Published in final edited form as:

Clin Liver Dis. 2008 August ; 12(3): 713–xi. doi:10.1016/j.cld.2008.03.002.

Host Genetic Factors and Antiviral Immune Responses to HCV

Chloe L. Thio, MD

Johns Hopkins University, Department of Medicine, Baltimore, MD

Abstract

Human genome variations explain some of the heterogeneity in the immune response to antigenic stimuli. Such differences in response to hepatitis C virus (HCV) antigens can account for the ability of the immune response to clear HCV after an acute infection or to develop more rapidly progressive liver disease. Several studies have examined polymorphisms in several candidate immune-response genes for their relationship to these HCV outcomes. Results of some of these studies complement knowledge gained from immunology studies and others offer new insights into HCV biology. This review summarizes published studies on variation in immune-response genes and HCV outcomes.

Keywords

human; genetics; polymorphisms; hepatitis C; fibrosis

Introduction

Infection with hepatitis C virus (HCV) results in a gamut of clinical outcomes ranging from viral elimination to the development of end stage liver disease or hepatocellular carcinoma. Viral elimination, which occurs in a minority (~20%) of acutely HCV-infected individuals, is the result of effective immune control of HCV replication (1). The 80% of people who do not eliminate HCV progress to a chronic HCV infection. Not all chronic infections are created equal since some have minimal liver disease whereas others develop cirrhosis or hepatocellular carcinoma. Epidemiological factors associated with these different clinical outcomes include age, ethnicity, other viral co-infections especially HIV, and gender (2–4). Even in a relatively epidemiologically homogeneous population, there are marked differences in the ability to eliminate the virus that are not related to viral characteristics. One example is a cohort of 704 Irish women who were accidentally infected with the same viral inoculum (contaminated anti-D immune globulin), and 314 (45%) of them cleared their infection (5). Likewise, 43% of 152 German women cleared their infection after being exposed to the same contaminated lot of anti-D immune globulin (6). Such data suggest that it is not the virus, but it is the interactions between the virus and the host immune response that are important for determining the natural history of an HCV infection (7).

Studies demonstrate that a strong, broad immune response favors viral clearance compared to one that is weak or narrowly-focused (8;9). Likewise, once a chronic infection is established, HCV is not cytopathic to the hepatocytes; rather, it is the immune response to the virus that is

Address for correspondence: Chloe L. Thio, MD, 1503 E Jefferson Street, Baltimore, MD 21231, Ph: 410-955-0349, Fax: 410-614-7564, Email: cthio@jhmi.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

believed to be responsible for the liver damage. A significant barrier to dissecting the components of the immune response that result in viral clearance or that lead to hepatocyte damage is the lack of small animal models or cell culture systems.

Since such models are not available, an alternative approach to understanding the immune response is to analyze whether host genetic variants or polymorphisms in immune response genes account for some of the heterogeneity in outcome. With the recent development of more rapid, cost-effective genotyping procedures, such large-scale studies are possible and have already improved our understanding of HCV pathogenesis. This review will briefly summarize the immune response to HCV in order to give some background to understand the candidate genes that will be discussed. Then, associations with polymorphisms in various immune response genes that affect the ability to achieve HCV clearance or affect HCV fibrosis progression will be reviewed.

Immune response to an acute HCV infection

After an HCV infection, the innate immune response is initially important for controlling viral replication (see article by Szabo in this issue) with the adaptive immune response peaking at 8– weeks after infection. Ultimately, a coordinated effort between the innate and adaptive immune responses is necessary to eliminate HCV from the liver. The innate immune system recognizes both single-stranded and double-stranded HCV RNA through its pattern recognition receptors, TLR3 on the hepatocyte cell surface and RIG-I (retinoic-acid-inducible gene I) in the cytoplasm of the hepatocyte. Engagement of either of these receptors activates a cascade of signals including interferon-regulatory factor 3 (IRF-3) that culminates in the induction of type 1 interferons such as interferon- α and $-\beta$. These interferons bind receptors that activate the Jak-STAT pathway, which then turns on hundreds of interferon-stimulated genes to control viral replication (reviewed Lloyd et al)(10).

The innate immune response also controls replication via its effector cells, the natural killer cells (NK). Since NK cells comprise ~30% of all T cells in the liver (11), they likely are an important component to the HCV immune response. Their role includes lysing infected cells, producing interferon- γ to control viral replication, and directing inflammatory cells to HCV-infected hepatocytes.

This initial innate immune response is an important host defense against the virus, but viral clearance is also dependent upon an effective adaptive immune response. The onset of the cellular immune response is clinically detected by a rise in serum transaminases marking immune-mediated liver injury. A strong, broad CD4+ and CD8+ T cell response is more likely to lead to HCV clearance than a weak, narrowly-focused response (8;9). Evidence also suggests that a CD4+ response that elicits Th1 cytokines is more likely to result in viral clearance. Those with a weak CD4+ response appear to have a functional impairment of these cells from exhaustion or anergy rather than a depletion of CD4+ cells. Similarly, acutely infected individuals who do not clear have CD8+ T cells that are functionally impaired compared to those who clear the virus. However, not all individuals with a robust response will ultimately clear HCV, which, in some instances, may be due to the development of viral escape mutants from immune pressure on the virus. Thus, HCV must balance viral fitness with immune escape. Several studies have documented viral escape mutations in HLA-restricted CD8+ T cell epitopes (12–14). In the cohort of Irish women who received the same viral inoculum, Ray et al demonstrated that those with amino acid substitutions in known HLA epitopes directed these mutations away from consensus in persons with the HLA allele associated with that epitope, and toward consensus in those lacking the allele (12).

