Expert Review of Gastroenterology & Hepatology

ISSN: 1747-4124 (Print) 1747-4132 (Online) Journal homepage: informahealthcare.com/journals/ierh20

Natural killer cells in hepatitis C virus recurrence following liver transplantation: what role do they play?

Alejandro Espadas de Arias, Simone Elizabeth Haworth, Tullia Maria Defeo & Francesca Poli

To cite this article: Alejandro Espadas de Arias, Simone Elizabeth Haworth, Tullia Maria Defeo & Francesca Poli (2009) Natural killer cells in hepatitis C virus recurrence following liver transplantation: what role do they play?, Expert Review of Gastroenterology & Hepatology, 3:4, 329-332, DOI: 10.1586/egh.09.29

To link to this article: https://doi.org/10.1586/egh.09.29

Natural killer cells in hepatitis C virus recurrence following liver transplantation: what role do they play?

Expert Rev. Gastroenterol. Hepatol. 3(4), 329–332 (2009)

Alejandro Espadas de Arias
Researcher, Department of Regenerative Medicine, Organ and Tissue Transplantation Immunology, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena – Via Francesco Sforza 35, 20122 Milan, Italy
alejandro.espadas@policlinico.mi.it

Simone Elizabeth Haworth
Researcher, Department of Regenerative Medicine, Organ and Tissue Transplantation Immunology, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena – Via Francesco Sforza 35, 20122 Milan, Italy
simonenicholson@hotmail.co.uk

Tullia Maria Defeo
Gastroenterologist, Department of Regenerative Medicine, Organ and Tissue Transplantation Immunology, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena – Via Francesco Sforza 35, 20122 Milan, Italy
tullia.defeo@policlinico.mi.it

Francesca Poli
Author for correspondence
Head, Histocompatibility and Immunogenetics Laboratory, Department of Regenerative Medicine, Organ and Tissue Transplantation Immunology, Ospedale Maggiore Policlinico, Mangiagalli, Regina Elena, IRCCS, Milan, Italy
Tel.: +39 025 503 4239
Fax: +39 025 501 2573
france.poli@policlinico.mi.it

"Following liver transplantation, hepatitis C virus reinfection is universal and hepatitis occurs in most patients, with a 30% cumulative probability of progression to cirrhosis at 5 years following transplant."

Natural killer (NK) cells are a type of lymphocyte that constitute a major component of the innate immune system [1]. NK cells play an important role in the rejection of tumors and cells infected by viruses through direct or indirect cytolytic mechanisms. The target cells die by apoptosis or necrosis due to the release of two proteins: perforin and granzyme. In this process, pro-inflammatory cytokines, such as IFN-γ, and cytoplasmic granules are also released [2].

In humans, NK cells express killer cell immunoglobulin-like receptors (KIRs) on their surface, which specifically recognize the HLA Class I antigens of the major histocompatibility complex present on target cells. The effector functions of NK cells are influenced by inhibitory receptors for self-HLA Class I ligands HLA-A, -B and -C, with HLA-C and Bw4 being the predominant ligands for most KIR receptors [3]. The dimorphism in amino acids present in the α_1 helix of the HLA-C molecules is characterized by Ser77/Asn80 and Asn77/Lys80 that define distinct allotypes of *HLA-C* (*C1* and *C2*, respectively). The alleles encoding the C1 epitope include *HLA-C*01*, **03*, **07* and **08*, which interact with the KIR receptors 2DL2/3 and 2DS2. The alleles encoding the C2 epitope include *HLA-C*02*, **04*, **05* and **06*, which interact with the 2DL1 and 2DS1 receptors [4,5]. The KIRs 3DL1 and 3DS1 interact with HLA-Bw4, and KIR 3DL2 recognizes HLA-A3 and -A11 [6].

Several studies have pointed out that hepatitis C virus (HCV) persistence might be associated with a defective NK cell response [7–10] and that therapy with IFN would re-establish NK cell activity [11]. In the last few years, several studies highlighting the possible role of KIR receptors and ligands in persistence of HCV infection have been published in the scientific literature [12–14].

