Hepatitis C Virus: Molecular Pathways and Treatments

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Published by **OMICS Group eBooks**

731 Gull Ave, Foster City. CA 94404, USA

Cover OMICS Group Design team

First published June, 2014

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Introduction

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Abstract

Hepatitis C Virus (HCV) was found as the causative agent of the Non-A, Non-B Hepatitis (NANBH) in 1989. HCV is an enveloped RNA virus that could be classified into six genotypes. The major host cell supporting HCV replication is the human hepatocyte. HCV is primarily transmitted by exposure to contaminated blood. HCV exists in host blood as quasispecies, a population of dynamic strains closely related to each other. Hepatitis C patients may have decreased appetite, abdominal pain, jaundice, fatigue and flu-like symptoms, or could be asymptomatic. Acute infection refers to the first six months of HCV infection, while more than 75% of patients develop chronic Hepatitis in the natural course of HCV infection. The interactions between host immune responses and virus immune evasion determine the outcome of HCV infection. The base of the current standard therapy of HCV is pegylated interferon and ribavirin, the usage of which is restricted due to its side effects. No HCV vaccine is currently available in clinics. However, recent research has shown that a single strain of HCV could elicit broad cross-neutralizing antibodies against all known major genotypes of HCV, and provides considerable encouragement for HCV vaccine developement.

General Background of Hepatitis C Virus (HCV)

After World War II (1939-1945), which induced a huge need for blood replacement, transfusion-associated Hepatitis became one of the major hazards of blood transfusion. Following the identification of Hepatitis B Virus (HBV) as an infectious agent causing Hepatitis, an all-volunteer donor system was adopted and multiple screening assays for HBV antigen were introduced in the 1970's. Although these interventions dramatically decreased rates of transfusion-associated Hepatitis, still many Hepatitis patients were infected by unidentified agents. A retrospective study showed that only 25% of transfusion-associated Hepatitis was caused by HBV, indicating that 75% of cases were tentatively classified as Non-B Hepatitis [1,2]. At that time, the only other identified Hepatitis virus was Hepatitis A Virus (HAV). However, surprisingly, there was not a single transfusion-associted Hepatitis case caused by HAV. Therefore, the Non-A, Non-B Hepatitis (NANBH) was designated to describe the new form of blood transfusion-associated Hepatitis caused by the unknown infectious agent [3]. The search for the causative agent of NANBH finally led to the identification of HCV.

Following the description of NANBH, international research efforts to identify the causative agent of this transfusion-associated Hepatitis failed for more than a decade. A major discovery was made in 1989, when Choo and colleagues under the direction of Dr. M. Houghton, collaborating with Dr. D. W. Bradley, utilized a novel molecular cloning method to identify the unknown infectious agent of NANBH. Using RNA extracted from the plasma of a chimpanzee freshly infected with the NANBH agent, a random-primed complementary DNA (cDNA) expression library was constructed. The cDNA library was constructed in the bacteriophage Xgtl 1 and screened with antibodies present in the serum of a patient with NANBH. This resulted in the isolation of a cDNA clone that encoded an antigen specifically related to NANBH [4]. The virus was later confirmed to be present in a panel of NANBH specimens by Dr. H. J. Alter and coworkers, and the virus was re-named "Hepatitis C Virus" or HCV [5]. The discovery of HCV has led to significant improvements in the diagnosis of Hepatitis, improved blood transfusion safety by screening assays, and a rapid expansion in epidemiology, pathogenesis and antiviral research.

The HCV Particle

HCV is a small (approximately 50 nm in size), enveloped, positive-stranded RNA virus. It is the only known member of the genus *Hepacivirus* of the *Flaviviridae* family. The *Flaviviridae* family also includes other viruses, e.g., Yellow Fever virus and West Nile virus [6].