Polymorphisms in immune response genes and outcome of acute HCV infection

As described above, both the innate and adaptive immune responses are important for clearance of an acute HCV infection. Published studies to date have not examined polymorphisms in the innate immune response pathway genes or the type I interferon genes to determine if they affect the ability to clear HCV. However, there is genetic epidemiological evidence to support the importance of the natural killer (NK) cells, the effector cell of the innate immune response, in HCV outcome. Khakoo et al examined polymorphisms in the genes for the killer immunoglobulin-like receptors (KIRs), which are receptors on NK cells (15). As the name implies, these receptors have either 2 or 3 immunoglobulin-like domains, a transmembrane domain, and either a long or short cytoplasmic tail. Those with the long tails (2DL, 3DL) inhibit the NK cells when bound and those with short tails (2DS, 3DS) activate NK cells upon binding. The ligands for the inhibitory KIRs are HLA class I molecules whereas the ligands for the activating KIRs have not been defined. The study by Khakoo et al focuses on the inhibitory KIRs 2DL1, 2DL2, and 2DL3, of which the latter two are alleles of each other. These three KIRs bind HLA-C alleles, which are divided into two groups based on the amino acid at position 80 of the HLA allele: HLA-C1 alleles have an asparagine whereas HLA-C2 alleles have a lysine at position 80. The HLA-C1 alleles bind KIR2DL2/2DL3 and the HLA-C2 alleles bind KIR2DL1. The strongest inhibitory signal is transduced to the NK cell when KIR2DL1 binds a HLA-C2 allele whereas the weakest signal is from KIR2DL3 binding a HLA-C1 allele (Table 1). Khakoo et al found that in individuals with low titer HCV inocula (i.e. injection drug users), homozygosity for the combination with the weakest inhibitory signal (KIR2DL3 and HLA-C1) favored HCV clearance compared to those without this compound genotype (OR 2.33, $P=0.001$). Presumably this association is due to those individuals with the protective genotype having the least inhibition of the NK cells giving rise to increased NK cell activity. The authors also found an independent association with the presence of the activating KIR3DS1 and its putative ligand, a group of *HLA-B* alleles known as *HLA-Bw4*.

Immune response genes of the adaptive immune response have been studied more extensively than those of the innate immune response in acute HCV infection. Several studies have tested the hypothesis that certain *HLA* class I alleles may present epitopes that lead to a more robust CD8⁺ T cell response to acute HCV infection. In the largest study, Thio et al examined individuals from three different cohorts that included 231 individuals with clearly documented HCV recovery and 444 matched persistently HCV infected individuals (16). They found that *HLA-A*1101* (OR 0.49, 95% CI 0.27–0.89), *B*57* (OR 0.62, 95% CI 0.39–1.0) and *Cw*0102* (OR 0.43, 95% CI 0.21–0.89) were associated with viral clearance. *HLA-Cw*04* (OR 1.78, 95% CI 1.21–2.59) and *A*2301* (OR 1.78, 95% CI 1.01–3.11) were associated with HCV persistence. The *HLA-C* data are consistent with the *HLA-KIR* data above since *HLA-Cw*0102* is a *HLA-C1* allele and *HLA-Cw*04* is a *HLA-C2* allele. This study did not find an association between *HLA* class I homozygosity and viral persistence, which is interesting since it has been hypothesized that heterozygosity would be advantageous for recognition of a broader array of epitopes. *HLA-B*57* has also been associated with HCV clearance in an African population (17) and with slower HIV progression (18), but whether it is a gene linked to this allele or an immune characteristic about this allele itself that is protective in these chronic viral infections is not known. The other large study with class I *HLA* data is from the cohort of Irish women described above who received the same viral inoculum. They found that the 86 subjects with viral clearance were more likely to have *HLA-A*03* (OR 2.8), *B*27* (OR 7.5), *B*07* (OR 2.0) or *Cw*01* (OR 7.1) compared to the 141 chronically infected women (19). *HLA-B*08* (OR 0.4) and *B*18* (OR 0.2) were more common in women with viral persistence. These results (except for the *HLA-Cw*01*) are different from the study by Thio et al., which

may be due to baseline differences between the cohorts such as ethnicity, gender, and viral inoculum.

Since the CD4⁺ T cell response is important for viral clearance, several groups have tested the hypothesis that *HLA* class II molecules, which present CD4 epitopes, are associated with HCV outcome. Several results are consistent, and two meta-analyses demonstrated that *DQB1*0301* (estimates of 3.0 (20) and 2.4 (21) and *DRB1*11* (estimates 2.5 and 2.0) are associated with HCV recovery. However, a bias of these meta-analyses is that studies without any *HLA* associations are not published or studies where these alleles are not significant do not report their specific odds ratios. The allele *HLA-DRB1*01* was protective in the Irish cohort as well as in the Caucasians in the study by Thio et al.; thus, it may not be protective in non-Caucasian ethnic groups.

Cytokines are small proteins secreted by a variety of T cells in response to an immune stimulus and have several roles including mediating the immune response to infectious agents such as HCV. Thus, several studies have examined polymorphisms in a variety of cytokine genes to determine their role in HCV clearance (Table 2). Chemokines are a specific set of cytokines that attract leukocytes to sites of infection. Several chemokines bind to the receptor, CCR5, and polymorphisms in this receptor have been examined in clearance of HCV infection. A 32 base-pair deletion in the *CCR5* gene (*CCR5Δ32*) leads to loss of CCR5 expression on the surface of the T cell, which is protective against HIV infection since CCR5 is an essential co-receptor for HIV (22). CCR5 also influences T cell trafficking and the immune response. A study of women with genotype 1b found *CCR5Δ32* to be more common in those with HCV clearance (23). This association could be due to an increased T cell response to the HCV antigens since *ccr5* knockout mice have increased T cell responses to a variety of antigens (24;25). The chemokines that bind CCR5 have not been extensively studied with regards to HCV clearance, but polymorphisms in one of them, RANTES, have been associated with HCV treatment response (26;27).

