

Innate immunity: a new chapter for hepatitis C

Theresa Hydes^a, Salim I. Khakoo^b

Poole Hospital NHS Foundation Trust; University of Southampton, UK

Abstract

The immune response to hepatitis C virus (HCV) and the mechanisms leading to successful resolution are complex. Much work has been done on understanding the adaptive immune response to HCV due to the imperative of trying to find a vaccine. However the importance of the innate immune system is being increasingly recognized. This has culminated in the discovery of IL-28B as a key genetic element in recovery from HCV in diverse populations. This association is one of the strongest in anti-viral immunity and represents a paradigm shift in how we view the immune response to HCV infection.

Keywords Hepatitis C, immunity, innate, interleukins

Ann Gastroenterol 2012; 25 (2): 232-240

Introduction

Hepatitis C virus (HCV) is a small enveloped positive strand RNA virus belonging to the flaviridae family. It currently infects up to 3% of the world's population [1]. However its incidence is continuing to rise and presents a serious global health concern. Reducing the disease burden is a significant therapeutic challenge as only 30% of people are able to spontaneously clear the virus [2]. Currently only 40-50% of patients chronically infected with the Genotype (G) 1 virus (the most prevalent HCV genotype) achieve a sustained virological response (undetectable HCV RNA in the serum at 6 months post treatment) with standard therapy consisting of pegylated interferon and ribavirin (IFN/Rib) [3]. Side effects can be severe and G1 patients are required to undergo therapy for up to 48 weeks [4]. Newer therapies are being devised which will improve this, however at present they are expensive and this may limit their use.

The influence of environmental factors including co-infection, cirrhosis, obesity and excessive alcohol intake in determining a patient's likelihood of responding to treatment is well described [5]. Research is now beginning to turn towards the exciting field of host genetic determinants of therapeutic response, as evidence continues to build supporting the importance of single nuclear polymorphisms (SNPs), which will no doubt lead to

targeted treatment for particular individuals in the near future. This paper presents an overview of important aspects of the innate immune system examining their influence on spontaneous clearance of the HCV, treatment response to IFN and roles they are likely to play in drug development in the future.

IFN

The IFN lambdas form a family of three cytokines: IL-28A, IL-28B and IL-29. They can be produced by both dendritic cells and macrophages. They all signal via a common receptor which is expressed on hepatocytes and is a heterodimer of the IL-10R and IL-28R chains. The significance of the role played by IL-28 within the innate immune response to the HCV became apparent following four landmark Genome Wide Association Studies (GWAS) in 2009 and 2010 [6-9]. The use of GWAS technology gives a non-hypothesis driven approach to the field of disease association studies which have previously relied on candidate gene studies, in which identification of disease related SNPs relies on testing a specific hypothesis. The GWAS approach allows sampling of the entire human genome for associations with HCV, with the remarkable finding that not only were SNPs upstream of the IL-28B gene significant in all four studies in ethnically diverse populations from America, Europe, Asia and Australia, but that no other genes reached GWAS significance in these studies.

Another important discovery was that this data correlates well with epidemiological studies which have shown that ethnicity strongly influences treatment response. There is a non-homogenous distribution of protective IL-28B genotypes across different populations, such that East Asian populations who achieve high SVR rates are commonly found to have the more favorable genotype and a large number of individuals of African descent, who are less likely to respond to treatment,

^aGastroenterology Registrar, Poole Hospital NHS Foundation Trust (Theresa Hydes); ^bFaculty of Medicine, University of Southampton (Salim I. Khakoo), UK

Conflict of Interest: None

Correspondence to: Salim Khakoo, Faculty of Medicine, University of Southampton, Mailpoint 811, Level E South Academic Block, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK, Tel: +44 23 8079 6671/5099, Fax: +44 23 8051 1761, e-mail: S.I.Khakoo@soton.ac.uk

Received 18 March 2012; accepted 28 March 2012

have the non-protective allele [6,8]. Furthermore protective SNPs within the IL-28B region have been found to occur more commonly in patients who become infected with HCV G2 or G3, and may partly account for their superior response rates to treatment [10-12]. This suggests a complex interplay between HCV and IL-28B in which SNPs within the gene may influence both the spontaneous and treatment-associated outcome of HCV infection, with a second layer of complexity provided by the virus genotype.

Treatment outcome with pegylated IFN and Rib

Two major protective SNPs for treatment of G1 individuals were identified by the GWAS studies to be associated with a SVR to IFN/Rib. These were the T allele of rs8099917 and the C allele of rs12979860 located 8 kilo bases (kb) and 3 kb respectively upstream of the IL-28B gene [6-9]. These SNPs are in linkage disequilibrium with each other and also with a coding SNP (rs8103142). Hence the causative variant has been difficult to discern and it may be that it is not a single SNP that confers protection but the whole IL-28B haplotype. Conversely, these may mark the coding SNP, which may confer an as yet unidentified functional importance. Nevertheless, the benefit of these SNPs was found to be more than two fold in all these studies.

The effect of genetic variants within this region is stronger than most other factors influencing treatment outcome. Rauch et al demonstrated an odds ratio of 5.19 in terms of treatment response, which was more significant than age of >40, advanced fibrosis, male gender and HCV viral load >800 k iu/mL, with an odds ratio of 2.9, 2, 1.6 and 1.4 respectively [9]. Only viral genotype was as or more significant than IL-28B genotype. This association was seen across a range of populations and geographical regions including Caucasians, East Asians and African Americans [6,13].

