Hepatology. Author manuscript; available in PMC 2014 October 20.

Published in final edited form as:
Hepatology. 2010 April ; 51(4): 1168–1175. doi:10.1002/hep.23477.

Consistent Beneficial Effects of Killer Cell Immunoglobulin-Like Receptor 2DL3 and Group 1 Human Leukocyte Antigen-C Following Exposure to Hepatitis C Virus

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Abstract

Natural killer cells are a key component in the immune control of viral infections. Their functions are controlled by inhibitory receptors for major histocompatability complex (MHC) class I, including the killer cell immunoglobulin-like receptors (KIR). KIR2DL3 in combination with its cognate human leukocyte antigen (HLA)-C ligand has been shown to be associated with spontaneous resolution of viremia following hepatitis C virus (HCV) infection. In order to determine if this gene combination is advantageous across all potential outcomes following HCV exposure, we studied individuals with apparent resistance to HCV infection who remain seronegative and aviremic despite long-term injection drug use and also individuals chronically infected with HCV who successfully clear HCV with treatment. Homozygosity for KIR2DL3 in combination with group 1 HLA-C allotypes was more frequent in exposed seronegative aviremic individuals as compared to those with chronic HCV (25.0% versus 9.7%, $P = 0.003$, odds ratio [OR] = 3.1, 95% confidence interval [CI] = 1.3-7.1) in a model similar to that found for those spontaneously resolving HCV. In individuals undergoing treatment for HCV, those with KIR2DL3 and group 1 HLA-C were more likely to make a sustained virological response (SVR) ($P = 0.013$, OR = 2.3, 95% CI = 1.1-4.5). KIR and HLA-C protection in both treatment response and spontaneously resolving HCV was validated at the allelic level, in which KIR2DL3-HLA-Cw*03 was associated with SVR ($P = 0.004$, OR = 3.4, 95% CI = 1.5-8.7) and KIR2DL3/

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Potential conflict of interest: Nothing to report.

Europe PMC Funders Author Manuscripts

KIR2DL3-HLA-Cw*03 was associated with spontaneous resolution of HCV infection ($P = 0.01$, OR = 2.3, 95% CI = 1.2-4.4).

Conclusion—KIR and HLA-C genes are consistently beneficial determinants in the outcome of HCV infection. This advantage extends to the allelic level for both gene families.

Hepatitis C virus (HCV) is a common chronic viral infection. The virus poses a significant challenge to the immune system as the majority of individuals exposed to HCV fail to spontaneously clear the virus, develop a chronic infection, and are predisposed to cirrhosis and hepatocellular carcinoma. This failure to mount a successful immune response is multifactorial in nature and includes abnormalities in T, B, and dendritic cell responses.

Natural killer (NK) cells are a subset of lymphocytes that interact directly with virus-infected cells, but can also activate dendritic cells and secrete Th1-type cytokines to augment antiviral cytotoxic T-cell responses. Their responses are controlled by multiple activating and inhibitory receptors and it is thought that the net inhibitory or activating signal derived from these receptors determines whether or not they become activated. NK cells are enriched in the liver in comparison to peripheral blood.[1] However, their role in the outcome of HCV infection remains controversial. Initial work demonstrated that NK cell killing was depressed in chronic HCV, that this could be restored with interferon treatment,[2,3] and that they had low levels of perforin.[4] Conversely, more recent work has suggested that NK cell cytotoxicity in chronic HCV may not be impaired,[5] and may even be augmented, with an impairment of Th1 type cytokine secretion, and augmented interleukin 10 (IL-10) secretion.[6-8] Phenotypic studies have also given conflicting results in terms of expression of natural cytotoxicity receptors.[7,9] Alterations of NK cell function have been related to the binding of the HCV envelope proteins to CD81. Cross-linking of this molecule can impair NK cell function *in vitro*.[10,11] However, this effect cannot be demonstrated for infectious HCV particles and so is thought unlikely to occur *in vivo*.[12] More recently, the changes in NK cell phenotype and function have been associated with chronic exposure to interferon-alpha (IFN-α).[8]