Interferon- γ (IFN- γ) is produced by effector T cells and NK cells and is critical to the defense against HCV infection as it can inhibit HCV replication in the replicon system (28). This gene does not have any coding region polymorphisms, but several non-coding region polymorphisms have been described (29;30). In one study, nine non-coding region polymorphisms were genotyped in a HCV treatment cohort and a HCV recovery cohort, and the uncommon G variant at position -764 in the interferon-gamma promoter was associated with recovery from HCV (OR = 3.51 (0.98–12.49) as well as with sustained response to HCV therapy (OR 3.37 (1.15–9.83) (31). This variant is more common in Caucasian-Americans consistent with the findings that spontaneous recovery is more common in Caucasians (4). The -764G variant has a higher binding affinity to the NF- κ B motif resulting in higher levels of promoter activity and thus perhaps explaining the association with HCV recovery and treatment response (31).

Interleukin-10 (IL-10) is a cytokine important to the immune response to HCV through its downregulation of the Th1 response and suppression of secretion of proinflammatory cytokines such as TNF- α and interferon- γ . Several studies have implicated that polymorphisms in this region of the genome are important in HCV clearance. One study found that a particular promoter haplotype (-1117A, -854T, -627A), which is associated with lower IL-10 expression, was more frequent in those with recovery (36%) than in persistent infection (23%) (32). A promoter polymorphism at -1082 that is associated with higher IL-10 levels has also been associated with HCV persistence in women (33). Olekys et al tested polymorphisms in the IL-10 gene region and found associations with HCV outcome in African-Americans (34). However, several other studies examined the IL-10 haplotypes and polymorphisms but did not find associations with HCV recovery (35–38).

Interleukin-12 (IL-12) is a cytokine important for the generation of a Th1 response, which favors HCV clearance. A polymorphism in interleukin-12 p40 gene (*IL-12B*) at position 1188 has been associated with both increased and decreased levels of IL-12 secretion (39;40). Homozygosity for the A allele has been associated with HCV persistence in Chinese patients (OR 0.34, $P = 0.014$) (41). This was also found in a second study from the United Kingdom where 66% of the persistently infected people were A/A homozygous compared to 50% of those with spontaneous recovery (42). However, this was not confirmed in a different population of German patients (43). We also failed to confirm this in our study from North America of 188 patients with HCV recovery matched to 360 persistently infected persons with HCV persistence. The A allele was found in 72.5% and 71% of persons with HCV recovery and persistence, respectively (our unpublished data).

Transforming growth factor- β (TGF- β) is a cytokine that inhibits the immune response by suppressing NK cell activity and inhibiting interferon- γ and IL-12 production. The C allele at -509 in the promoter, which leads to lower levels of TGF- β 1, was associated with HCV recovery in a study of Japanese patients (44). Similarly, Barrett et al. studied two coding region polymorphisms in TGF- β and found that the haplotype associated with low TGF- β was associated with HCV clearance (35). In this study, Barrett also found that homozygosity for the low producing IL-6 promoter -174 variant was associated with HCV recovery.

Although TNF- α is an important pro-inflammatory cytokine with the best known functional polymorphisms at positions -308 and -238 in the promoter, none of the studies to date have found an association with these polymorphisms and HCV recovery or persistence (32;35).

Although the importance of the humoral immune response to HCV is just beginning to be uncovered, there is one study showing that certain immunoglobulin GM and KM allotypes affect HCV outcome. GM and KM allotypes are antigenic markers of the IgG heavy chains and κ -light chains, respectively. GM allotypes are strongly associated with IgG subclass concentrations. In a study of African-American injection drug users, Pandey et al found that subjects with GM 1, 17 5, 13 and KM 1,3 phenotype are over three times as likely to clear HCV as those without that phenotypes (45).

Polymorphisms in immune response genes and HCV disease progression

Of the individuals with a persistent HCV infection, ~30% progress to end stage liver disease or cirrhosis. The mean time to development of cirrhosis is 20–30 years (2); however, in some individuals, it occurs more quickly. Such variation in fibrosis progression rates has been associated with epidemiological factors such as age, gender, HIV status, and alcohol use (2). Another factor is the strength of the immune response, which is initially stimulated to eliminate HCV. However, in a persistent infection, the continued inflammatory state leads to hepatocyte necrosis and deposition of extracellular matrix proteins (ECM) by hepatic stellate cells. Activators of hepatic stellate cells include the Th1 response (46), the family of proteolytic enzymes known as matrix metalloproteases (MMPs), and the tissue inhibitors (TIMPs) of MMPs. Since the continued immune response generates an inflammatory state, genetic variation of the immune system may affect fibrosis progression rates.

One major limitation to genetic epidemiologic studies of HCV disease progression is accurately defining the severity and the progression of fibrosis. The gold standard for determining the amount of fibrosis, which is often used in genetic epidemiology studies, is a liver biopsy. Unfortunately, this gold standard is problematic because it is imprecise due to differences in size of the biopsy and in regional differences of fibrosis. Furthermore, there is reader error with studies showing inadequate inter and intra-reader concordance (47). A second limitation is that the studies are cross-sectional, so a liver biopsy at one particular point is used along with an estimated date of infection to determine the progression rate. This is problematic since the

classification of disease state and/or progression rate can be unreliable since it is dependent upon an estimated date. A third limitation is that some genetic epidemiology HCV studies define disease stage based on liver enzyme elevations, but it is known that liver enzymes do not correlate well with the stage of disease. An advantage of this approach is that repeated measurements are more easily obtained than with a liver biopsy, so the problems associated with a cross-sectional study design are less applicable. Taken together, these limitations result in misclassification that can contribute to false associations, especially in small studies. With these caveats in mind, we will review the immune-response genes that have been studied in liver disease progression.

