

Review

Hepatitis C virus (HCV) and the genome-wide association studies: The role of IL-28B in HCV chronicity

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It is estimated that more than 170 million persons are infected with hepatitis C virus (HCV) world-wide. Of persons acutely infected with HCV, 20–30% recover spontaneously and clear viremia shortly following seroconversion, whereas 70–80% develop persistent infection. However, for a given infected individual, there is currently no way of predicting prognosis. Various studies have shown a clear difference in rates of HCV clearance based on race, host's immunogenetic factors, cytokine genotypes and expression, HLA types and NK activity. The genetic tools and the knowledge derived from the Human Genome Project, as well as availability of powerful and relatively inexpensive whole human genome analysis systems have led to the development of powerful genetic approach called genome-wide association study (GWAS). In such studies, one can examine common genetic variations across large spans of the human genome by analyzing hundreds of thousands of single nucleotide polymorphisms (SNPs) spanning all chromosomes. Several studies have shown cytokine genes or expression as immune mediators of HCV clearance. This review gives an overview of HCV molecular virology and highlight the growing evidence of the role of IL-28B in determining the outcome of HCV infection.

Keywords: Hepatitis c virus, genome-wide association studies, IL-28B

INTRODUCTION

Hepatitis C virus (HCV) was identified and cloned in 1989 (Choo et al., 1989). Immunoassays were subsequently developed to detect antibodies to this virus and HCV was found to be the agent responsible for the majority of cases of post-transfusion hepatitis (Ashfaq et al., 2011). The most striking features of HCV are its propensity to persist in a large proportion of infected individuals and the broad spectrum of liver disease that result from infection. However, the natural history of this viral infection and the mechanism of liver injury in acute and chronic hepatitis C remain largely unknown.

HCV protein epitopes, recognised by antibodies in blood donor specimens, have been used to identify individuals with HCV infection (Ashfaq et al., 2011). Testing for anti-HCV is usually performed using an enzyme linked immunosorbent assay (ELISA). The most commonly applied supplemental assay is the recombinant immunoblot assay (RIBA). Currently, the

only definitive test for diagnosis of HCV infection is a positive PCR result (Albeldawi et al., 2010; Rubin et al., 1994). HCV RNA detection is particularly useful during the "window period" before the antibodies appear (Chou et al., 2004). More recently, HCV Core antigen assays were described (Obeid and Alzahrani, 2011; Alzahrani and Obeid, 2004; Alzahrani, 2009).

It is estimated that more than 170 million persons are infected with HCV world-wide. In developed countries, HCV seroprevalence rates are generally less than 3% in the general population, whilst among volunteer blood donors, they are less than 1% (Ashfaq et al., 2011).

HCV infection has been associated with parenteral exposure, although the virus can be detected in other human body fluids such as saliva, urine and semen (Abe et al., 1991; Numata et al., 1993). Risk factors for acquiring HCV infection include intravenous drug use, blood transfusion or use of blood products,

haemodialysis, multiple sexual partners, sexual or household exposure, occupational exposure and organ transplantation (Zeuzem et al., 1996).

Of persons acutely infected with HCV, 20–30% recover spontaneously and clear viremia shortly following seroconversion, whereas 70–80% develop persistent infection. Of the chronically infected individuals, 30% will have progressively worsening liver disease potentially culminating in cirrhosis or hepatocellular carcinoma, 40% will have slowly evolving infections with only modest liver damage and 30% will have viremia in the absence of clinically detectable liver damage. However, for a given infected individual, there is currently no way of predicting prognosis (Selvarajah et al., 2010).

Morphology, genome and proteins

HCV is an enveloped RNA virus with a diameter of about 50 nm. It is a Hepacivirus that belong to the Flaviviridae family. The viral particle consists of a nucleocapsid surrounded by a lipid bilayer, which anchors heterodimers between the two envelope glycoproteins E1 and E2 (Hwang et al., 1994; Barrera et al, 1995; Sharma, 2010; Moradpour et al, 2001; Chevaliez and Pawlotsky, 2007).

Although its means of transmission are well documented, the hepatitis C virus itself still remains an enigma. HCV contains a single-stranded, positive-sense RNA molecule of 9.6 kb with one long open reading frame coding for a large polyprotein of about 3000 amino acids (Sharma, 2010; Moradpour et al, 2001; Chevaliez and Pawlotsky, 2007). The N-terminal quarter of the genome encodes the core and structural proteins. The rest of the genome encodes the nonstructural proteins NS2-5. NS proteins have been localized to the membrane of the ER, suggesting that it is the site of polyprotein maturation and viral particle assembly. Little is known about the three dimensional structure of the HCV proteins. The HCV polyprotein is processed by a combination of host and virus-encoded proteases into at least 10 individual proteins. These proteins are C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B (Bartenschlager et al, 1993). Viral genome replication with the error-prone RNA-dependent RNA polymerase results in frequent mutations, some of which are tolerated resulting in a new variant RNA genome. It lacks efficient proofreading ability as it replicates. Several closely related sequences may co-exist in a single patient. HCV undergoes rapid mutation in a hypervariable region of the genome coding for the envelope proteins (E2) and escapes immune surveillance by the host. Moreover, HCV inhibits several innate immune mechanisms of the host (Sharma, 2010; Ashfaq et al, 2011). As a consequence, most HCV-infected people develop chronic infection.