One family of receptors that make a substantial contribution to the NK cell receptor repertoire are the killer-cell immunoglobulin-like receptors (KIR). The ligands for these receptors are human leukocyte antigen (HLA) class I. Nearly all NK cells express an inhibitory receptor for self-HLA class I, which are important for both NK cell development and function.[13,14] The KIRs are expressed exclusively on the $CD56^{dim}$ subset of NK cells,[22] and this perforin and granzyme-rich subset is reduced in individuals with chronic HCV infection.[15] One feature of KIR, in comparison to other NK cell receptors, is their substantial genetic diversity.[16] The KIR locus is rapidly evolving and exhibits significant population diversity.[17] Thus, different individuals can have different numbers of KIR genes. Furthermore, the ligand for the inhibitory KIR are subsets of the polymorphic HLA class I molecules, HLA-A,-B, and -C, and this generates a further level of diversity.[18]

Exposure to HCV leads to a number of clinical profiles that may be determined by the host immune response. Individuals may remain HCV Ab-negative and HCV RNA-negative (seronegative, aviremic), HCV Ab-positive HCV RNA-negative (spontaneous resolver), and HCV Ab-positive HCV RNA-positive (chronically infected). This latter group may be

further subdivided into those who clear HCV RNA following therapy (sustained virological responders) and those who do not. KIR2DL3 binds a subset of HLA-C allotypes that include HLA-Cw*01, -Cw*03, -Cw*07, -Cw*08 and Cw*12, Cw*14 and Cw*16. Collectively, these are called the group 1 HLA-C (HLA-C1) allotypes. HLA-Cw*01 was shown to be protective against HCV infection, and it was subsequently shown in two independent studies that individuals who spontaneously resolve infection are more likely to have the gene KIR2DL3 in combination with its cognate HLA-C1 ligands than those who remain chronically infected.[19,20] This receptor:ligand combination is thought to provide weaker inhibitory signals than other inhibitory KIR:HLA-C receptor:ligand pairings and thus permit a more responsive NK cell phenotype.[21,22] Furthermore, although KIR3DS1 is protective against HCV-associated hepatocellular carcinoma, it is not known if the HLA-C-specific KIR influences the course of the other stages of infection.[23]

Seronegative aviremic individuals with a long history of likely HCV exposure through high-risk injection drug use have detectable HCV-specific T-cell responses in up to 60% of cases, despite the absence of these conventional markers of infection.[24,25] These individuals have been previously termed “exposed uninfected”[31] with “uninfected” indicating the absence of demonstrable infection, as defined by conventional antibody or RNA testing, at the time of recruitment. The presence of T-cell responses to nonstructural antigens may indicate that at some stage they have had a replicative infection the magnitude of which is not clear.[25] Nevertheless, they appear to have a favorable immune response to HCV infection and, consistent with an augmented T-cell response being important to the HCV status of these individuals, they also have an increased frequency of a high IL-12-producing allele.[26] It is noteworthy that IL-12 is also an NK cell activating cytokine.

Another potent NK cell activating cytokine is interferon-alpha (IFN-α). It increases both NK cell cytotoxicity and IFN-γ production. IFN-α in combination with ribavirin forms the cornerstone of current anti-HCV therapies. Treatment with IFN-α and ribavirin is effective in over 50% of treated individuals, with the most important determinant of outcome being viral genotype. However, there is a considerable heterogeneity in response to IFN-α and host genetic factors also play a role.[27-29] Moreover, it has also been shown that treatment-induced or enhanced cellular immune responses are important in the outcome of the treatment of HCV infection.[30-32] Successful treatment with IFN-α has been associated with augmented cytotoxicity and up-regulation of activating receptors, implying a normalization of NK cell function.[3,9] These findings are controversial because of recent work showing that cytotoxicity and activating receptor expression are already up-regulated in chronic HCV infection.[6-8] However, IFN-α stimulated expression of MIC-A and -B, the ligand for the activating NK cell receptor NKGD, is impaired on monocyte-derived dendritic cells from HCV-infected individuals.[33] Therefore, the balance of activating and inhibitory interactions may be important in determining the outcome of IFN-α treatment in HCV infection, and this may be related to effects on target cells, in addition to the NK cells themselves. The role of NK cell receptors in HCV infection is thus complex. The aim of this study was to study one aspect of this complexity, namely, to determine whether the gene combination of KIR2LD3 and group 1 HLA-C is beneficial across the spectrum of potential clinical outcomes following HCV exposure.