This benefit appears to occur at an early stage in chronic

hepatitis C (CHC) viral clearance following treatment. Rosso et al examined rapid viral response (RVR) (undetectable HCV RNA at 4 weeks) rates in patients with HCV G1 and G4 and found that of those patients achieving an RVR, 100% carried the protective rs12979860 C allele, whereas 64% of non-RVR individuals expressed the non-protective genotype [14]. A number of other haplotypes of SNPs have also been linked to treatment outcomes in further fine mapping of the IL-28B region (rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs7248668, rs12980275, rs10853727 and rs8109886) [7]. This finding further supports the implication that the observed benefit is produced by the IL-28B haplotype rather than individual polymorphisms.

The association of IL-28B polymorphisms and treatment response in patients infected with HCV G2/3 is less clear than for G1 and findings differ between investigatory groups (Table 1). For the rs12979860 SNP, Sarrazin et al found the protective genotype to significantly influence SVR following treatment with IFN/Rib in G2/3 infection [15]. However, this SNP was individually significant only in the G3 population. Conversely, Montes-Cano et al failed to demonstrate any association between the two [12]. No association in G2/3 infection was found between rs8099917 and treatment outcome in the study of Rauch et al [9]. Both rs12979860 and rs8099917 have however been shown to influence RVR rates in patients infected with HCV G2/3 [14,16,17], although without always resulting in an SVR [16,17]. To confuse matters further rs23979860 has been shown by another group to exert a strong influence on SVR in G2/3 patients but only where patients failed to achieve a RVR [18]. It is as yet unclear whether larger studies are required to resolve the matter or whether in non-G1 patients, polymorphisms within the IL-28B region influence viral kinetics differently during the different stages of treatment response as this data appears to suggest. Clearly, however, viral genotype does appear to influence the protective effects of the IL-28B polymorphism.

Table 1 Summary of IL-28B associations

Strong Evidence of Association	Possible Association	No Association
Spontaneous resolution in adults and children	Treatment response with IFN/Rib in G2/3 infected patients	Acute HCV treated with IFN/Rib
Treatment response to IFN/Rib in G1/4 infected patients	Treatment response with triple therapy regimes that include protease inhibitors	Susceptibility to HIV and HBV
Post-transplant HCV treatment response and rate of fibrosis	HIV/HCV coinfection treatment response in G2/3 patients	Exposed seronegative individuals and HCV resistance
HIV/HCV coinfection treatment response in G1 patients		Risk of vertical transmission from mother to child
Treatment response for IFN/Rib in children with CHC		

IFN/Rib, pegylated interferon / ribavirin; HIV, human immunodeficiency virus; HCV, hepatitis C virus; G, genotype

Treatment outcome with new agents

Unfortunately, data is still sparse in this area and larger trials using triple therapy with IL-28B stratification are still awaited. Akuta et al examined 72 Japanese patients infected with HCV G1b treated with telaprevir, Rib and IFN and found a positive association. This finding was reproduced by Gaultier et al who demonstrated a greater reduction in HCV viral load in individuals with the protective IL-28B genotype following treatment with standard therapy (IFN/Rib) plus a protease inhibitor (PI) (either ABT-072 or ABT-333), although not with the more potent PI, ABT-450 [19]. They concluded that genetic variations near the IL-28B gene strongly predicted SVR although reanalysis by Thompson et al suggested this data was confounded by an uneven distribution of IL-28B genotypes [20].

Chu et al studied the influence of IL-28B polymorphisms on viral kinetics following treatment with the nucleoside polymerase inhibitor, mericitabine, and the PI danoprevir in HCV G1 patients who were either treatment naïve or had failed standard therapy [21]. They concluded that the CC rs12979860 allele only gave a slight advantage to achieving lower HCV viral loads within the first 2 weeks of treatment when compared to the effect it promotes when IFN therapy is used. Likewise, Aerssens et al failed to demonstrate an association with SVR when using an oral HCV NS3/A4 PI, TMC435, in combination with IFN/Rib for 24 weeks in treatment naïve G1 patients, despite an obvious association when using standard therapy alone [22].

Finally, early studies have examined the role of quadruple therapy (standard therapy plus BMS-790052, a potent HCV NS5A replication complex inhibitor and BMS-650032, a potent HCV NS3 PI) in HCV G1 null responders with unfavorable IL-28B SNPs (rs12979860 CT/TT) and revealed a 100% SVR rate at 12 weeks [23]. It appears that the disadvantage encountered by patients with non-protective IL-28B genotypes can be overcome by potent newer treatments and a poly-pharmacy approach. While ongoing studies are required to clarify this matter IL-28B genotyping may prove a vital tool in selecting patients for newer treatments, especially given the observations that the newer agents will more than triple, and may even quadruple, the drug costs associated with treating a patient with CHC.

Co-infection with human immunodeficiency virus (HIV)

Small studies have revealed an influence of both rs8099917 and rs12979860 on HCV treatment outcomes in patients co-infected with HIV, although it remains unclear as to whether this applies to G2/3 patients [24-28]. Aparicio et al demonstrated a much higher response rate to IFN/Rib in patients carrying the rs8099917 TT genotype in patients infected with HCV G1 ($p < 0.0001$). They failed however to prove any significant difference when examining patients infected with G3. Payer et al reported a protective effect of the IL-28B SNP rs12979860 in co-infected individuals but noted

that the predictive model was enhanced when combined with serum IFN inducible protein (IP)-10 levels [29], which are a marker of the level of activation of the type I IFN pathway in the liver. Indeed, the presence of a favorable IL28B genotype in addition to serum IP-10 levels of < 400 pg/mL resulted in an SVR rate of 97% across all genotypes. Of further interest, the IL-28B genotype does not appear to impact on the susceptibility of hepatitis C patients becoming co-infected with HIV and hepatitis B [30,31], or on HIV disease progression [31].