In a study that appeared in *Science* in 2004, Khakoo *et al.* demonstrated that *KIR2DL3* and homozygosity for its ligand C1 are associated with resolving HCV infection in patients with a low viral load [12]. In another paper, the same group postulated that different genotypes confer either susceptibility or resistance to infectious diseases [13].

Lopez Vazquez *et al.* identified a protective effect of HLA-Bw4I80 receptor 3DS1 against the development of hepatocellular carcinoma [14]. Paladino *et al.* have shown that NK receptors are associated with the elimination of HCV. In particular, they found that the frequency of the *KIR2DL3* gene was significantly higher in HCV RNA-positive patients with serum transaminase levels above the normal limit [15]. In addition, they found that lower KIR2DL2 and 2DS2 frequencies were associated with increased frequencies of KIR2DS5 in subjects who cleared the virus.

In contrast to the studies described previously, Rauch *et al.* did not find any implication of *KIR* genes in resolving HCV infection. However, these results might be biased owing to a low sample size and/or the lack of information on patient viral load [16].

 10.1586/EGH.09.29 ISSN 1747-4124

Worldwide, the prevalence of HCV infection is 2% [17] and nearly 30% of HCV-positive subjects develop complications, such as cirrhosis and hepatocellular carcinoma. For these individuals, liver transplantation is the best available treatment [18]. Commonly, the criteria for liver allocation do not include donor/recipient HLA compatibility, the immunological factors being less relevant than the clinical ones. Nevertheless, recent reports suggest that alloreactivity of NK cells does exist [19–21].

Does alloreactivity lead to liver rejection?

The role of NK cells in liver rejection is controversial. In animal models, it has been demonstrated that innate immunity (NK/IFN-γ) is activated after liver transplantation with consequent liver damage and inhibition of hepatocyte proliferation [22].

In humans, a study has indicated that compatibility between the HLA-C ligand and KIR receptors would significantly reduce the number of rejection episodes [20], while another study has evidenced that the expression of the C2 epitope is associated with host NK cell inhibition [21]. Such inhibition would lead to an impaired graft outcome.

> "In humans, a study has indicated that the compatibility between HLA-C ligand and KIR receptors would significantly reduce the number of rejection episodes..."

In contrast to these data, Ortel *et al.* concluded that, for liver transplanted patients under standard immunosuppressive therapy, NK alloreactivity did not have an impact on liver acute rejection [19]. Tran [23] and Hanvesakul [21], in two independent studies, analyzed the possible correlation between the donor HLA-C2 epitope and liver survival and have arrived at divergent conclusions. Even though there were some notable differences between the two cohorts, it can be hypothesized that the alloreactivity of NK cells in liver transplantation is not relevant for all liver recipients and that the relative role of NK cells could be obscured by original disease or by the immunosuppressive treatment that the recipients underwent.

The analysis of the NK cell role in different subsets of patients would be an important area of study. In our paper, no correlation was found between the occurrence of rejection episodes and HCV progression [24].

Do NK cells & their ligands affect the risk of severe HCV recurrence?

Following liver transplantation, HCV reinfection is universal [25] and hepatitis occurs in most patients, with a 30% cumulative probability of progression to cirrhosis at 5 years following transplant [18,26,27]. Its progression to liver fibrosis seems to be modulated by NK alloreactivity.

Patients with low pretransplant concentrations of NK $CD56^+$ cells develop a more severe HCV recurrence than that of subjects with a high concentration [28]. Moreover, a high viral load is correlated with impaired graft survival and function [27]. Rosen has postulated that the pretransplant innate immune response is linked with post-transplant HCV recurrence [28], while Golden-Mason has proposed a model in which NK cells might be the primary target for HCV immune-evasion strategies [29].

We have shown how the presence of KIR2DL3 in liver transplant recipients correlated with the progression to liver fibrosis and that mismatching of HLA–KIR ligands favored the progression to fibrosis only in the presence of KIR2DL3 [24]. We have also postulated that, in immunocompetent individuals, NK cells with the receptor KIR2DL3 would have a protective effect only during the first stage of the infection [12] but not later when the viral load increases and the innate immune response is possibly overwhelmed [30,31].