Patients and Methods

Seronegative Aviremic Cohort

Forty-eight HCV exposed but seronegative aviremic cases were recruited from Dartmoor prison (n = 19) and from various needle exchange and community drug services in Plymouth (n = 29), between 2001 to 2007, with Ethical Committee approval. Individuals with a substantial history of past or present intravenous drug use, sharing of needles or other drug injection equipment, and who tested negative for both HCV antibody and HCV RNA were included. No other selection criteria were applied. The absence of HCV antibodies was determined by third-generation enzyme-linked immunosorbent assay (ELISA; Abbott IMx, Abbott Diagnostics, Maidenhead, UK), and HCV RNA by commercially available qualitative polymerase chain reaction (PCR; Amplicor, Roche, Basel, Switzerland). All were HIV-and hepatitis B surface antigen (HBsAg)-negative. Detailed information about drug-injecting behavior was ascertained by means of a structured questionnaire (Table 1). Mean duration of drug use was 8.4 years with an estimated median number of injection episodes of over 4,000.

Intravenous Drug Use (IDU) Cohort

A total of 257 Caucasian individuals were recruited from the hepatology clinics at Southampton General Hospital from 2003 to 2007, with Ethical Committee approval. These individuals were selected on the basis of race and having intravenous drug usage as their only parenteral risk factor for acquiring HCV (Table 1). HBsAg-and HIV-positive individuals were excluded. All individuals were positive by second-generation ELISA and confirmed viremic using the HCV COBAS Amplicor system (Roche). The mean age was 43.9 years, 187 (72.8%) were male, and seven had biopsy-proven cirrhosis.

Interferon-Treated Cohort

In all, 208 individuals with chronic HCV underwent treatment for chronic HCV infection. These individuals were selected on the basis of having completed >80% of a course of IFN-α-based treatment, and of these 32, individuals had dosage modifications to their treatment regimen. A total of 165 were from the IDU cohort and the remainder denied IDU as a risk factor for HCV infection. Of these, 18 had received blood products, 20 had an unknown route of infection, and five had potential exposure by needlestick injury or tattooing. Individuals coinfected with HBV or HIV were excluded. These individuals were also recruited from the hepatology clinics at Southampton General Hospital, with Ethical Committee approval between 2003 and 2007. All had viremia confirmed by the COBAS Amplicor system. HCV genotyping was performed using quantitative PCR (iQur, Southampton, UK). Details of this cohort are given in Table 2. Individuals were determined to have a sustained response to therapy if they had a negative HCV-RNA test at the end of treatment and a second negative test at least 6 months after cessation of treatment using the same assays.

Retrospective Analysis

For the retrospective analysis of resolved against chronically infected individuals the previously described UK cohort of HCV exposed individuals was used.[19] This group consisted of 341 individuals (122 HCV Ab-positive, RNA-negative, and 219 HCV Ab-positive, RNA-positive). The mean age was 41.9 years, 339 (99%) were Caucasian, and 227 (66.5%) were male. There was no significance in these parameters between the Ab-positive, RNA-negative, and Ab-positive, RNA-positive groups.[19] These individuals were recruited between 1999 to 2003.

KIR and HLA Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes conventionally using a salt precipitation method or the QIAamp blood kit (Qiagen, Crawley, UK). KIR genotyping was performed using the Lifematch kit (Tepnel, Stamford, CT) and by PCR using sequence specific primers as described.[19] HLA typing was performed by direct sequencing of PCR products.[34] *HLA* types that were not resolved by sequencing or which gave unusual results were also tested by sequence specific oligonucleotide probe typing (PCR-SSOP), using commercial kits (Dynal, RELI SSO, Wirral, UK).