HLA has been studied as a candidate gene in the disease progression hypothesis. In a study by Asti et al, *DRB1*1103* and *DRB1*1104* were associated with normal ALT; whereas, *DRB1*1101* was more common in those with more chronic hepatitis especially those with more advanced disease, as defined by liver biopsy (48). A French study by Renou et al examined 83 patients with normal ALT over a 6 month period and 233 patients with elevated ALT and found *HLA-DRB1*11* to be overrepresented in those with a normal ALT (43% versus 24%, OR 2.36) (49). In this study, liver biopsies were performed and milder disease was also associated with *HLA-DRB1*11*. Subtyping of the *HLA-DRB1*11* was not performed as in the Asti et al study. A Polish study also found *HLA-DRB1*11* to be more common in those with milder disease (50), and a German study found that it protected from cirrhosis (51). The German study also found protection from *HLA-DQB1*03*. A study from Japan examined HLA class I and II antigens in those with normal ALT compared to those without and found that haplotypes with *HLA-B*54* are more common in those with high ALT (52). In this study, *HLA* haplotype *DRB1*1302-DQB1*0604* was also associated with normal ALT. Several other studies have yielded inconsistent results (53–55). Patel et al also tested the hypothesis that *HLA* allelic diversity affects fibrosis progression rates, but they did not find differences in median progression rates in patients heterozygous or homozygous at all three *HLA* class I loci (56). They did not find any individual class I associations, but they used a serological assay to define HLA types making it more difficult to discover weaker associations.

An approach to examining the inherited genetic variability of the presence or severity of diseases, which has become possible with the HapMap and with the decrease in genotyping cost, is performing a genome-wide scan (57;58). The advantage of such an approach is that it obviates the need for *a priori* knowledge of candidate genes. For HCV, early findings from a genome-wide scan of disease severity have been published (59). This scan included 433 patients from one center and then significant results were validated in 483 patients from a second center. The DNA from the first center was pooled based on the following fibrosis stages from a baseline biopsy: no or minimal fibrosis (stages 0 or 1), mild (stage 2), and advanced (stages 3 or 4). The advanced fibrosis group was compared to no or mild disease. Of the 24,823 SNPs genotyped, 1609 (6.5%) had a two-fold association with liver disease stage and 100 of these have been tested in samples from the second center. Two SNPs remained associated with disease stage were Dead box polypeptide 5 (*DDX5*) and carnitine palmitoyltransferase 1A (*CPT1A*). The *DDX5* SNP was a non-synonymous change that was associated with more advanced fibrosis and on analysis of SNPs in close proximity, two SNPs in the *POLG2* gene (polymerase DNA-directed gamma 2) were also associated with advanced fibrosis. *DDX5* is a RNA helicase that is expressed in the liver and may interact with HCV RNA to activate stellate cells. The *CPT1A* SNP was also a non-synonymous change, and it was more frequent in subjects with milder disease. This SNP (A275T) may lead to less oxidative stress potentially explaining the association with decreased fibrosis.

Cytokines are believed to be important in fibrogenesis, and rodent models confirm that they can affect fibrosis (reviewed in Bataller et al)(60). Tumor necrosis factor (TNF)- α is a proinflammatory Th1 cytokine that is upregulated in chronic HCV so is a profibrogenic

candidate (61). Yee et al compared polymorphisms in TNF- α in 33 patients with cirrhosis and 114 without cirrhosis, although the precise stage of the non-cirrhotics is not stated (62). Both the -308A and -238A promoter variants increased the risk for cirrhosis in this study. A Japanese study had similar results and found that these variants were associated with increased type IV collagen 7S, which is a marker of hepatic fibrosis (63), but they were not associated with liver enzyme elevations. A second Japanese study also found that these promoter variants were not associated with liver enzyme elevations (55).

One study examined seven CC chemokines and their receptors and found an association between a polymorphism in monocyte chemoattractant protein 2 (*MCP-2*), Q46K, and severe fibrosis (OR 2.29, $P=0.018$) (64). In addition, *CCR5Δ32* was associated with reduced portal inflammation but increased fibrosis (OR 1.97, $P=0.015$). It is not clear why reduced portal inflammation and more fibrosis are associated with the same genotype especially since in autoimmune hepatitis mouse models and infectious models, *CCR5Δ32* is associated with an increased inflammatory response (65;66). Studies from Spain and India also contradicted this result since there was no association between *CCR5Δ32* and fibrosis stage (67;68).

Interleukin-10 (IL-10) is a Th1 cytokine that mediates its effects through the IL-10 receptor, which is a heterodimer consisting of IL-10R1, required for binding, and IL-10R2, required for signaling. IL-10 is an anti-inflammatory cytokine which downregulates collagen 1 expression and upregulates collagenase. One study found that the minor allele in G330R IL-10R1 was associated with cirrhosis but not with inflammation suggesting that the profibrotic effect of this allele was independent of the inflammatory response (69).

TGF- β 1 is one of the major profibrogenic cytokines and has been implicated in disease progression. The proline to arginine switch at codon 25 in *TGFB1* increases production of the cytokine and leads to increased fibrosis ($P=0.023$) (70). Angiotensinogen II is another profibrogenic cytokine. The promoter G to A SNP at -6 leads to increased transcription of the gene and is also associated with increased fibrosis. Together, SNPs in these two genes have a dose response effect since individuals with polymorphisms in both of these genes have more fibrosis progression than those with either one alone.

Interleukin-12 (IL-12) is a cytokine that induces production of interferon- γ . A SNP in the gene for one of the subunits of IL-12 (IL-12p40) has been associated with immune mediated diseases. Homozygosity for the minor allele of this SNP, 1188C/C, was more common in patients with mild (fibrosis score ≤ 2) compared to severe fibrosis (fibrosis score >2) (23.7% vs. 6.25%, $P = 0.004$), but there was no association with inflammation (71).