> "Patients with low pretransplant concentrations of natural killer $CD56^+$ cells develop a more severe hepatitis C virus recurrence than that of subjects with a high concentration."

The recipient incapacity to eliminate HCV, would lead to liver damage caused by the immune response. In fact, HCV *per se* is not cytopathic and viral load does not correlate with the degree of liver fibrosis. However HCV induces the production of cytokines (e.g., IFN-γ, IL-12, IL-18 and IL-15) from hepatocytes that activate NK cells, which may result in liver damage.

The weak inhibitory receptor–ligand interaction, such as KIR2DL3–HLA-C1, allows a more rapid NK-cell activation than that of a strong inhibitory receptor–ligand interaction, such as KIR2DL2–HLA-C1 or KIR2DL1–HLA-C2 [32]. The NK cells develop and activate $CD4^+$ and $CD8^+$ T cells. If persistent, this activation damages the liver due to the accumulation of these cells in liver parenchyma [33].

The absence of the KIR2DL3 receptor and matching for the HLA-KIR epitopes in HCV-positive liver-transplant recipients might avoid a continuous activation of NK cells. Conversely, the NK cell receptor KIR2DL3 might modify cell activity in the presence of the mismatch, facilitating the progression to fibrosis.

Does the presence of a particular KIR haplotype favor the development of liver fibrosis?

So far, no correlation has been found between the different KIR haplotypes and liver fibrosis [24]. However, because of the rapid evolution of KIR genes and haplotype diversity, the role of this parameter is difficult to assess.

Are there other factors that correlate with liver graft outcomes for HCV-positive patients?

In addition to NK-cell alloreactivity, other factors correlate with liver graft outcomes. Genotype 1b [34] and cytomegalovirus-positive patients [35] are at a greater risk of developing HCV recurrence. There is also some evidence that HLA-DRB1 donor/recipient matching plays a significant role; especially in the presence of *HLA-DRB1*11* in the recipient which correlated with a decreased risk of HCV recurrence [36]. Several clinical factors, including those related to the virus, the host and the donor, are probably implicated in post-transplantation HCV-related disease

progression. For instance, a donor age of over 50 years is unanimously recognized as a detrimental factor in the progression to liver fibrosis [37,38].

Is it possible to improve liver graft outcomes in HCV-positive recipients?

There is a compelling need for improving liver graft outcome in HCV-positive recipients. To pursue this goal, we have to take into consideration all factors already known and associated with donor and recipient clinico–pathological features. In addition, we have to consider the increasing evidence that NK cell alloreactivity may play an important role. Therefore, we should further investigate this factor in the current matching and selection of HCV-positive recipients of liver transplantation. If larger studies confirm that genetic components contribute to both NK cell-mediated control of HCV and to liver injury in the transplant setting, this will help to develop more appropriate allocation policies and to ensure the optimal use of the limited organs available.

Acknowledgements

The authors thank Ferruccio Bonino and Mario Scalamogna for their critical review of the manuscript.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest

1 Moretta A, Bottino C, Mingari MC, Biassoni R, Moretta L. What is a natural killer cell? *Nat. Immunol.* 3(1), 6–8 (2002).

2 Krug A, French AR, Barchet W *et al.* TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. *Immunity* 21(1), 107–119 (2004).

3 Vilches C, Parham P. KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. *Annu. Rev. Immunol.* 20(1), 217–251 (2002).

4 Mandelboim O, Reyburn HT, Vales-Gomez *et al.* Protection from lysis by natural killer cells of group 1 and 2 specificity is mediated by residue 80 in human histocompatibility leukocyte antigen C alleles and also occurs with empty major histocompatibility complex molecules. *J. Exp. Med.* 184(3), 913–922 (1996).

5 Falk CS, Schendel DJ. HLA-C revisited. Ten years of change. *Immunol. Res.* 16(2), 203–214 (1997).

6 Pende D, Biassoni R, Cantoni C *et al.* The natural killer cell receptor specific for HLA-A allotypes: a novel member of the p58/p70 family of inhibitory receptors that is characterized by three immunoglobulin-like domains and is expressed as a 140-kD disulphide-linked dimer. *J. Exp. Med.* 184(2), 505–518 (1996).