Statistical Analysis

Statistical analysis was performed using SPSS v. 17. The data presented were analyzed by chi-squared analysis unless otherwise stated. The Bonferroni correction was applied where relevant. Binomial logistic regression was performed using SPSS version 17 using the ENTER method.

Results

Group 1 HLA-C and KIR2DL3 Is Overrepresented in Exposed Seronegative Aviremic Individuals

Forty-eight individuals with apparent resistance to HCV infection from injection drug use were typed for HLA-C and for KIR2DL2 and KIR2DL3. Twenty-four of these had been tested for T-cell responses by ELISPOT assay,[24] of whom 15 had a positive response to at least one of the HCV antigens studied. The KIR genotypes in these 48 cases were compared to the 257 individuals with chronic HCV acquired from IDU. Both groups had similar frequencies of KIR2DL2 and KIR2DL3 alleles, and group 1 and 2 HLA-C alleles (Table 3). Furthermore, there was no difference in the frequency of KIR2DL3 or KIR2DL2 in combination with one group 1 HLA-C allele. However, the combination of homozygosity for KIR2DL3 and 2 group 1 HLA-C alleles was found at a significantly greater frequency in the exposed seronegative aviremic individuals as compared to chronically infected individuals (25.0% versus 9.7%, $P = 0.003$, odds ratio [OR] = 3.1, 95% confidence interval [CI] = 1.3-7.1). This recessive model of protection from HCV infection is similar to that observed for antibody-positive HCV-exposed individuals.[19,20]

Group 1 HLA-C in Combination with KIR2DL3 Is Beneficial in Chronic HCV Infection

In all, 165 of the IDU with chronic HCV infection and 43 additional individuals with chronic HCV infection had been treated with IFN-α-based therapy in our outpatient clinics. The frequencies of KIR2DL2 and KIR2DL3 were similar between those with and those without a sustained virological response (SVR). Individuals who had an SVR to IFN-α therapy were more likely to have at least one group 1 HLA-C allotype than those without SVR (87.9% versus 75.2%, $P = 0.02$, OR = 2.4, 95% CI = 1.1-5.3) (Table 4). Consideration of HLA-C with their cognate KIR receptors demonstrated that the beneficial effect of group 1 HLA-C was mediated in combination with KIR2DL3 (82.2% versus 67.3%, $P = 0.01$, OR = 2.3, 95% CI = 1.2-4.3), but not KIR2DL2 (43% versus 39.6%, $P > 0.1$). Furthermore, binomial logistic regression in a model considering group 1 HLA-C in the presence or the absence of KIR2DL3 demonstrated that SVR was associated with group 1 HLA-C only in combination with KIR2DL3 ($P = 0.016$, OR = 2.49, 95% CI = 1.19-5.22). In contrast to our findings for the exposed seronegative aviremic individuals, we observed no statistically significant association with homozygosity of KIR2DL3: HLA-C group 1 being protective ($P > 0.1$). Logistic regression analysis demonstrated that the effect of KIR2DL3:HLA-C group 1 was independent of genotype, age, ALT, dose modifications, duration, cirrhosis, and gender within our cohort (Table 5). It was also independent of whether the individual had received the current gold standard of therapy (pegylated interferon and ribavirin versus other protocols, $P = 0.028$, OR = 2.34, 95% CI = 1.01-5.00), the use of pegylated versus standard interferon ($P = 0.033$, OR = 2.27, 95% CI = 1.07-4.82), and more weakly independent of the use of ribavirin ($P = 0.057$, OR = 2.13, 95% CI = 0.98-4.66). Standardized viral load data on the cohort was not available and therefore could not be included in this analysis.