Treatment outcomes following liver transplant

Recurrence is the most common cause of death following transplant for HCV [32] and while treatment is possible, SVR rates are low [33]. IL-28B polymorphisms have been shown by a number of groups to significantly influence SVR rates in this context [34-37], in addition to pre-treatments levels of viremia, inflammation [36], fibrosis and overall survival [35]. An interesting paper by Charlton et al brought attention to the fact that the donor's as well as the host's IL-28B rs12979860 CC genotype status plays an important role in determining treatment outcome in the post-transplant setting, with an apparent additive beneficial effect [35]. This study suggests that IL-28B protection functions at the levels of both the liver and the peripheral tissue. It also raises the argument that CC donor livers should be preferentially allocated to patients with CHC [35].

Acute hepatitis C

The GWAS of Rauch et al performed in 347 European patients who had cleared HCV spontaneously highlighted the association of this outcome with same SNPs in the IL28B gene that were associated with enhanced SVR rates [9]. These findings are supported by Tillmann et al who found rs12979860 to be protective against developing CHC in 190 women infected with HCV G1. In addition the predictive value of both rs12979860 and rs8099917 favorable polymorphisms in terms of reaching spontaneous resolution, has been shown to be enhanced when combined with serum IP-10 levels [38].

Di Lulio et al attempted to investigate the net effect of IL-28B genetic variation on HCV spontaneous clearance more precisely by controlling for viral and demographic effects [39]. They reported that haplotypes carrying major alleles at IL-28B SNPs were found at a much higher frequency in patients who cleared the virus. The mechanism relating to this has been previously studied in a chimpanzee model by Bigger et al who used DNA microarray technology to characterize HCV-host interactions and reported that acute hepatitis C infection produces a strong induction of ISGs early on leading to viral clearance around weeks 6 to 8 [40]. It is worth noting that while the majority of studies highlight the importance of rs12979860 and rs8099917, Rao et al demonstrated associations of four SNPs with respect to spontaneous resolution in a Chinese population although rs12979860 was not one of them [41].

The protection observed is therefore most likely related to the presence of the protective haplotype.

In spite of the wealth of data supporting a role of IL-28B and the outcome of IFN/Rib treatment for HCV infection, it does not appear to impact on the outcome of treatment in the setting of acute hepatitis C (presence of HCV RNA less than 6 months) even within the cohort of HCV G1 patients in both mono-infected and co-infected individuals [25,42]. In these studies SVR rates were between 60-70%, although it should be noted that patients receiving regimens including IFN monotherapy and also combination therapy with Rib were included. IL-28B genotype should therefore not be used to stratify these patients for therapy.

Exposed seronegative individuals

Another area of interest surrounds the possible role of IL-28B polymorphisms in protecting active intravenous drug users who are at high risk of exposure to the HCV without detectable HCV RNA or antibodies. Knapp et al analyzed a group of 74 exposed seronegative individuals and demonstrated a significantly lower frequency of the favorable rs1979860 allele compared to patients who spontaneously cleared the virus [43]. The frequency was similar to that seen in patients chronically infected with hepatitis C. This suggests that the mechanism by which IL-28B SNPs protect against CHC development differs from the role IL-28B plays in this subgroup. It also highlights the observation that there are probably multiple discrete mechanisms by which individuals can resolve HCV infection.

Children infected with hepatitis C

The predominant mode of contracting hepatitis C in children is vertical transmission. Ruiz et al examined 100 mothers with CHC and demonstrated that while the rs12979860 CC allele was strongly associated with spontaneous clearance of the virus among infected infants, IL-28B status had no influence in terms of susceptibility of children acquiring the virus via vertical transmission [44]. A second group has published evidence suggesting that polymorphisms within rs12979860 and rs8099917 are highly predictive of treatment outcomes following IFN/Rib in children, as is observed in adults [45].

Conflicting issues

Despite a wealth of evidence describing the protective role of IL-28B polymorphisms many unresolved issues remain. Firstly, the protective allele rs12979860 has repeatedly been found to be associated with a higher pre-treatment viral load [6,11], a factor well established to predict a poor outcome to standard therapy. In addition, the same allele has been linked to increased levels of alanine aminotransferase (ALT) [12,16]

and higher inflammatory activity in the liver [46], although rs12979860 has not as yet been shown to be associated with fibrosis [47].

Another area of contention lies in the relationship between IL-28B protective genotypes and the induction of IFN stimulated genes (ISGs). Strong IL-28B expression followed by a strong induction of ISGs associated with the protective IL-28B genotype is associated with spontaneous resolution [48]. However this mechanism is much more complex for treatment outcomes for CHC. A number of groups have demonstrated higher levels of IL-28B in peripheral blood mononuclear cells (PBMCs) prior to treatment in individuals carrying the protective IL-28B allele [7,8]. Others have found the same patients to have very low levels of IL-28B in the liver [49]. Bigger et al conducted DNA microarray analysis in livers from 10 chimpanzees chronically infected with hepatitis C and demonstrated an up regulation of 162 genes, many of them ISGs, compared to uninfected controls [50]. This shows that HCV induces the type I IFN response, but can persist in its presence. A similar scenario is found in the livers from individuals with CHC infection [51,52]. Paradoxically, the induction of ISGs in the liver has been found by several centers to be associated with non-response to standard therapy [53,54].

In an attempt to explain this observation, Sarosin-Filipowicz et al looked at ISG expression in both the liver and PBMCs before and after IFN treatment [55]. They demonstrated two key findings. Firstly, weak ISG induction prior to treatment results in a strong ISG response with IFN and subsequent viral clearance. Secondly, pre-activation occurs in the liver, not the PBMCs and therefore it may not be possible to study pre-activation in these cells as a means of predicting an individual's treatment outcome. Serum IP-10 is the product of an ISG and is secreted by the liver into the circulation. In a large study focusing on IP-10, Darling et al revealed a positive predictive value for SVR of 69% where IP-10 levels were <600 pg/mL [56]. The combined use of IL-28B polymorphisms and IP-10 levels significantly enhanced the predictive power of both tests alone.