7 Corado J, Toro F, Rivera H, Bianco NE, Deibis L, De Sanctis JB. Impairment of natural killer (NK) cytotoxic activity in hepatitis C virus (HCV) infection. *Clin. Exp. Immunol.* 109(3), 451–457 (1997).

8 Bonavita MS, Franco A, Paroli *et al.* Normalization of depressed natural killer activity after interferon-α therapy is associated with a low frequency of relapse in patients with chronic hepatitis C. *Int. J. Tissue React.* 15(1), 11–16 (1993).

9 Deignan T, Curry MP, Doherty DG *et al.* Decrease in hepatic $CD56^+$ T cells and $V\alpha 24^+$ natural killer T cells in chronic hepatitis C viral infection. *J. Hepatol.* 37(1), 101–108 (2002).

10 Kawarabayashi N, Seki S, Hatsuse K *et al.* Decrease of $CD56^+$ T cells and natural killer cells in cirrhotic livers with hepatitis C may be involved in their susceptibility to hepatocellular carcinoma. *Hepatology* 32(5), 962–969 (2000).

11 Yamagiwa S, Matsuda Y, Ichida T *et al.* Sustained response to interferon-α plus ribavirin therapy for chronic hepatitis C is closely associated with increased dynamism of intrahepatic natural killer and natural killer T cells. *Hepatol. Res.* 38(7), 664–672 (2008).

• **Provides evidence that *KIR2DL3* and homozygosity for its ligand C1 are directly involved in resolving hepatitis C virus (HCV) infection.**

12 Khakoo SI, Thio CL, Martin MP *et al.* HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 305 (5685), 872–874 (2004).

13 Carrington M, Martin MP. The impact of variation at the *KIR* gene cluster on human disease. *Curr. Top. Microbiol. Immunol.* 298, 225–257 (2006).

14 Lopez-Vazquez A, Rodrigo L, Martinez-Borra J *et al.* Protective effect of the HLA-Bw4I80 epitope and the killer cell immunoglobulinlike receptor *3DS1* gene against the development of hepatocellular carcinoma in patients with hepatitis C virus infection. *J. Infect. Dis.* 192(1), 162–165 (2005).

15 Paladino N, Flores AC, Marcos CY *et al.* Increased frequencies of activating natural killer receptors are associated with liver injury in individuals who do not eliminate hepatitis C virus. *Tissue Antigen* 69(Suppl. 1), 109–111 (2007).

16 Rauch A, Laird R, McKinnon E *et al.* The Swiss HIV Cohort Study. Influence of inhibitory killer immunoglobulin-like receptors and their HLA-C ligands on resolving hepatitis C virus infection. *Tissue Antigen* 69(Suppl. 1), 237–240 (2007).

17 Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect. Dis.* 5(9), 558–567 (2005).

18 Berenguer M, Lopez-Labrador FX, Wright TL. Hepatitis C and liver transplantation. *J. Hepatol.* 35(5), 666–678 (2001).

19 Oertel M, Kohlhaw K, Diepolder HM *et al.* Alloreactivity of natural killer cells in allogeneic liver transplantation. *Transplantation* 72(1), 116–122 (2001).

20 Bishara A, Brautbar C, Zamir G, Eid A, Safadi R. Impact of HLA-C and Bw epitopes disparity on liver ransplantation outcome. *Hum. Immunol.* 66(11), 1099–1105 (2005).

• **Reveals the importance of natural killer cells in chronic rejection and graft cirrhosis.**

21 Hanvesakul R, Spencer N, Cook M *et al.* Donor HLA-C genotype has a profound impact on the clinical outcome following liver transplantation. *Am. J. Transplant.* 8(9), 1931–1941 (2008).

• **Demonstrates that the innate immunity contributes to liver injury.**

22 Shen K, Zheng SS, Park O, Wang H, Sun Z, Gao B. Activation of innate immunity (NK/IFN-γ) in rat allogeneic liver transplantation: contribution to liver injury and suppression of hepatocyte proliferation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294(4), G1070–G1077 (2008).