The hepatitis C virus life cycle

The full details of the replication cycle of HCV are not well established. Understanding of the molecular basis of viral infection was hampered by the lack of convenient infectious model systems.

In the past decade, the characterization of HCV replicons, HCV pseudoparticles (HCVpp), an infectious cell culture model (HCVcc), and small animal models based on chimeric uPA-SCID or other mice have resulted in a better understanding of the lifecycle, pathogenesis and the potential for development of better antiviral strategies (Georgel et al., 2010).

The virus enters the cell and is uncoated in the cytoplasm. The viral genome is transcribed to form a complementary negative-sense RNA molecule, which, in turn, serves as a template for the synthesis of progeny positive-strand RNA molecules^{9,10}. The newly translated polyprotein is cleaved by a host-cell signalase as well as virus-specific non-structural proteins, NS-2 and NS-3. The enzyme capable of performing both steps of RNA synthesis is the virally encoded RNA-dependent RNA polymerase NS5b. The NS-3 of HCV also has helicase (unwindase) activity. HCV replicates by a negative-strand RNA intermediate and has no reverse transcriptase activity. Over time, this endless cycle of reproduction results in significant damage to the liver. Millions upon millions of cells are destroyed by viral reproduction or by the immune system's attacks on infected cells (Chevaliez and Pawlotsky, 2007).

HCV Genotypes

The overall mutation rate of the entire HCV genome is estimated at between 1.4×10^{-3} and 1.92×10^{-3} base substitutions/genome site/year (Chevaliez and Pawlotsky, 2007). Phylogenetic analysis of the NS5 gene, in a series of HCV isolates from Europe, North and South America and the Far East, showed that HCV can actually be structured into six groups based on genome sequence (Types 1-6). Similar relationships may also be found between variants if analysis is carried out using the E1, NS4 or core regions.

HCV is classified into eleven major genotypes (designated 1-11), many subtypes (designated a, b, c, etc.), and about 100 different strains (numbered 1,2,3, etc.) based on the genomic sequence heterogeneity. The variability is distributed throughout the genome. However, the non-coding regions at either end of the genome (5'-UTR and 3'-UTR; UTR-untranslated region) are more conserved and suitable for virus detection by PCR. The genes coding for the envelope E1 and E2 glycoproteins are the most variable. Amino acid changes may alter the antigenic properties of the proteins, thus allowing the virus to escape neutralizing antibodies (Chevaliez and

Pawlotsky, 2007).

Genotypes 1-3 have a worldwide distribution. Types 1a and 1b are the most common, accounting for about 60% of global infections. They predominate in Northern Europe and North America, and in Southern and Eastern Europe and Japan, respectively. Type 2 is less frequently represented than type 1. Type 3 is endemic in south-east Asia and is variably distributed in different countries. Genotype 4 is principally found in the Middle East, Egypt, and central Africa. Type 5 is almost exclusively found in South Africa, and genotypes 6-11 are distributed in Asia (Moradpour et al., 2001).

The influence of viral genotype in the pathogenesis of liver disease is still controversial. Environmental, genetic, and immunological factors may contribute to the differences in disease progression, so characteristic of HCV infection, observed among patients. The determination of the infecting genotype is however important for the prediction of response to antiviral treatment: genotype 1 is generally associated with a poor response to interferon alone, whereas genotypes 2 and 3 are associated with more favorable responses (Pereira et al., 2009).

The current standard of care treatment consists of pegylated interferon- α in combination with ribavirin that must be given for 24–48 weeks depending on genotype and virological response (Selvarajah et al., 2010; Pereira et al., 2010; McHutchison et al., 2010). Using this regime, 54–63% of individuals with persistent infection exhibited a sustained virological response (SVR), which is believed to represent true eradication of HCV infection (Maheshwari et al., 2010; Farnik et al., 2009). However, the SVR rates for genotype 1-infected individuals are lower at 42–52% as compared with that for individuals infected with genotype 2 or 3 at 76–84%.

Host genetics and HCV infection

The outcome of many human diseases including infection is influenced by host genetic factors. Human genome sequence and HapMap projects helped in studying role of host genetic factors in HCV pathogenesis (Georgel et al., 2010). The first studies have led to the identification of the individual genes regulating host immune responses. Thomas et al. (2000) showed a clear difference in rates of HCV clearance based on race, with white patients more likely to clear virus spontaneously than African-American patients. The differences observed in either spontaneous or treatment-induced HCV elimination suggested that there may be a strong genetic contribution, perhaps manifested phenotypically in more or less robust host.