The degree to which ISGs are pre-induced in CHC is subject to the influence of polymorphisms within the population, accounting for wide inter-individual variations in treatment response, and may be associated with IL-28B. A popular hypothesis is that patients with the protective IL-28B allele who failed to spontaneously clear hepatitis C express low levels of IL-28B in the liver, reflecting a weak induction of ISGs, but following IFN treatment mount an excellent ISG response leading to viral eradication. Unfortunately, important aspects of this theory are unsupported. Firstly, low pre-treatment levels of IL-28B in the liver in patients carrying the major allele have never been demonstrated. In fact, Dil et al recently described the opposite, although these individuals still had low levels of ISG pre-activation [57]. The same group also published evidence demonstrating that increased ISG expression pre-treatment in non-responders was independent of IL-28B, and in fact that ISG expression was a better predictor of treatment outcome than IL-28B genotype in a multivariate analysis [56]. Younossi et al published similar findings, revealing the

ISG, SOCS1, to be a good predictor of SVR, independent of IL-28B status [58]. The opposite has however been observed in HCV/HIV coinfecting patients, i.e. patients expressing the protective rs12979860 CC allele responded well to treatment, and despite increased plasma levels of IL-28B, SVR rates could not be predicted according to IL-28B levels alone [59].

One explanation for this discrepancy may be that favorable IL-28B genotypes exert their protective effect via a number of mechanisms other than ISG induction, in addition to the contributory role of other genetic factors towards determining therapeutic response (Fig. 1). Younossi et al identified downregulation of genes associated with other aspects of both the innate and adaptive immune systems including B cell signaling, toll-like receptor (TLR) pathways and hepatocyte apoptosis in patients carrying the rs12979860 CC allele [60]. All these 3 pathways either returned to normal or were up-regulated following IFN treatment. Another factor which may account for the lack of correlation between IL-28B and ISG expression may be the linkage disequilibrium which exists between rs12979860 and rs8099917. It is likely that they are both “tagging” haplotypes which include polymorphisms within coding, promoter, and 3’ untranslated regions of the IL-28B gene [39]. There may therefore be a combined effect of several SNPs affecting IL-28B expression and associated IFN-induced cytokines [61] in addition to an independent role for genetic variations within ISGs themselves.

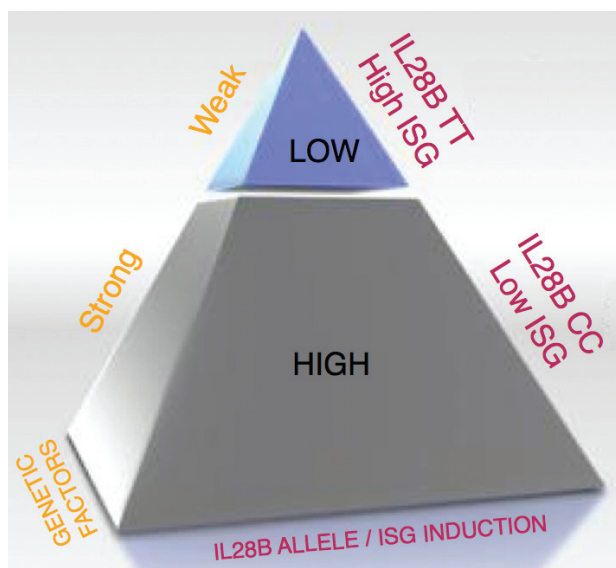


Figure 1 The influence of genetic variants in determining treatment response in patients with chronic hepatitis C: The pyramid represents the probability of an individual clearing HCV as a continuum determined by the presence of the protective IL28-B CC allele / low pre-treatment ISG induction, in addition to other genetic factors. “Low” indicates a low probability of clearance and “high” a high probability of clearance. Patients may attain resolution with a favorable IL-28B status and weak or strong additional protective genetic factors, but if they have an unfavorable IL-28B genotype the additional protective genetic factors must be strongly favorable

IL28B as a therapeutic target for the future

While the mechanism remains unresolved there is overwhelming evidence to suggest that the IFN family plays a significant role in the clearance of hepatitis C, inevitably leading to interest in their therapeutic potential, both as post exposure prophylaxis and CHC treatment. While the role of protective and non-protective genotypes is complex, on a simple note individuals with the favorable genotype have greater IL-28B mRNA expression prior to treatment [7,8], suggesting that the introduction of IL-28B as a therapeutic agent would be beneficial. Trials have so far only focused on the use of IL29 and are at the early stages of development with a phase 2 trial currently underway comparing IL-29 to IFN. However the preliminary results appear promising. A phase 1b trial has recently been completed in which seven treatment naive patients with HCV G1 were treated with pegylated IL-29 and Rib for 4 weeks [62]. At this point, six patients achieved a more than 2-log drop in HCV RNA of which 2 had HCV RNA levels which were undetectable. While the numbers are small they suggest IL-29 has the potential to out-perform IFN therapy in the management of G1 patients potential in combination with the newer directly acting anti-viral drugs. Adverse events occurred at a similar rate to those seen with IFN therapy, although disturbingly liver toxicity was seen more frequently.

Other innate genes: the type I IFN pathway

The entry of the HCV into a hepatocyte immediately leads to activation of the innate immune system and a type I IFN response rapidly unfolds (Fig. 2). This is followed by the induction of type III interferons and activation of both natural killer (NK) and T cells [18,63]. Two main recognition systems are involved in this initial intracellular response; the first consists of signaling proteins retinoic acid inducible gene-1 (RIG-1) and melanoma differentiation association gene 5 (MDA5), and the second using TLRs. These pathways go on to induce type I IFN (alpha and beta) which triggers the rest of the IFN signaling cascade including ISGs. IFN signal transduction occurs via Janus kinase 1 (Jak1) and tyrosine-protein kinase 2 (Tyk2), leading to phosphorylation of signal transducers and activators of transcription (STAT) 1, and STAT2 molecules, in a similar manner to type I IFNs [64,65].