The HCV particle consists of an RNA genome in an icosahedral nucleocapsid, which is surrounded by a lipid bilayer. The HCV RNA genome consists of a single Open Reading Frame (ORF) that is approximately 9600 nucleotide bases long. The lipid bilayer of HCV is of cellular origin, and two viral envelope glycoproteins, E1 and E2, are embedded in the lipid bilayer on the envelope [6]. The HCV genome contains a 5' Noncoding Region (NCR), followed by an ORF that codes for structural and Nonstructural (NS) proteins and a 3' NCR required for HCV replication.

The translation product of the HCV genome is a polyprotein approximately 3000 amino acids long, which is cleaved by viral and cellular enzymes into individual proteins including three structural proteins (core, E1, E2) and six NS proteins (P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) [6]. The three HCV structural proteins include, besides E1 and E2, a core protein. E1 and E2 glycoproteins play crucial roles in mediating HCV binding to and entry into target host cells. E2 contains three hypervariable regions (HVRs), called HVR1, HVR2 and HVR3. They are considered as hotspots for extreme sequence variability. Epitopes localized to these HVRs evolve rapidly, and are targets of host-developed neutralizing antibodies [7-9].

The six NS proteins of HCV include the p7 ion channel, the NS2 protease, the NS3 serine protease and RNA helicase, the NS4A polypeptide, the NS4B and NS5A proteins, and the NS5B RNA-dependent RNA polymerase (RdRp). The small hydrophobic protein p7 consists of 63 amino acids. Following its release from the HCV polyprotein, p7 oligomerizes on the Endoplasmic Reticulum (ER) membrane to form calcium ion-conductive pores [10]. While deletion of p7 does not appear to affect HCV entry and replication, it significantly abrogates infectious virion assembly and release of HCV [11]. The NS2-3 cysteine protease consists of the C-terminal domain of NS2 in conjunction with the N-terminal region of NS3. It is responsible for the autocatalytic cleavage of the NS2-NS3 junction in HCV polypeptides [12]. NS3 is a multifunctional protein with serine protease activity located in its N-terminal domain, as well as

RNA helicase and Nucleotide Triphosphatase (NTPase) activity provided by its C-terminus domain. The NS3 N-terminal serine protease domain, which engages the NS4A polypeptide as a cofactor, is responsible for the cleavage of all remaining junctions in the NS portions of the HCV polyprotein [13]. NS4A is required for efficient processing of the HCV polyprotein by functioning as a viral protease cofactor and provides stability to NS3 [14]. NS4B is an integral membrane protein that for many years was characterized mostly as a protein of unknown function. Recently, however, NS4B has been identified to be responsible for the formation of a novel intracellular membrane structure, termed the "membranous web", which appears to be the platform upon which HCV replication occurs. Furthermore, NS4B is implicated in modulation of the RdRp activity of NS5B [15,16]. The NS5A phosphoprotein has generated wide interest in HCV research because of its ability to modulate the Interferon (IFN) response of host cells. NS5A harbors a potential Interferon Sensitivity Determining Region (ISDR) and sequence variations within this cluster may contribute to the resistance to IFN-α therapy which is often observed in patients with HCV [17]. While no known enzymatic function has currently been ascribed to NS5A, it is still believed to be an essential component of HCV replication. Furthermore, NS5A has been identified to be an RNA-binding protein [18] and a major determinant of viral assembly [19]. Being the RdRp for HCV, NS5B is critical for RNA replication in the host cells and is membrane-associated [20]. These NS proteins together with structural proteins are important targets of specific antiviral agents for HCV.

In addition to the HCV polyprotein, an Alternate Reading Frame (ARF) protein or Frameshift Protein (F) is also produced in host cells [21]. The F protein was found to be in the HCV core-coding region that has the potential to encode a protein of up to 160 amino acids. Specific antibodies [22] and T cells [23] active against the F protein have been detected in patients with chronic HCV infection, which indicates that the F protein is expressed during HCV infection. The precise function of the F protein is unclear, although it may play a role in HCV replication. Indeed, mutations in the F protein delay HCV RNA replication and reduce virus titers [24,25].