Candidate genes have been identified using transcriptome approaches. These include Human leukocyte antigens (HLA) and the inhibitory NK cell Killer immunoglobulin-like receptor (KIR2DL3) (Georgel et al.,

2010; Yee, 2004; Khakoo et al., 2004).

Effect of host type III interferon lambda (IFN- λ) ON the outcome of HCV infection

Most innate or adaptive immune genes associated with HCV pathogenesis have been identified in single nucleotide polymorphism (SNP) association studies. Such approaches, termed genome-wide association studies (GWAS), are based on high-throughput assays that simultaneously provide data on millions of SNPs (Rauch et al., 2010; Tanaka et al., 2009). The comparison of the frequency of common (i.e. present in more than 5% of a population) variants (SNPs) in thousands of patients compared with a comparable number of controls permits the detection of low-risk variants. Independent groups have recently reported GWAS data from ethnically different cohorts (Mosbrugger et al., 2010). These studies provided a comparative analysis between responders to the standard treatment upon HCV infection and non-responders. Viral clearance occurs in only 40–50% of patients receiving standard-of-care treatment who are infected by HCV genotype 1. One SNP, located upstream of the IL-28B gene, is identified in the four reports, which provides stunning evidence that type III IFN- λ (encoded by the IL-28B gene) affects the response to treatment in chronic HCV infection. Furthermore, IL-28B mRNA expression is higher in responders than non-responders. Individuals across several ethnic groups could be separated between those who spontaneously (without treatment) clear HCV infection and those in which HCV remains persistent by their IL-28B genotype. These results unambiguously implicate IL-28B in the spontaneous resolution of acute HCV infection. Type III (λ) IFNs, which include IFN- λ 1, 2 and 3 (also known as IL-29, IL-28A and IL-28B), are cytokines distantly related to type I (α / β) IFNs. Both classes of molecules have similar antiviral activities and are produced upon the activation of common innate sensors (Rauch et al., 2010; Tanaka et al., 2009).

Ge et al. (2009) reported a genetic polymorphism in the IL-28B gene on chromosome 19, the SNP rs12979860, associated with HCV treatment-induced clearance.

The GWAS by Suppiah et al. (2009) found a SNP rs8099917 in the intergenic region between IL-28A and IL-28B was associated with positive response to treatment-induced recovery. Tanaka et al. (2009) reported results of a study in a Japanese cohort and found that the same SNP as in the study by Suppiah et al. (rs8099917) was associated with response to treatment.

Thomas et al. (2009) examined the association of SNP rs12979860 with spontaneous HCV clearance. They genotyped individuals who spontaneously cleared infection for the SNP rs12979860, compared with a control group of persistently infected individuals (this

study included a large number of blood donors with resolved and persistent HCV infections). Interestingly, the same IL-28B SNP rs12979860 shown to be associated with treatment-induced viral clearance was also associated with spontaneous clearance of HCV. Rauch et al. (2010) performed a GWAS for Swiss whites individuals with different HCV disease outcomes that included those who spontaneously cleared the virus, responded to treatment (pegylated interferon- α and ribavirin), or were chronically infected. The SNP rs8099917 (in the IL-28B promoter region) gave the strongest association with spontaneous HCV clearance and no other SNP outside the IL-28B/A locus reached genome-wide significance.

Several studies have examined other cytokine genes or expression as immune mediators of spontaneous HCV resolution (Lemon, 2010; Rauch et al., 2009). The most studied of these are IFN- γ , TNF- α , IL-10, IL-12B, and TGF- β (Thio et al., 2004; Kimura et al., 2006; Haung et al., 2007; Mueller et al., 2004; Selzner and McGilvray, 2008).

CONCLUDING REMARKS

The molecular characterization of viral factors important for HCV infection provides new opportunities to develop antiviral therapies. The translation of IL-28B data to clinical practice remains a long way off for several reasons. There is a need to investigate patients infected with non-type 1 genotypes and to explore the molecular mechanism for IL-28B in the pathogenesis of HCV infection in HCV model systems and HCV-infected patients. Nevertheless, it is reasonable to consider using IFN- λ - based therapies to treat chronic HCV infection. Indeed, a clinical trial to evaluate the effect of IL-29 (or IFN- λ 3) on HCV infection reported a decrease in viral load in HCV infected patients (website references). Furthermore, IFN- λ treatment seems to be well tolerated. Thus, type III IFN (or IFN- λ) therapy could be as (and possibly even more) effective than the standard of care with fewer side effects. Alternatively, IFN- λ could be combined with current treatment because they could cooperatively suppress HCV infection.

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