Allelic Specificity of Protection by HLA-C and KIR

Although the specificity of KIR2DL3 for HLA-C is determined by residue 80 of the MHC class I heavy chain, the binding of KIR to HLA-C is determined by several additional residues. Recent work has shown that HLA-C allelic diversity can affect binding of KIR to HLA-C, and hence different HLA-C alleles within the C1 grouping may have different protective effects in HCV infection.[22] To test this model we determined the frequency with which specific group 1 HLA-C alleles were beneficial in combination with KIR2DL3 in the IFN-treated population. HLA-Cw*03 alleles were found more frequently in combination with KIR2DL3 in individuals with SVR than in those without SVR (22.4% versus 7.9%, $P = 0.004$, OR = 3.4, 95% CI = 1.5-8.7) (Table 6). This trend was observed in individuals treated with pegylated IFN-α and ribavirin (21.8% with SVR versus 11.3% without SVR, $P = 0.06$), and also in those with other regimens (3 out 11 with SVR versus 1 out of 39 without SVR $P = 0.03$, Fisher's exact test). By logistic regression analysis the beneficial effect of KIR2DL3-Cw*03 was also independent of genotype, age, ALT, dose modifications, duration, cirrhosis, and gender and the following treatment regimens: pegylated interferon and ribavirin versus other protocols ($P = 0.028$, OR = 3.06, 95% CI = 1.13-8.31), the use of pegylated versus standard interferon ($P = 0.032$, OR = 2.94, 95% CI = 1.10-7.84), and the use of ribavirin ($P = 0.019$, OR = 3.41, 95% CI = 1.23-9.49). Overall, HLA-Cw*12 in combination with KIR2DL3 was also more common in those with SVR (12.1% versus 4.0%, $P = 0.03$, OR = 3.4, 95% CI = 1.0-14.6). The most frequent group 1 HLA-C allele,

HLA-Cw*07 (27% of all HLA-C alleles in this population), was not associated with SVR, indicating that the described associations are not related purely to an effect of study power.

As both treatment-induced and spontaneously resolving HCV infection are associated with KIR2DL3 and group 1 HLA-C alleles, we determined if the same HLA-C alleles were associated with spontaneously resolving HCV infection in our previously described UK cohort.[19] Data were available on 341 individuals (122 with resolved infection and 219 persistently infected). Individuals with spontaneous resolution of HCV, as compared to those remaining persistently infected, were more likely to have the combination of HLA-Cw*03 and KIR2DL3 (31.2% versus 19.2%, $P = 0.01$ OR = 1.9, 95% CI = 1.1-3.2) (Table 7). Furthermore, this was only beneficial in individuals homozygous for KIR2DL3 (18.9% versus 9.1%, $P = 0.01$, OR = 2.3, 95% CI = 1.2-4.4), indicating that this is not purely an effect of HLA and so unlikely to be due to linkage disequilibrium with another gene at the MHC. HLA-Cw*12 in combination with KIR2DL3 was also found more frequently in those with resolved HCV infection, but this did not reach statistical significance. No specific effects of HLA-C allelism were noted in the exposed seronegative aviremic cohort, with the frequency of HLA-Cw*03 in combination with KIR2DL3 being 16.7% in the exposed seronegative aviremic individuals as compared to 18.3% in the chronically infected IDU. The increase in group 1 HLA-C allotypes as compared to the chronically infected individuals is thus related to small changes across the range of group 1 HLA-C alleles.

Discussion

This work shows that KIR2LD3 and its group 1 HLA-C ligand are associated with favorable outcomes across a spectrum of clinical profiles following HCV exposure. These data, combined with our previous work and that of Romero et al.,[20] show a beneficial influence of these genes in HCV infection for the following subgroups of individuals: those having an apparent degree of resistance to HCV infection becoming established; those with spontaneous resolution of established infection; and those who resolve chronic infection with IFN-α-based treatment.