HCV Genotypes

HCV isolates are classified into six (or seven) major genotypes and more than 100 subtypes. Genotype 7 is often classified as part of genotype 6, which explains the difference between HCV genotyping methods.

HCV main genotypes, identified as genotypes 1 through 6 (or 7), have more than 30% sequence divergence between them. Each genotype is further divided into subtypes designated by lowercase letters, with each subtype differing from one another by 10-30% sequence divergence. Subtypes are further clustered into quasispecies based on their genetic diversity, generated by the low-fidelity level of HCV RdRp [26].

The distribution of HCV genotypes varies worldwide. In North America, genotype 1a predominates, followed by genotypes 1b, 2a, 2b and 3a [27]. Genotypes are clinically important in that they are among the principal determinants of potential response to IFN-based therapy for HCV and also determine the required duration of such therapy. For example, patients infected with genotype 1 and 4 are less responsive to standard IFN-based therapy than those infected with other HCV genotypes [27]. The duration of IFN-based HCV therapy for genotypes 1 and 4 infections is 48 weeks, while it can be reduced to 24 weeks for patients infected with genotypes 2 or 3.

Besides the effects of HCV genotype on standard HCV therapy (pegylated IFNα and ribavirin), HCV genotype also affects the clinical usage of Direct-Acting Antiviral Agents (DAAs), which are novel selective inhibitors of the HCV protease and nearly double the cure rates for this infection when combined with the standard HCV therapy. Boceprevir and telaprevir are the first DAAs that selectively target HCV [28]. Telaprevir is a valuable new treatment for use in combination with pegIFNα and ribavirin in genotype 1 chronic Hepatitis C, and it is effective for treatment-naive and previously treated patients with genotype 1 in whom the pegIFNα and ribavirin dual therapy may not be successful [29].

HCV Lifecycle

The HCV lifecycle comprises viral entry, uncoating and release of the viral genome into the cytoplasm, translation and replication of the RNA, cleavage of the polyprotein, assembly into new particles and egress of new HCV particles. The natural host of HCV infection is human, and the major host cell supporting HCV infection and replication *in vivo* is the human hepatocyte.

HCV entry is the first step of interaction between the virus and the host target cell. As a complex multi-step process, HCV entry occurs in a pH- and clathrin-dependent manner and involves several host receptors. The current model of HCV entry includes (1) initial attachment of HCV to Glycosaminoglycans (GAG) and/or Low-Density Lipoprotein Receptor (LDL-R) on target cells, (2) binding of HCV to CD81 and /or the scavenger receptor class B type I (SR-BI) and (3) re-location to tight junctions via binding of HCV to members of the Claudin Family (CLDN1, 6 and 9) and occludin [6,30] on target cells.

In the serum of Hepatitis patients, HCV is mostly (more than 85%) associated with lipoproteins and thus has been termed as "Lipo-Viro-Particles" (LVP) [31]. Density gradient analysis of patient serum reveals that HCV-RNA is found in both low (1.06-1.15 g/ml) and high (1.17-1.25 g/ml) density fractions. HCV in the low-density fractions demonstrates a higher infectivity than HCV in the high-density fractions [32,33]. Observed in transmission electron microscopy, the LVP of HCV with the unusual low-density is spherical and up to 100 nm in diameter [31]. After delipidation with detergents, the HCV LVP is converted to smaller particles resembling nucleocapsids with a diameter of 30-40 nm [31]. Analysis of the unusual low-density LVP from patient serum demonstrated that they contain apolipoprotein B and apolipoprotein E proteins, triglycerides, and cholesterol [31], which are components of Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) particles. Furthermore, blocking LDL-R with antibodies can block HCV entry [34], indicating that LDL-R is a co-receptor for HCV entry.

