated IFN therapy,¹⁴ functionally competent HCV-specific CD8⁺ T cells after IFN-free therapy may confer protection against reinfection. This has recently been questioned by work in multiply exposed injecting drug users (IDU) in whom the frequency, magnitude, and breadth of HCV-specific CD4 and CD8 T-cell responses did not differ among exposed uninfected versus incident cases of HCV infection in seronegative IDU.²¹ Importantly, NK cells were recognized to play a major role in protection from infection, because exposed uninfected subjects had higher frequencies of IFN- γ producing NK cells, and lower frequencies of CD107a expression compared with incident cases of acute hepatitis C, suggesting that significant STAT1 phosphorylation did not occur in exposed uninfected individuals and that IFN- γ is a major determinant of protection against HCV infection and reinfection.

Future avenues of research should address NK cell responses in those few patients who do not respond to DAA treatment, because failure may not entirely depend upon selection of resistance-associated variants. Because the kinetics of virus decay are unlikely to differ among next-generation DAAs, work should be focused on difficult to treat genotypes such as genotype 3, for which no specific efficient antiviral is in sight. Another important mission of NK cells is the control of liver fibrosis. Indeed, despite advancements in the field, no treatment options currently exist to halt fibrogenesis, except eradicating the etiological factor. Hepatic stellate cell (HSC) activation during liver injury is a key step in the development of liver fibrosis and previous studies demonstrated that NK cells selectively kill activated HSC. In addition, IFN- γ produced by NK cells induces HSC apoptosis and cell cycle arrest and subsequently inhibits liver fibrosis (Figure 1).²² Thus, rapid reconstitution of NK cell function after DAA treatment could also contribute to more efficient reversal of severe liver fibrosis and cirrhosis than previously shown with Peg-IFN/ribavirin therapy.²³ The pioneer study by Serti et al is leading the way to an exciting new area of translational research in the field.

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Conflicts of interest

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