The exposed seronegative aviremic individuals have no detectable anti-HCV Ab or RNA, but up to 60% of these individuals will have detectable T-cell responses to HCV antigens, implying anti-HCV immunoreactivity.[24,35] The finding of a high frequency of individuals homozygous for KIR2DL3 and group 1 HLA-C alleles implies that they are a population selected out by exposure to HCV. Indeed, the frequency of this combination of genes is remarkably similar to that found for Ab-positive RNA-negative individuals.[19] Thus, they appear to be distinct from this subgroup of individuals on the basis of a lack of anti-HCV Ab, but not KIR:HLA genotype. This implies that that they do not have an additional NK cell protection above that observed with the Ab-positive, RNA-negative group of individuals. Consistent with the beneficial association in the exposed seronegative aviremic cases, the advantageous effect of the KIR:HLA genotype in spontaneous clearers was strongest in those who acquired HCV infection from IDU, suggesting that the size of inoculum is important. The chronically infected IDU comparator population for the exposed seronegative aviremic individuals had a frequency of KIR2DL3-C1 similar to that of a US cohort of Caucasians with chronic HCV.[36] This implies that the KIR:HLA genotypes in our

population are representative of Caucasians with chronic HCV infection, and not due to a selection bias.

KIR2DL3-C1 was associated with SVR in our IFN-α-treated individuals, but homozygosity for these genes was not. One possibility is that the activation of NK cells by pharmacological doses of IFN-α can overcome the inhibition mediated by other NK cell receptors and hence NK cells that coexpress KIR2DL3 and KIR2DL1 may be antiviral in this context, but not in spontaneously resolving infection. Alternatively, KIR protection could also be related to an effect on KIR-positive T cells in IFN-α-induced resolution, but NK cells in spontaneous resolution, as individuals who are homozygous for KIR2DL3-C1 would be predicted to have more NK cells that expressed KIR2DL3 than those who were heterozygous for either gene. However, we cannot rule out that in all stages of chronic infection it is related to an effect of KIR2DL3 on the KIR-positive T cells, which are usually of the effector memory phenotype, and can be found in chronic HCV at similar numbers to healthy controls.[37] Furthermore, the odds ratio is smaller for this group of individuals than for the exposed seronegative aviremic cohort, implying that although these alleles are associated with viral clearance they may be less important than in spontaneously resolving infection. Thus, they are one of several factors that determine outcome following IFN-α-based therapy, of which the most important is viral genotype. One complicating factor in the interpretation of the data is that cirrhosis is an adverse factor in response to antiviral therapy for HCV. Recent work has suggested that homozygosity for KIR2DL3-C1 is associated with the development of cirrhosis in chronic HCV infection and that KIR and HLA-C may affect the severity of liver disease following transplantation for HCV infection.[36,38] Thus, the beneficial effect of KIR2DL3-C1 homozygosity on viral clearance may be balanced by the adverse effect that this gene combination has on disease stage. This could be one explanation as to why we have not observed KIR2DL3-C1 homozygosity as an advantageous factor in these individuals.

Our observations of KIR:MHC extend to the allelic level for HLA-C for the IFN-treated patients and also those resolving infection. Overall, across all groups we found a trend toward most group 1 HLA-C alleles apart from Cw*07 being beneficial. There is no clear data that shows that HLA-Cw*07 is a stronger binder of KIR than other HLA-C allotypes, but it may be crossreactive with KIR2DL1 and this crossreactivity may be relevant as a mechanism for generating alternative inhibitory signals.[39] MHC has a complex influence on both NK cell education and function[40] and NK cells from HLA-Cw*07-positive individuals make significantly more IFN-γ when challenged with an MHC class I-negative target than those with other HLA-C allotypes.[40] These data suggest that NK cells from HLA-Cw*07-positive individuals may be “licensed” differently from those with other group 1 HLA-C allotypes. The effect of this may be to respond to activating signals with a different magnitude of IFN-γ secretion. One effect of this could be a change in the NK cell response to HCV infection. Furthermore, KIR2DL2 and KIR2DL3 segregate as alleles at a single locus and protection in HCV is only observed with KIR2DL3. Thus, allelism at both the KIR and the HLA-C loci appear to influence the outcome of HCV infection. As KIR2DL3 is sensitive to the peptide bound by HLA-C this raises the possibility that HCV-derived peptides may also affect NK cell reactivity. Although functional work is required to tease

out the precise mechanisms of inhibitory KIR-mediated protection in viral infections, our data underscore the relevance of this gene family in the immune response to HCV.