The initial attachment of HCV is probably mediated by apolipoproteins E and B on viral particles binding the LDL-R expressed on target cells [34,35]. Pretreatment of HCV LVP with heparin or pretreatment of cells with heparinase could decrease HCV attachment, which indicates the involvement of GAGs [35]. Following the initial attachment of HCV to LDL-R and GAGs, CD81 [36,37] and SR-BI [38-40] are involved in the uptake of HCV. The E2 envelope protein of HCV interacts with the large extracellular loop of CD81, a post-attachment entry co-receptor [37]. E2 can also interact with SR-BI [40]. The role of CD81 [41,42] and SR-BI [38,39,43] in HCV entry is post-binding, as antibodies against either CD81 [37] or SR-BI [40] could inhibit infection after HCV attachment. Following the interaction of HCV with CD81 and SR-BI, HCV re-locates to tight junctions as mentioned above [6,30]. Claudin-1, a tight junction component highly expressed in hepatocytes, is essential for HCV entry at a late step. Antibodies against claudin-1 block HCV infection [44,45]. Another tight junction protein, occludin, was also identified as a receptor required for HCV entry. These studies on tight junction protiens highlight the importance of the tight junction complex in HCV entry [46].

HCV entry involves multiple host factors, such as human kinase, iron uptake receptor and cholesterol uptake receptor. A Mitogen-Activated Protein Kinase (MAPK)-related kinase, MAPK interacting serine/threonine kinase 1 (MKNK1), is a host factor required for HCV entry [47]. Transferrin Receptor 1 (TfR1) is an iron uptake receptor which acts during HCV entry at a post binding step after CD81. TfR1 plays a role at the level of glycoprotein-mediated entry [48]. A cellular cholesterol uptake receptor, Niemann-Pick C1-Like 1 (NPC1L1), is identified as a HCV entry factor [49]. These factors provide novel targets for HCV treatment.

After HCV comes into contact with the tight junction proteins claudin-1 and occludin, it undergoes clathrin-dependent endocytosis [50]. Clathrin is a protein involved in the formation of coated vesicles. Clathrin is one of the coat-proteins that can be used to build small vesicles in order to safely transport molecules between cells. Small interfering RNA (siRNA)-mediated depletion of clathrin inhibits HCV entry and infection [50].

Following internalization via endocytosis, the HCV genome is delivered from the endosome to the target cell's cytoplasm in a pH-dependent fusion process. Being a positive sense RNA, the HCV genome acts as mRNA and is therefore directly translated into a polyprotein. Upon release of the viral genome into the host cell, the HCV polyprotein is produced by an Internal Ribosome Entry Site (IRES)-dependent translation mechanism in the infected cell cytoplasm [51]. Translation of HCV genome RNA occurs in the Rough Endoplasmic Reticulum (RER) of cells. Translation of the HCV Open Reading Frame (ORF) yields a polyprotein precursor, which is co-translationally and post-translationally processed by cellular and viral proteases into several mature structural and NS proteins. The structural proteins and the p7 polypeptide are processed by the Endoplasmic Reticulum (ER) signal peptidase, while the NS proteins are processed by the viral NS2-3 protease and NS3-4A serine protease. NS2-3 protease also works as an autoprotease. Its auto-catalytic activity resides in the C-terminal half region of NS2 and the N-terminal one-third region of NS3 [6]. With the NS4A polypeptide functioning as a cofactor for the NS3 serine protease, the NS3-4A serine protease complex processes all other NS proteins [6,30].

During or shortly after the polyprotein processing, the replication complex of HCV is formed. This complex presumably involves viral proteins, viral RNA, and host cell factors. The NS5B RdRp protein initiates synthesis of the complementary negative-strand RNA immediately after infection, which then serves as a template to generate genomic positive-strand RNA. Newly synthesized positive-stand RNAs are used for translation, further RNA production, or become the genetic material packaged in new virions. HCV replication induces the synthesis of the membranous web, which concentrates lipid-rich structures facilitating HCV replication and assembly [6].