Solute carrier family 11 member 1 (SLC11A1) protein is involved in macrophage function as well as in upregulation of chemokines important in the immune response to HCV (TNF- α , IL-1 β , inducible nitric oxide synthase). Several mutations result in variable mRNA expression including a GT tandem repeat promoter polymorphism. Four functionally different alleles have been described in the population, which are designated 1–4. These alleles have been associated with other diseases such as tuberculosis (72). Individuals who were homozygous for allele 2 had lower inflammation, less fibrosis (2.4% versus 18.1%, OR 8.85), and lower HCV RNA levels (336 versus 1290×10^3 IU/ml) compared to the other alleles (73). Further work is needed to understand the biological basis for this association.

Myeloperoxidase (MPO) is an oxidant-generating enzyme found in macrophages and neutrophils. A functional promoter polymorphism (-463A) in this gene has less MPO production but was associated with more fibrosis (74). The reason for this association is unclear but MPO has been associated with inhibition of NK activity and T cell proliferation (75).

Summary

This review has concentrated on summarizing several published *HLA* and non-*HLA* associations in immune-response genes with various HCV outcomes. Some have been repeated in more than one study or have a biological basis for the association. The associated SNPs increase our understanding of HCV clearance and liver disease progression.

However, a limitation of this review and of the current published studies is that studies where associations are not found often go unreported. In order to maximize the utility of such studies to offer insight into how the immune response may account for the heterogeneity in HCV outcomes, studies that do not find associations should also be published. Another limitation is that epistatic interactions between polymorphisms in different genes are often not assessed.

In the future, as large-scale genotyping becomes more affordable, there will be an increasing number of studies examining polymorphisms in various genes. As the ability to scan the genome improves, such studies will also offer clues to important genes involved in HCV pathogenesis including ones that may not be obvious candidates. However, such studies will still not be able to evaluate interactions of variants in different genes. Nonetheless, insights into the immune response from such studies can lead to rational drug development to improve therapeutics for chronic hepatitis C. In addition, human genetic variations can also affect response to HCV therapies, so future therapeutic trials may involve studying polymorphisms and treatment response.

Acknowledgements

This work was supported by NIH grant DA 13324 and the Burroughs Wellcome Fund Investigators in the Pathogenesis of Infectious Diseases Award

Reference List

1. Villano SA, Vlahov D, Nelson KE, Cohn S, Thomas DL. Persistence of viremia and the importance of long-term follow-up after acute hepatitis C infection. *Hepatology* 1999;29:908–914. [PubMed: 10051497]
2. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997;349:825–832. [PubMed: 9121257]
3. Eyster ME, Diamondstone LS, Lien JM, Ehmann WC, Quan S, Goedert JJ. Natural history of hepatitis C virus infection in multitransfused hemophiliacs: effect of coinfection with human immunodeficiency virus. The Multicenter Hemophilia Cohort Study. *J Acquir Immune Defic Syndr* 1993;6:602–610. [PubMed: 8098752]
4. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, et al. The natural history of hepatitis C virus infection: host, viral, and environmental factors. *JAMA* 2000;284:450–456. [PubMed: 10904508]
5. Kenny-Walsh E. Irish Hepatology Research Group. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Engl J Med* 1999;340:1228–1233. [PubMed: 10210705]
6. Muller R. The natural history of hepatitis C: clinical experiences. *J Hepatol* 1996;24:52–54. [PubMed: 8836889]
7. Rehermann B. Interaction between the hepatitis C virus and the immune system. *Semin Liver Dis* 2000;20:127–141. [PubMed: 10946419]
8. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nature Reviews Immunology* 2005;5:215–229.
9. Cooper S, Erickson AL, Adams EJ, Kansopon J, Weiner AJ, Chien DY, et al. Analysis of a successful immune response against hepatitis C virus. *Immunity* 1999;10:439–449. [PubMed: 10229187]