Acknowledgments

We thank Karen Parker and all the hepatology specialist nurses at Southampton General Hospital for their assistance in performing this study.

Supported by a Wellcome Trust Senior Clinical fellowship (to S.I.K.) and a grant from the DANA foundation.

Abbreviations

HCV	hepatitis C virus
HLA	human leukocyte antigen
HLA-C1	group 1 HLA-C allotype
IFN	interferon
KIR	killer cell immunoglobulin-like receptor
MHC	major histocompatability complex
PEG-IFN	pegylated-interferon
SVR	sustained virological response

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Table 1

Demographics and Drug-Injecting Behavior of Exposed Seronegative Aviremic and Chronically Infected IDU Cohorts

	Exposed Seronegative Aviremic n (%)	Chronic IDU n (%)	*P* Value
Number of patients	48	257	
Sex (male)	39 (81%)	187 (72.8%)	0.22
Age (years)	28.8 ± 5.2	43.9 ± 9.8	<0.001
Caucasian	48 (100)	243 (94.6%)	0.14
Age at first injection drug use (years)	19 ± 5	–	
Duration of injection drug use (years)	8.4 ± 7.5 (0.5-24)	–	
Lifetime injection episodes (median n)	4380 (36-17,520)	–	
Frequency of injection drug use (n)			
4-6 times a day	2 (4%)	–	
2-3 times a day	33 (69%)		
Daily	8 (17%)		
Every few days/weekly	3 (6%)		
Monthly or less	2 (4%)		
Currently using (n)	13 (27%)	–	
Sharing behavior (n)			
Any sharing	48 (100%)	–	
Shared needles	44(91.7%)		
Shared syringes	42 (87.5%)		
Shared other drug injecting paraphernalia	46 (95.8%)		
Shared with someone known to have HCV infection	19 (39.5%)		

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Table 2
Demographics of the Interferon-Treated Cohort

	SVR n (%)	No SVR n (%)	*P* Value
Number of patients	107	101	
Mean age	43.6 ± 9.2	48.0 ± 8.2	<0.001
Male sex	74 (69.2)	71 (70.3)	0.88
Caucasian	99 (92.5)	96 (95.0)	0.45
Mean ALT	69.8 ± 60.0	79 ± 54.8	0.36
Dose reduction Genotype	14 (13.1)	17 (16.8)	0.34
1	30 (28.0)	56 (55.4)	<0.001
2/3	68 (63.6)	29 (28.7)	
Other	1 (0.9)	1 (1.0)	
Not available	8 (7.5)	15 (14.9)	
Cirrhosis Treatment:	9 (8.4)	4 (4.0)	0.25
IFN	3 (2.8)	29 (28.7)	<0.001
IFN + Rib	7 (6.5)	6 (5.9)	
PEG-IFN	1 (0.9)	4 (4.0)	
PEG-IFN + Rib	96 (89.7)	62 (61.4)	

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Table 3
KIR and HLA-C Associations in the Exposed Seronegative Aviremic Individuals

Characteristic	Seronegative Aviremic n (%)	Chronic n (%)	*P* Value (*Pc*)	OR (95% CI)
Number of patients	48	257		
KIR2DL2	22 (45.8)	136 (52.9)	036	
KIR2DL3	43 (89.6)	223 (86.8)	0.59	
Group 1 HLA-C	40 (83.3)	214 (83.3)	0.99	
Group 2 HLA-C	28 (58.3)	181 (70.4)	0.10	0.59 (0.30-1.16)
KIR2DL2-C1	18 (37.5)	114 (44.4)	0.38	
KIR2DL3-C1	36 (77.0)	187 (72.8)	0.75	
KIR2DL3-C1 homozygous	12 (25.0)	25 (9.7)	0.003 (0.02)	3.1 (1.3-7.1)
KIR2DL3-C1 heterozygous	24 (50.0)	162 (63.0)	0.09	0.59 (0.30-1.14)