Comprehensive analysis of the genes of the IFN signaling pathway, has found that the gene encoding for the protein TRAF family member-associated NK-κB activator (TANK) may be associated with outcome following infection [66]. This protein can influence both the RIG-I and TLR signaling pathways. A number of polymorphisms in other ISGs have similarly been associated with spontaneous resolution and treatment response, namely interleukin-1 receptor associated kinase (IRAK), IFN, IFN regulatory factor (IRF)-3 and IRF-9 [65] as well as IFN stimulated genes: Myxovirus resistance protein A (MxA), protein kinase R (PKR) and 2’-5’ oligoadenylate

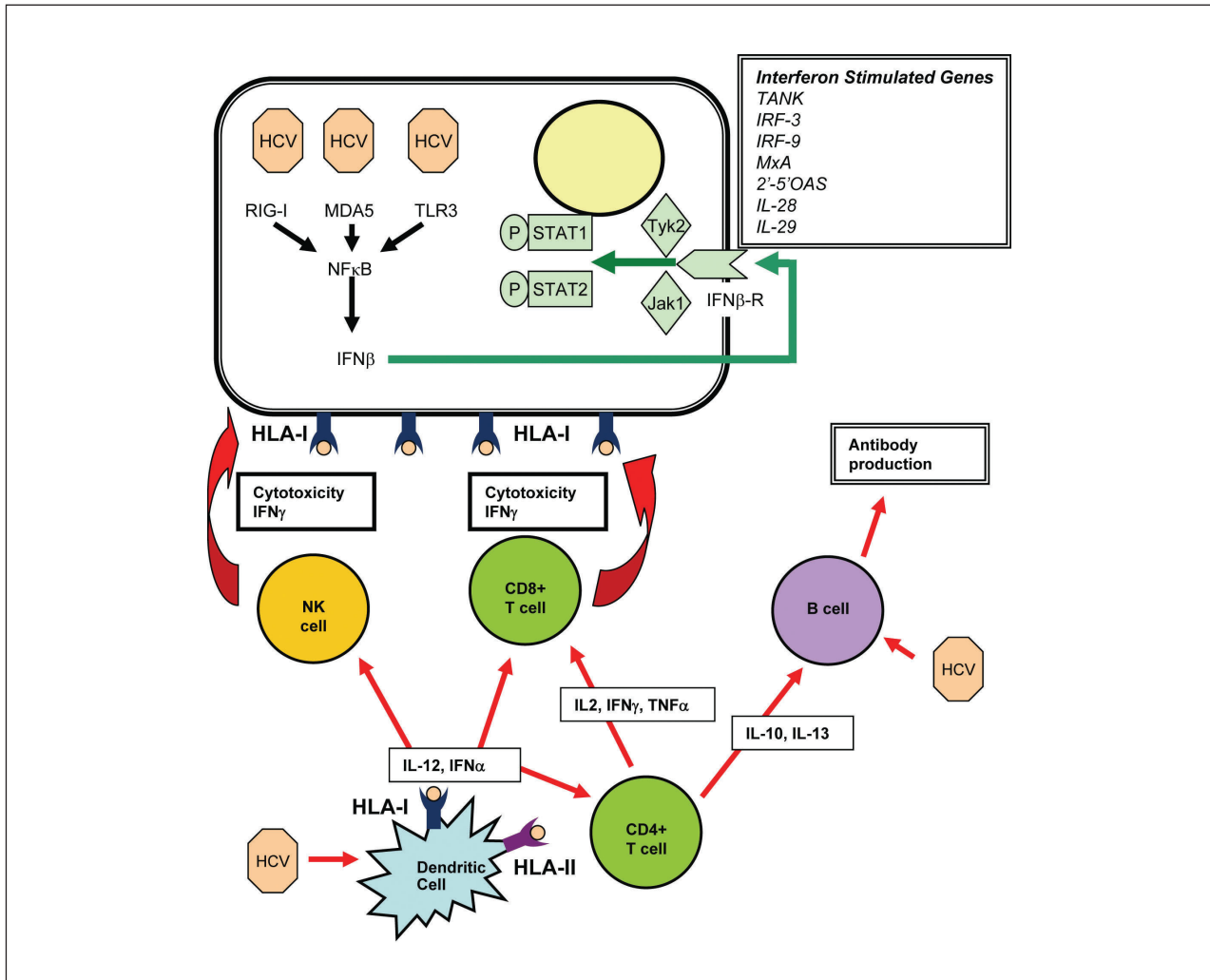


Figure 2 The influence of interferon signaling on the immune response to HCV
 HCV, hepatitis C virus; RIG-1, retinoic acid inducible gene-1; MDA5, melanoma differentiation association gene 5; TLR, toll-like receptors; NK-κB, nuclear factor kappa light chain enhancer of activated B cells; IFN, interferon; STAT, signal transducers and activators of transcription; Tyk, tyrosine kinase; jak, janus kinase; TANK, TRAF family member-associated nk-κb activator; IRAK, interleukin-1 receptor associated kinase; IRF, interferon regulatory factor; MxA, myxovirus resistance protein A; PKR, protein kinase R; 2'-5'OAS, 2'-5' oligoadenylate synthetase; IL-28, interleukin-28 B; IL-29, interleukin-29; HLA, human leukocyte antigen; APCs, antigen presenting cells; NK cell, natural killer cell; KIR, killer cell immunoglobulin-like receptor; TNF, tumor necrosis factor

synthetase (2'-5'OAS) [67,68]. The hepatitis C Virus itself can perturb RIG-1 and IFN signaling pathways and hence this may be one mechanism for its escape from clearance in the face of such strong ISG induction [69].