10. Lloyd AR, Jagger E, Post JJ, Crooks LA, Rawlinson WD, Hahn YS, et al. Host and viral factors in the immunopathogenesis of primary hepatitis C virus infection. *Immunol Cell Biol* 2007;85:24–32. [PubMed: 17130897]
11. Racanelli V, Rehermann B. The liver as an immunological organ. *Hepatology* 2006;43:S54–S62. [PubMed: 16447271]
12. Ray SC, Fanning L, Wang XH, Netski DM, Kenny-Walsh E, Thomas DL. Divergent and convergent evolution after a common-source outbreak of hepatitis C virus. *J Exp Med* 2005;201:1753–1759. [PubMed: 15939791]
13. Cox AL, Mosbrugger T, Mao Q, Liu Z, Wang XH, Yang HC, et al. Cellular immune selection with hepatitis C virus persistence in humans. *J Exp Med* 2005;201:1741–1752. [PubMed: 15939790]
14. Timm J, Li B, Daniels MG, Bhattacharya T, Reyor LL, Allgaier R, et al. Human leukocyte antigen-associated sequence polymorphisms in hepatitis C virus reveal reproducible immune responses and constraints on viral evolution. *Hepatology* 2007;46:339–349. [PubMed: 17559151]
15. Khakoo SI, Thio CL, Martin MP, Brooks C, Gao X, Astemborski J, et al. HLA and NK Cell Inhibitory Receptor Genes in Resolving Hepatitis C Virus Infection. *Science* 2004;305:872–874. [PubMed: 15297676]
16. Thio CL, Gao X, Goedert JJ, Vlahov D, Nelson KE, Hilgartner MW, et al. HLA-Cw*04 and hepatitis C virus persistence. *J Virol* 2002;76:4792–4797. [PubMed: 11967296]
17. Chuang WC, Sarkodie F, Brown CJ, Owusu-Ofori S, Brown J, Li C, et al. Protective effect of HLA-B57 on HCV genotype 2 infection in a West African population. *J Med Virol* 2007;79:724–733. [PubMed: 17546694]
18. Migueles SA, Sabbaghian MS, Shupert WL, Bettinotti MP, Marincola FM, Martino L, et al. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *Proc Natl Acad Sci U S A* 2000;97:2709–2714. [PubMed: 10694578]
19. McKiernan SM, Hagan R, Curry M, McDonald GS, Kelly A, Nolan N, et al. Distinct MHC class I and II alleles are associated with hepatitis C viral clearance, originating from a single source. *Hepatology* 2004;40:108–114. [PubMed: 15239092]
20. Yee LJ. Host genetic determinants in hepatitis C virus infection. *Genes Immun* 2004;5:237–245. [PubMed: 15103322]
21. Hong X, Yu RB, Sun NX, Wang B, Xu YC, Wu GL. Human leukocyte antigen class II DQB1*0301, DRB1*1101 alleles and spontaneous clearance of hepatitis C virus infection: a meta-analysis. *World J Gastroenterol* 2005;11:7302–7307. [PubMed: 16437632]
22. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets RGJ, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CCR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* 1996;273:1856–1862. [PubMed: 8791590]
23. Goulding C, McManus R, Murphy A, MacDonald G, Barrett S, Crowe J, et al. The CCR5-delta32 mutation: impact on disease outcome in individuals with hepatitis C infection from a single source. *Gut* 2005;54:1157–1161. [PubMed: 15863470]
24. Algood HM, Flynn JL. CCR5-deficient mice control *Mycobacterium tuberculosis* infection despite increased pulmonary lymphocytic infiltration. *J Immunol* 2004;173:3287–3296. [PubMed: 15322191]
25. Zhou Y, Kurihara T, Ryseck RP, Yang Y, Ryan C, Loy J, et al. Impaired macrophage function and enhanced T cell-dependent immune response in mice lacking CCR5, the mouse homologue of the major HIV-1 coreceptor. *J Immunol* 1998;160:4018–4025. [PubMed: 9558111]
26. Promrat K, McDermott DH, Gonzalez CM, Kleiner DE, Koziol DE, Lessie M, et al. Associations of chemokine system polymorphisms with clinical outcomes and treatment responses of chronic hepatitis C. *Gastroenterology* 2003;124:352–360. [PubMed: 12557141]
27. Wasmuth HE, Werth A, Mueller T, Berg T, Dietrich CG, Geier A, et al. Haplotype-tagging RANTES gene variants influence response to antiviral therapy in chronic hepatitis C. *Hepatology* 2004;40:327–334. [PubMed: 15368437]

28. Frese M, Schwarzle V, Barth K, Krieger N, Lohmann V, Mihm S, et al. Interferon-gamma inhibits replication of subgenomic and genomic hepatitis C virus RNAs. *Hepatology* 2002;35:694–703. [PubMed: 11870386]
29. Pravica V, Asderakis A, Perrey C, Hajeer A, Sinnott PJ, Hutchinson IV. In vitro production of IFN-gamma correlates with CA repeat polymorphism in the human IFN-gamma gene. *Eur J Immunogenet* 1999;26:1–3. [PubMed: 10068907]
30. Bream JH, Carrington M, O'Toole S, Dean M, Gerrard B, Shin HD, et al. Polymorphisms of the human IFNG gene noncoding regions. *Immunogenetics* 2000;51:50–58. [PubMed: 10663562]
31. Huang Y, Yang H, Borg BB, Su X, Rhodes SL, Yang K, et al. A functional SNP of interferon-gamma gene is important for interferon-alpha-induced and spontaneous recovery from hepatitis C virus infection. *Proc Natl Acad Sci U S A* 2007;104:985–990. [PubMed: 17215375]
32. Mangia A, Santoro R, Piattelli M, Pazienza V, Grifa G, Iacobellis A, et al. IL-10 haplotypes as possible predictors of spontaneous clearance of HCV infection. *Cytokine* 2004;25:103–109. [PubMed: 14698136]
33. Paladino N, Fainboim H, Theiler G, Schroder T, Munoz AE, Flores AC, et al. Gender susceptibility to chronic hepatitis C virus infection associated with interleukin 10 promoter polymorphism. *J Virol* 2006;80:9144–9150. [PubMed: 16940525]
34. Oleksyk TK, Thio CL, Truelove AL, Goedert JJ, Donfield SM, Kirk GD, et al. Single nucleotide polymorphisms and haplotypes in the IL-10 region associated with HCV clearance. *Genes Immun* 2005;6:347–357. [PubMed: 15815689]
35. Barrett S, Collins M, Kenny C, Ryan E, Keane CO, Crowe J. Polymorphisms in tumour necrosis factor-alpha, transforming growth factor-beta, interleukin-10, interleukin-6, interferon-gamma, and outcome of hepatitis C virus infection. *J Med Virol* 2003;71:212–218. [PubMed: 12938195]
36. Constantini PK, Wawrzynowicz-Syczewska M, Clare M, Boron-Kaczmarek A, McFarlane IG, Cramp ME, et al. Interleukin-1, interleukin-10 and tumour necrosis factor-alpha gene polymorphisms in hepatitis C virus infection: an investigation of the relationships with spontaneous viral clearance and response to alpha-interferon therapy. *Liver* 2002;22:404–412. [PubMed: 12390476]
37. Knapp S, Hennig BJ, Frodsham AJ, Zhang L, Hellier S, Wright M, et al. Interleukin-10 promoter polymorphisms and the outcome of hepatitis C virus infection. *Immunogenetics* 2003;55:362–369. [PubMed: 12942209]
38. Minton EJ, Smillie D, Smith P, Shipley S, McKendrick MW, Gleeson DC, et al. Clearance of hepatitis C virus is not associated with single nucleotide polymorphisms in the IL-1, -6, or -10 genes. *Hum Immunol* 2005;66:127–132. [PubMed: 15694997]
39. Seegers D, Zwiers A, Strober W, Pena AS, Bouma G. A TaqI polymorphism in the 3'UTR of the IL-12 p40 gene correlates with increased IL-12 secretion. *Genes Immun* 2002;3:419–423. [PubMed: 12424624]
40. Morahan G, Huang D, Wu M, Holt BJ, White GP, Kendall GE, et al. Association of IL-12B promoter polymorphism with severity of atopic and non-atopic asthma in children. *Lancet* 2002;360:455–459. [PubMed: 12241719]
41. Yin LM, Zhu WF, Wei L, Xu XY, Sun DG, Wang YB, et al. Association of interleukin-12 p40 gene 3'-untranslated region polymorphism and outcome of HCV infection. *World J Gastroenterol* 2004;10:2330–2333. [PubMed: 15285014]
42. Houldsworth A, Metzner M, Rossol S, Shaw S, Kaminski E, Demaine AG, et al. Polymorphisms in the il12B gene and outcome of HCV infection. *J Interferon Cytokine Res* 2005;25:271–276. [PubMed: 15871664]
43. Mueller T, Mas-Marques A, Sarrazin C, Wiese M, Halangk J, Witt H, et al. Influence of interleukin 12B (IL-12B) polymorphisms on spontaneous and treatment-induced recovery from hepatitis C virus infection. *J Hepatol* 2004;41:652–658. [PubMed: 15464247]
44. Kimura T, Saito T, Yoshimura M, Yixuan S, Baba M, Ji G, et al. Association of transforming growth factor-beta 1 functional polymorphisms with natural clearance of hepatitis C virus. *J Infect Dis* 2006;193:1371–1374. [PubMed: 16619184]
45. Pandey JP, Astemborski J, Thomas DL. Epistatic effects of immunoglobulin GM and KM allotypes on outcome of infection with hepatitis C virus. *J Virol* 2004;78:4561–4565. [PubMed: 15078937]