23 Tran TH, Middleton D, Döhler B *et al*. Reassessing the impact of donor HLA-C genotype on long-term liver transplant survival. *Am. J. Transplant*. DOI: 10.1111/j.1600-6143.2008.02341.x (2009) (Epub ahead of print).

• **Evidences the role of receptor KIR2DL3 and HLA-C ligands in the outcome of HCV liver transplant.**

24 Espadas de Arias A, Haworth SE, Belli *et al*. Killer cell immunoglobulin-like receptor genotype and killer cell immunoglobulin-like receptor-human leukocyte antigen C ligand compatibility affect the severity of hepatitis C virus recurrence after liver transplantation. *Liver Transpl*. 15(4), 390–399 (2009).

25 Davis GL. Chronic hepatitis C and liver transplantation. *Rev. Gastroenterol. Disord*. 4(1), 7–17 (2004).

26 Feray C, Caccamo L, Alexander GJ *et al.*; European Concerted Action on Viral Hepatitis (EUROHEP) Group. European collaborative study on factors influencing outcome after liver transplantation for hepatitis C. *Gastroenterology* 117(3), 619–625 (1999).

27 Charlton M, Seaberg E, Wiesner R *et al*. Predictors of patient and graft survival following liver transplantation for hepatitis C. *Hepatology* 28(3), 823–830 (1998).

28 Rosen HR, Doherty DG, Madrigal-Estebas L, O'Farrelly C, Golden-Mason L. Pretransplantation $CD56^+$ innate lymphocyte populations associated with severity of hepatitis C recurrence. *Liver Transpl.* 14(1), 31–40 (2008).

• **Proposes a theoretical model for HCV persistence that places the natural killer cell at the center of HCV immune evasion strategies.**

29 Golden-Mason L, Rosen HR. Natural killer cells: primary target for hepatitis C virus immune evasion strategies? *Liver Transpl.* 12(3), 363–372 (2006).

30 Ciccorossi P, Maina AM, Oliveri F *et al*. Viral load 1 week after liver transplantation, donor age and rejections correlate with the outcome of recurrent hepatitis. *Liver Int*. 27(5), 612–619 (2007).

31 Massaguer A, Ramírez S, Carrión JA *et al*. Evolution of the NS3 and NS5B regions of the hepatitis C virus during disease recurrence after liver transplantation. *Am. J. Transplant*. 7(9), 2172–2179 (2007).

32 Winter CC, Gumperz JE, Parham P, Long EO, Wagtmann N. Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. *J. Immunol*. 161(2), 571–577 (1998).

33 Bonorino P, Leroy V, Dufeu-Duchesne T *et al*. Features and distribution of CD8 T cells with human leukocyte antigen class I-specific receptor expression in chronic hepatitis C. *Hepatology* 46(5), 1375–1386 (2007).

34 Bruno S, Crosignani A, Maisonneuve P, Rossi S, Silini E, Mondelli MU. Hepatitis C virus genotype 1b as a major risk factor associated with hepatocellular carcinoma in patients with cirrhosis: a seventeen-year prospective cohort study. *Hepatology* 46(5), 1350–1356 (2007).

35 Rosen HR, Chou S, Corless CL *et al*. Cytomegalovirus viremia: risk factor for allograft cirrhosis after liver transplantation for hepatitis C. *Transplantation* 64(5), 721–726 (1997).

36 Belli LS, Burra P, Poli F *et al*. HLA-DRB1 donor–recipient mismatch affects the outcome of hepatitis C disease recurrence after liver transplantation. *Gastroenterology* 130(3), 695–702 (2006).

37 Berenguer M, Prieto M, San Juan F *et al*. Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. *Hepatology* 36(1), 202–210 (2002).

38 McCaughan GW, Shackel NA, Bertolino P, Bowen DG. Molecular and cellular aspects of hepatitis C virus reinfection after liver transplantation: how the early phase impacts on outcomes. *Transplantation* 87(8), 1105–1111 (2009).