Finally the induction of these ISGs leads to activation of the adaptive immune system which is driven by a number of cytokines. A strong CD8 cytotoxic T cell response is invoked following the release of IL-12, IFN-gamma and TNF-alpha, creating an environment which is conducive to HCV clearance. As expected, SNPs within these cytotoxic genes have been shown to influence spontaneous HCV clearance and treatment response [25-28]. Cytokines invoking a CD4 T cell response including IL-10 are similarly subject to genetic variants impacting on these two end-points but to a lesser

degree [24,32-34]. Thus, at a cytokine and cellular level SNPs may synergize to augment the probability of HCV clearance.

NK cells and NK cell receptors

In addition to innate cytokines, there are a number of cells of the innate immune system which play a role in HCV eradication, one important group being NK cells. These cells can directly lyse infected hepatocytes and also shape the downstream adaptive immune response. NK receptor genes and their ligands can influence the outcome of HCV infection. This association of KIR2DL3 and its group 1 HLA-C ligands, was originally demonstrated in spontaneously resolving

HCV infection in Caucasians and African Americans, but only in those individuals thought to have acquired HCV through injection drug usage or needle-stick injuries, not in those receiving blood products [70]. The association has been subsequently confirmed in an independent cohort [71]. Furthermore, the same association may be protective in treatment induced resolution and also against chronic infection in those individuals that have been exposed to HCV infection, but remain Ab and RNA negative (“exposed seronegative”) [72]. Interestingly, there seems to be little synergy between NK receptor genes and IL-28B genes in protecting individuals against CHC [43]. Conversely, they do seem to interact in determining susceptibility to infection. Thus, individuals with a susceptible NK cell receptor genotype, either 2 group 2 HLA-C alleles (the ligands for KIR2DL1) or KIR2DS3, in combination with a susceptible IL-28B allele are significantly more likely to remain chronically infected than those with only one susceptibility factor [73,74]. Thus, these innate pathways can act in a complex and unpredictable way to determine the outcome of HCV infection.

Conclusion

HCV commonly causes chronic infection. The IL-28B polymorphism has an effect on the outcome of HCV that is virtually unprecedented in a viral infection. Other innate polymorphisms may play a more minor role. However, different systems both cytokine and cellular may interact in a complex, and not necessarily predictable way to determine the outcome of HCV infection. Above all, it appears that there are several independent ways to clear HCV that do not necessarily synergize. Unraveling these pathways and understanding their interactions may present a greater challenge than may have originally appeared from the landmark IL-28B genome-wide association studies.

References

1. Lavanchy D. The global burden of hepatitis C. *Liver Int* 2009; **29**:174-181.
2. Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. *J Viral Hepat* 2006; **13**:34-41.
3. Feld JJ, Hoofnagle JH. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. *Nature* 2005; **436**:967-972.
4. Calvaruso V, Craxi A. European Association of the Study of the Liver hepatitis C virus clinical practice guidelines. *Liver Int* 2012; **32**:12-18.
5. Management of hepatitis C. *NIH Consensus Statement* 1997; **15**:1-41.
6. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**:399-401.
7. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**:1100-1104.
8. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**:1105-1109.
9. Rauch A, Kutalik Z, Descombes P, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010; **138**:1338-1345.
10. Huang CF, Dai CY, Huang JF, Chuang WL, Yu ML. Linkage of the hepatitis C virus genotype and interleukin-28B genetic polymorphisms in Asian patients. *Hepatology* 2011; **53**:367-368.
11. McCarthy JJ, Li JH, Thompson A, et al. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. *Gastroenterology* 2010; **138**:2307-2314.
12. Montes-Cano MA, García-Lozano JR, Abad-Molina C, et al. Interleukin-28B genetic variants and hepatitis virus infection by different viral genotypes. *Hepatology* 2010; **52**:33-37.
13. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**:798-801.
14. Rosso C, Abate ML, Ciancio A, Smedile A, Rizzetto M. IL28B genotyping for 2 SNPs associated with chronic hepatitis C therapy responses. *J Hepatol* 2011; **54**: S530.
15. Sarrazin C, Susser S, Doehring A, et al. Importance of IL28B gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. *J Hepatol* 2011; **54**:415-421.
16. Moghaddam A, Melum E, Reinton N, et al. IL28B genetic variation and treatment response in patients with hepatitis C virus genotype 3 infection. *Hepatology* 2011; **53**:746-754.
17. Scherzer TM, Hofer H, Staettermayer AF, et al. Early virologic response and IL28B polymorphisms in patients with chronic hepatitis C genotype 3 treated with peginterferon alfa-2a and ribavirin. *J Hepatol* 2011; **54**:866-871.
18. Mangia A, Thompson AJ, Santoro R, et al. An IL28B polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. *Gastroenterology* 2010; **139**:821-827.
19. Akuta N, Suzuki F, Hirakawa M, et al. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. *Hepatology* 2010; **52**:421-429.
20. Thompson AJ, Muir AJ, Sulkowski MS, et al. Hepatitis C trials that combine investigational agents with pegylated interferon should be stratified by interleukin-28B genotype. *Hepatology* 2010; **52**:2243-2244.
21. Chu TW, Kulkarni R, Gane EJ, et al. Effect of IL28B Genotype on Early Viral Kinetics During Interferon-Free Treatment of Patients With Chronic Hepatitis C. *Gastroenterology* 2012 (in press)
22. Aerssen J, Fanning G, Scholliers A, et al. Impact of IL28B genotype and pre-treatment naive genotype 1 HCV patients treated with TMC435 in combination with pegylated interferon alfa 2A and ribavirin in pillar study. *J Hepatol* 2011; **54**:S5.
23. Lok A, Gardiner D, Lawitz E, et al. Quadruple therapy with BMS-790052, BMS-650032 and peg-IFN RBV for 24 weeks results in 100% SVR12 in HCV genotype 1 null responders. *J Hepatol* 2011; **54**:S536.
24. Pineda JA, Caruz A, Rivero A, et al. Prediction of response to pegylated interferon plus ribavirin by IL28B gene variation in patients coinfecting with HIV and hepatitis C virus. *Clin Infect Dis* 2010; **51**:788-795.
25. Nattermann J, Vogel M, Nischalke HD, et al. Genetic variation in IL28B and treatment-induced clearance of hepatitis C virus in HIV-positive patients with acute and chronic hepatitis C. *J Infect Dis* 2011; **203**:595-601.