46. Baroni GS, Pastorelli A, Manzin A, Benedetti A, Marucci L, Solforosi L, et al. Hepatic stellate cell activation and liver fibrosis are associated with necroinflammatory injury and Th1-like response in chronic hepatitis C. *Liver* 1999;19:212–219. [PubMed: 10395041]
47. Maharaj B, Maharaj RJ, Leary WP, Cooppan RM, Naran AD, Pirie D, et al. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. *Lancet* 1986;1:523–525. [PubMed: 2869260]
48. Asti M, Martinetti M, Zavaglia C, Cuccia MC, Gusberti L, Tinelli C, et al. Human leukocyte antigen class II and III alleles and severity of hepatitis C virus-related chronic liver disease. *Hepatology* 1999;29:1272–1279. [PubMed: 10094975]
49. Renou C, Halfon P, Pol S, Cacoub P, Jouve E, Bronowicki JP, et al. Histological features and HLA class II alleles in hepatitis C virus chronically infected patients with persistently normal alanine aminotransferase levels. *Gut* 2002;51:585–590. [PubMed: 12235085]
50. Kryczka W, Brojer E, Kalinska A, Urbaniak A, Zarebska-Michaluk D. DRB1 alleles in relation to severity of liver disease in patients with chronic hepatitis C. *Med Sci Monit* 2001;7:217–220. [PubMed: 12211723]
51. Tillmann HL, Chen DF, Trautwein C, Kliem V, Grundey A, Berning-Haag A, et al. Low frequency of HLA-DRB1*11 in hepatitis C virus induced end stage liver disease. *Gut* 2001;48:714–718. [PubMed: 11302974]
52. Kuzushita N, Hayashi N, Moribe T, Katayama K, Kanto T, Nakatani S, et al. Influence of HLA haplotypes on the clinical courses of individuals infected with hepatitis C virus. *Hepatology* 1998;27:240–244. [PubMed: 9425943]
53. Kondo Y, Kobayashi K, Kobayashi T, Shiina M, Ueno Y, Satoh T, et al. Distribution of the HLA class I allele in chronic hepatitis C and its association with serum ALT level in chronic hepatitis C. *Tohoku J Exp Med* 2003;201:109–117. [PubMed: 14626512]
54. Yoshizawa K, Ota M, Saito S, Maruyama A, Yamaura T, Rokuhara A, et al. Long-term follow-up of hepatitis C virus infection: HLA class II loci influences the natural history of the disease. *Tissue Antigens* 2003;61:159–165. [PubMed: 12694584]
55. Tokushige K, Tsuchiya N, Hasegawa K, Hashimoto E, Yamauchi K, Komatsu T, et al. Influence of TNF gene polymorphism and HLA-DRB1 haplotype in Japanese patients with chronic liver disease caused by HCV. *Am J Gastroenterol* 2003;98:160–166. [PubMed: 12526952]
56. Patel K, Norris S, Lebeck L, Feng A, Clare M, Pianko S, et al. HLA class I allelic diversity and progression of fibrosis in patients with chronic hepatitis C. *Hepatology* 2006;43:241–249. [PubMed: 16440356]
57. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, et al. Genomewide association analysis of coronary artery disease. *N Engl J Med* 2007;357:443–453. [PubMed: 17634449]
58. Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, et al. A whole-genome association study of major determinants for host control of HIV-1. *Science* 2007;317:944–947. [PubMed: 17641165]
59. Huang H, Shiffman ML, Cheung RC, Layden TJ, Friedman S, Abar OT, et al. Identification of two gene variants associated with risk of advanced fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2006;130:1679–1687. [PubMed: 16697732]
60. Bataller R, North KE, Brenner DA. Genetic polymorphisms and the progression of liver fibrosis: a critical appraisal. *Hepatology* 2003;37:493–503. [PubMed: 12601343]
61. McGuinness PH, Painter D, Davies S, McCaughan GW. Increases in intrahepatic CD68 positive cells, MAC387 positive cells, and proinflammatory cytokines (particularly interleukin 18) in chronic hepatitis C infection. *Gut* 2000;46:260–269. [PubMed: 10644323]
62. Yee LJ, Tang J, Herrera J, Kaslow RA, van Leeuwen DJ. Tumor necrosis factor gene polymorphisms in patients with cirrhosis from chronic hepatitis C virus infection. *Genes Immun* 2000;1:386–390. [PubMed: 11196686]
63. Kusumoto K, Uto H, Hayashi K, Takahama Y, Nakao H, Suruki R, et al. Interleukin-10 or tumor necrosis factor-alpha polymorphisms and the natural course of hepatitis C virus infection in a hyperendemic area of Japan. *Cytokine* 2006;34:24–31. [PubMed: 16698276]