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Table 4
KIR and HLA-C Associations in the Interferon-Treated Individuals

	SVR n (%)	No SVR n (%)	*P* Value (*Pc*)	OR (95% CI)
Number of patients	107	101		
KIR2DL2	54 (50.5)	60 (59.4)	0.20	
KIR2DL3	98 (91.6)	90 (89.1)	0.54	
Group 1 HLA-C	94 (87.9)	76 (75.2)	0.02 (>0.1)	2.4 (1.1-5.3)
Group 2 HLA-C	63 (58.9)	67 (66.3)	0.27	
KIR2DL2-C1	46 (43.0)	40 (39.6)	0.62	
KIR2DL3-C1	88 (82.2)	68 (67.3)	0.01 (0.1)	2.3 (1.2-4.3)
KIR2DL3-C1 homozygous	18 (16.8)	13 (12.9)	0.64	
KIR2DL3-C1 heterozygous	70 (65.4)	55 (54.4)	0.11	

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Table 5

Logistic Regression Analysis of the Association of KIR2DL3-C1 with the Outcome of Interferon Treatment

			95% CI	
	P	**OR**	**Lower**	**Upper**
Age <40	.001	4.44	1.85	10.64
Pegylated IFN + Ribavirin	.003	7.66	1.98	29.67
Genotype 1	.021	0.18	0.04	0.77
KIR2DL3-C1	.028	2.34	1.01	5.00
Normal ALT	.063	1.95	0.97	3.93
Race	.218	0.38	0.08	1.76
Dose reduction	.323	0.64	0.26	1.56
Cirrhosis	.514	0.64	0.17	2.46
Duration 24 versus 48	.663	0.99	0.92	1.05
Sex	.935	1.03	0.50	2.13

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Table 6
KIR2DL3 and Group 1 HLA-C Associations in Treatment Cohort

	SVR n (%)	No SVR n (%)	*P* Value (*Pc*)	OR (95% CI)
Number of patients	107	101		
2DL3+Cw*01	6 (5.6)	6 (5.9)	0.92	
2DL3+Cw*03	24 (22.4)	8 (7.9)	0.004 (0.03)	3.4 (1.5-8.7)
2DL3+Cw*07	48 (44.9)	45 (44.6)	0.96	
2DL3+Cw*08	13 (12.1)	6 (5.9)	0.12	
2DL3+Cw*12	13 (12.1)	4 (4.0)	0.03 (>0.1)	3.4 (1.0-14.6)
2DL3+Cw*14	4 (3.7)	2 (2.0)	0.57	
2DL3+Cw*16	5 (4.7)	9 (8.9)	0.22	

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Table 7
KIR2DL3 and Group 1 HLA-C Associations in the Spontaneously Resolving Cohort

	Resolved n (%)	Persistent n (%)	*P* Value (*Pc*)	OR (95% CI)
Number of patients	122	219		
KIR2DL3 and HLA-C alleles				
2DL3+Cw*01	7 (5.7)	10 (4.6)	0.42	
2DL3+Cw*03	38 (31.2)	42 (19.2)	0.01 (0.08)	1.9 (1.1-3.2)
2DL3+Cw*07	70 (57.4)	115 (52.5)	0.38	
2DL3+Cw*08	12 (8.2)	11 (5.0)	0.09 (>0.1)	2.06 (0.82-5.21)
2DL3+Cw*12	11 (9.0)	13 (5.9)	0.26	
2DL3+Cw*14	1 (0.8)	3 (1.4)	0.65	
2DL3+Cw*16	10 (8.2)	18 (8.2)	0.99	
KIR2DL3 number and HLA-Cw*03				
2DL2/2DL3+Cw*03	14 (11.4)	20 (9.1)	0.49	
2DL3/2DL3+Cw*03	23 (18.9)	20 (9.1)	0.01 (0.02)	2.3 (1.2-4.4)