26. Medrano J, Neukam K, Rallón N, et al. Modeling the probability of sustained virological response to therapy with pegylated interferon plus ribavirin in patients coinfecting with hepatitis C virus and HIV. *Clin Infect Dis* 2010;**51**:1209-1216.
27. Aparicio E, Parera M, Franco S, et al. IL28B SNP rs8099917 is strongly associated with pegylated interferon- α and ribavirin therapy treatment failure in HCV/HIV-1 coinfecting patients. *PLoS One* 2010;**5**:e13771.
28. Rallón NI, Naggie S, Benito JM, et al. Association of a single nucleotide polymorphism near the interleukin-28B gene with response to hepatitis C therapy in HIV/hepatitis C virus-coinfecting patients. *AIDS* 2010;**24**:F23-29.
29. Payer BA, Reiberger T, Aberle J, et al. IL28B and interferon-gamma inducible protein 10 for prediction of rapid virologic response and sustained virologic response in HIV-HCV-coinfecting patients. *Eur J Clin Invest* 2012 (in press)
30. Martin MP, Qi Y, Goedert JJ, et al. IL28B polymorphism does not determine outcomes of hepatitis B virus or HIV infection. *J Infect Dis* 2010;**202**:1749-1753.
31. Rallón NI, Restrepo C, Naggie S, et al. Interleukin-28B gene polymorphisms do not influence the susceptibility to HIV-infection or CD4 cell decline. *AIDS* 2011;**25**:269-271.
32. Sallie R, Cohen AT, Tibbs CJ, et al. Recurrence of hepatitis C following orthotopic liver transplantation: a polymerase chain reaction and histological study. *J Hepatol* 1994;**21**:536-542.
33. Angelico M, Petrolati A, Lionetti R, et al. A randomized study on Peg-interferon alfa-2a with or without ribavirin in liver transplant recipients with recurrent hepatitis C. *J Hepatol* 2007;**46**:1009-1017.
34. Fukuhara T, Taketomi A, Motomura T, et al. Variants in IL28B in liver recipients and donors correlate with response to peg-interferon and ribavirin therapy for recurrent hepatitis C. *Gastroenterology* 2010;**139**:1577-1585.
35. Charlton MR, Thompson A, Veldt BJ, et al. Interleukin-28B polymorphisms are associated with histological recurrence and treatment response following liver transplantation in patients with hepatitis C virus infection. *Hepatology* 2011;**53**:317-324.
36. Eurich D, Boas-Knoop S, Ruehl M, et al. Relationship between the interleukin-28b gene polymorphism and the histological severity of hepatitis C virus-induced graft inflammation and the response to antiviral therapy after liver transplantation. *Liver Transpl* 2011;**17**:289-298.
37. Lange CM, Moradpour D, Doebering A, et al. Impact of donor and recipient IL28B rs12979860 genotypes on hepatitis C virus liver graft reinfection. *J Hepatol* 2011;**55**:322-327.
38. Beinhardt S, Aberle JH, Strasser M, et al. Serum level of IP-10 increases predictive value of IL28B polymorphisms for spontaneous clearance of acute HCV infection. *Gastroenterology* 2012;**142**:78-85.
39. Di Iulio J, Ciuffi A, Fitzmaurice K, et al. Estimating the net contribution of interleukin-28B variation to spontaneous hepatitis C virus clearance. *Hepatology* 2011; **53**:1446-1454.
40. Bigger CB, Brasky KM, Lanford RE. DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection. *J Virol* 2001;**75**:7059-7066.
41. Rao HY, Sun DG, Jiang D, et al. IL28B genetic variants and gender are associated with spontaneous clearance of hepatitis C virus infection. *J Viral Hepat* 2012;**19**:173-181.
42. Grebely J, Petoumenos K, Hellard M, et al. Potential role for interleukin-28B genotype in treatment decision-making in recent hepatitis C virus infection. *Hepatology* 2010;**52**:1216-1224.
43. Knapp S, Warshaw U, Ho KM, et al. A polymorphism in IL28B distinguishes exposed, uninfected individuals from spontaneous resolvers of HCV infection. *Gastroenterology* 2011;**141**:320-325.
44. Ruiz-Extremera A, Muñoz-Gómez JA, Salmerón-Ruiz MA, et al. Genetic variation in interleukin 28B with respect to vertical transmission of hepatitis C virus and spontaneous clearance in HCV-infected children. *Hepatology* 2011;**53**:1830-1838.
45. Hierro L, Alvarez L, Andueza S, et al. Influence of IL28B gene polymorphisms on sustained response to peginterferon plus ribavirin in children with chronic hepatitis C. *J Hepatol* 2011;**54**:s524.
46. Abe H, Ochi H, Maekawal T, et al. Common variation of IL28 affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. *J Hepatol* 2010;**53**:439-443.
47. Costa JM, Telehin D, Munteanu M, et al. HCV-GenoFibrotest: a combination of viral, liver and genomic (IL28b, ITPA, UGT1A1) biomarkers for predicting treatment response in patients with chronic hepatitis C. *Clin Res Hepatol Gastroenterol* 2011;**35**:204-213.
48. Langhans B, Kupfer B, Braunschweiger I, et al. Interferon-lambda serum levels in hepatitis C. *J Hepatol* 2011;**54**:859-865.
49. Abe H, Hayes CN, Ochi H, et al. IL28 variation affects expression of interferon stimulated genes and peg-interferon and ribavirin therapy. *J Hepatol* 2011;**54**:1094-1101.
50. Bigger CB, Guerra B, Brasky KM, et al. Intrahepatic gene expression during chronic hepatitis C virus infection in chimpanzees. *J Virol* 2004;**78**:13779-13792.
51. MacQuillan GC, Mamotte C, Reed WD, Jeffrey GP, Allan JE. Upregulation of endogenous intrahepatic interferon stimulated genes during chronic hepatitis C virus infection. *J Med Virol* 2003;**70**:219-227.
52. Asselah T, Bièche I, Laurendeau I, et al. Liver gene expression signature of mild fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2005;**129**:2064-2075.
53. Chen L, Borozan I, Feld J, et al. Hepatic gene expression discriminates responders and nonresponders in treatment of chronic hepatitis C viral infection. *Gastroenterology* 2005;**128**:1437-1444.
54. Asselah T, Bieche I, Narguet S, et al. Liver gene expression signature to predict response to pegylated interferon plus ribavirin combination therapy in patients with chronic hepatitis C. *Gut* 2008;**57**:516-524.
55. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, et al. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc Natl Acad Sci USA* 2008;**105**:7034-7039.
56. Darling JM, Aerssens J, Fanning G, et al. Quantitation of pretreatment serum interferon- γ -inducible protein-10 improves the predictive value of an IL28B gene polymorphism for hepatitis C treatment response. *Hepatology* 2011;**53**:14-22.
57. Dill MT, Duong FH, Vogt JE, et al. Interferon-induced gene expression is a stronger predictor of treatment response than IL28B genotype in patients with hepatitis C. *Gastroenterology* 2011;**140**:1021-1031.
58. Younossi ZM, Bireddinc A, Estep M, Stepanova M, Afendy A, Baranova A. The impact of IL28B genotype on the gene expression profile of patients with chronic hepatitis C treated with pegylated interferon alpha and ribavirin. *J Transl Med* 2012;**10**:25.
59. Rallón NI, Soriano V, Naggie S, et al. Impact of IL28B gene polymorphisms on interferon- λ 3 plasma levels during pegylated interferon- α /ribavirin therapy for chronic hepatitis C in patients coinfecting with HIV. *J Antimicrob Chemother* 2012 (in press)
60. Younossi ZM, Estep JM, Bireddinc A, et al. IL28B-CC Genotype-related gene expression during treatment of chronic hepatitis C (CH-C) with pegylated interferon- α (Peg-IFN) and ribavirin (RBV): potential mechanisms for sustained virological response (SVR). *J Hepatol* 2011;**54**:S534.
61. Ahlenstiehl G, Booth DR, George J. Clinical significance of IL28B gene variation in hepatitis C virus infection. *Hot Topics in Viral Hepatitis* 2011;**7**:17-24.
62. Muir AJ, Shiffman ML, Zaman A, et al. Phase 1b study of pegylated interferon lambda 1 with or without ribavirin in patients with chronic genotype 1 hepatitis C virus infection. *Hepatology* 2010;**52**:822-832.