64. Hellier S, Frodsham AJ, Hennig BJ, Klenerman P, Knapp S, Ramaley P, et al. Association of genetic variants of the chemokine receptor CCR5 and its ligands, RANTES and MCP-2, with outcome of HCV infection. *Hepatology* 2003;38:1468–1476. [PubMed: 14647058]
65. Ajuebor MN, Wondimu Z, Hogaboam CM, Le T, Proudfoot AE, Swain MG. CCR5 deficiency drives enhanced natural killer cell trafficking to and activation within the liver in murine T cell-mediated hepatitis. *Am J Pathol* 2007;170:1975–1988. [PubMed: 17525265]
66. Ajuebor MN, Aspinall AI, Zhou F, Le T, Yang Y, Urbanski SJ, et al. Lack of chemokine receptor CCR5 promotes murine fulminant liver failure by preventing the apoptosis of activated CD1d-restricted NKT cells. *J Immunol* 2005;174:8027–8037. [PubMed: 15944310]
67. Ruiz-Ferrer M, Barroso N, Antinolo G, Aguilar-Reina J. Analysis of CCR5-Delta 32 and CCR2-V64I polymorphisms in a cohort of Spanish HCV patients using real-time polymerase chain reaction and fluorescence resonance energy transfer technologies. *J Viral Hepat* 2004;11:319–323. [PubMed: 15230854]
68. Goyal A, Suneetha PV, Kumar GT, Shukla DK, Arora N, Sarin SK. CCR5Delta32 mutation does not influence the susceptibility to HCV infection, severity of liver disease and response to therapy in patients with chronic hepatitis C. *World J Gastroenterol* 2006;12:4721–4726. [PubMed: 16937446]
69. Hofer H, Neufeld JB, Oesterreicher C, Grundtner P, Wrba F, Gangl A, et al. Bi-allelic presence of the interleukin-10 receptor 1 G330R allele is associated with cirrhosis in chronic HCV-1 infection. *Genes Immun* 2005;6:242–247. [PubMed: 15729365]
70. Powell EE, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, et al. Host genetic factors influence disease progression in chronic hepatitis C. *Hepatology* 2000;31:828–833. [PubMed: 10733535]
71. Suneetha PV, Goyal A, Hissar SS, Sarin SK. Studies on TAQ1 polymorphism in the 3'untranslated region of IL-12P40 gene in HCV patients infected predominantly with genotype 3. *J Med Virol* 2006;78:1055–1060. [PubMed: 16789008]
72. Bellamy R, Ruwende C, Corrah T, McAdam KP, Whittle HC, Hill AV. Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. *N Engl J Med* 1998;338:640–644. [PubMed: 9486992]
73. Romero-Gomez M, Montes-Cano MA, Otero-Fernandez MA, Torres B, Sanchez-Munoz D, Aguilar F, et al. SLC11A1 promoter gene polymorphisms and fibrosis progression in chronic hepatitis C. *Gut* 2004;53:446–450. [PubMed: 14960532]
74. Reynolds WF, Patel K, Pianko S, Blatt LM, Nicholas JJ, McHutchison JG. A genotypic association implicates myeloperoxidase in the progression of hepatic fibrosis in chronic hepatitis C virus infection. *Genes Immun* 2002;3:345–349. [PubMed: 12209361]
75. El Hag A, Clark RA. Down-regulation of human natural killer activity against tumors by the neutrophil myeloperoxidase system and hydrogen peroxide. *J Immunol* 1984;133:3291–3297. [PubMed: 6092470]

Table 1

Strength of inhibitory signal for selected KIRs important in HCV clearance

Inhibitory KIR	Ligand [*]	Relative strength of NK cell inhibitory signal
2DL1	HLA-C2	Strong
2DL2	HLA-C1	Intermediate
2DL3	HLA-C1	Weak

^{*} HLA-C1 and -C2 are HLA-C alleles with lysine or asparagine at position 80, respectively.

Table 2

Associations between polymorphisms in chemokine genes and HCV clearance or persistence

Chemokine gene	Outcome	Comments
<i>CCR5Δ32</i>	Clearance	Deletion may lead to increased T cell response
<i>IFNG</i> -764G (promoter mutation)	Clearance	Increases promoter activity
<i>IL-10</i>	Clearance	Several polymorphisms lower IL-10 expression which can increase Th1 response
<i>IL-12</i> 1188 A/A	Persistence	Homozygosity for the A allele is associated in some but not all studies
<i>TGFB</i>	Clearance	Two separate polymorphisms leading to lower TGF-B, which can increase NK cell activity
<i>TNFA</i> (-238, -308)	No associations	