63. Rehermann B. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. *J Clin Invest* 2009;**119**:1745-1754.
64. Dumoutier L, Tounsi A, Michiels T, Sommereyns C, Kotenko SV, Renauld JC. Role of the interleukin (IL)-28 receptor tyrosine residues for antiviral and antiproliferative activity of IL-29/interferon-lambda 1: similarities with type I interferon signaling. *J Biol Chem* 2004;**279**:32269-32274.
65. Doyle SE, Schreckhise H, Khuu-Duong K, et al. Interleukin-29 uses a type I interferon-like program to promote antiviral responses in human hepatocytes. *Hepatology* 2006;**44**:896-906.
66. Mosbrugger TL, Duggal P, Goedert JJ, et al. Large-scale candidate gene analysis of spontaneous clearance of hepatitis C virus. *J Infect Dis* 2010;**201**:1371-1380.
67. Knapp S, Yee LJ, Frodsham AJ, et al. Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of MxA, OAS-1 and PKR. *Genes Immun* 2003;**4**:411-419.
68. Hijikata M, Ohta Y, Mishiro S. Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt-88) correlated with the response of hepatitis C patients to interferon. *Intervirology* 2000;**43**:124-127.
69. Horner SM, Gale M. Intracellular innate immune cascades and interferon defenses that control hepatitis C virus. *J Interferon Cytokine Res* 2009;**29**:489-498.
70. Khakoo SI, Thio CL, Martin MP, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 2004;**305**:872-874.
71. Romero V, Azocar J, Zuniga J, et al. Interaction of NK inhibitory receptor genes with HLA-C and MHC class II alleles in Hepatitis C virus infection outcome. *Mol Immunol* 2008;**45**:2429-2436.
72. Knapp S, Warshow U, Hegazy D, et al. Consistent beneficial effects of killer cell immunoglobulin-like receptor 2DL3 and group 1 human leukocyte antigen-C following exposure to hepatitis C virus. *Hepatology* 2010;**51**:1168-1175.
73. Suppiah V, Gaudieri S, Armstrong NJ, et al. IL28B, HLA-C, and KIR variants additively predict response to therapy in chronic hepatitis C virus infection in a European Cohort: a cross-sectional study. *PLoS Med* 2011;**8**:e1001092.
74. Dring MM, Morrison MH, McSharry BP, et al. Innate immune genes synergize to predict increased risk of chronic disease in hepatitis C virus infection. *Proc Natl Acad Sci USA* 2011;**108**:5736-57341.