Although little is known about the late steps of HCV lifecycle, HCV may use the assembly and secretion mechanism of VLDL to exit the infected cells. Inhibition of apolipoprotein B by short hairpin RNA could inhibit the production of infectious HCV [52]. Furthermore, inhibition of VLDL secretion leads to the accumulation of intracellular HCV-RNA and inhibits HCV secretion [53]. Therefore, the association of HCV nucleocapsid with lipid droplets has been proposed to play a crucial role in HCV release [30,53].

As HCV encodes only a few viral proteins, this virus depends mostly on host factors to propagate. A large number of host factors are required for HCV translation, replication and production. For example, the autophagy proteins Beclin-1, Atg4B, Atg5 and Atg12 have been demonstrated to be proviral factors required for the translation of incoming HCV RNA and thereby for the initiation of HCV replication [54]. Lipid Droplets (LDs) in the vicinity of HCV active replication sites are essential for HCV assembly in liver-derived cells. The capsid protein core and NS5A interact with LDs, which allows HCV assembly [55]. Screens with siRNA have further identified dozens of host factors required for HCV replication [56]. The identification of these host factors has advanced our understanding of virus-host interactions and indicated potential new targets for preventive and therapeutic interventions.

HCV Epidemiology

With approximately 170 million infected individuals worldwide, representing 3% of the world's population, HCV is the major cause of chronic Hepatitis and is a huge burden for public health.

HCV transmission primarily happens by blood-to-blood contact. Any practice or activity that involves exposure to contaminated blood can potentially lead to HCV infection. Most patients with chronic HCV infection have been infected through transfusion with HCV-contaminated blood or blood products, via Injection Drug Use (IDU), or through sexual exposure, although transmission by sexual contact is low [57]. Being at a particular high risk of HCV exposure and Infection, Injection Drug Users (IDUs) constitute one half to two thirds of HCV-infected patients in Canada [58].

Vertical transmission of HCV from an infected mother to her child could happen during the birth process. However, transmission occurs only among women who are HCV RNA positive at the time of delivery, and the risk of transmission in this setting is relatively low. Only less than 5% of infants born to HCV positive mothers become infected [59].

Occupational exposure could lead to HCV transmission in health care workers, with the highest proportion of occupational transmission being from percutaneous injury via hollow-bore needles used in vascular access [60].

Signs and Symptoms of HCV Infection

Acute HCV refers to the first 6 months of infection. Symptoms of patients with acute HCV infection can be decreased appetite, abdominal pain, jaundice, fatigue, and flu-like clinical signs. However, more than 70% of infected patients do not develop symptoms during the acute phase, which makes acute HCV infection difficult to be diagnosed. In terms of laboratory assays, acute Hepatitis C is marked by appearance of HCV RNA in patient serum within 1 to 2 weeks after infection followed by elevated serum levels of liver-associated enzymes, such as Alanine Aminotransferase (ALAT) and Aspartate Aminotransferase (ASAT) [61]. The appearance of antibodies against HCV is rather late, ranging from 3 to 15 weeks after infection [62].

In the natural course of Hepatitis C infection, approximately 75-85% of infected patients become chronic Hepatitis patients [62]. Chronic HCV infection is defined as an HCV infection persisting for more than six months. It is often asymptomatic clinically and discovered accidentally, e.g. by routine examination, blood donation or insurance screening. Most patients have evidence of liver inflammation on liver biopsy, while the rate of liver fibrosis varies significantly between individuals [61]. Although HCV viremia may reach a stable level and remain constant over years after infection, chronic HCV infection is usually associated with persistently elevated and frequently fluctuating elevations in ALAT and ASAT levels [62].

The most common severe sequelae of chronic HCV infection include progressive hepatic fibrosis, liver cirrhosis, and hepatocellular carcinoma. Once chronic HCV infection has progressed to cirrhosis, decreased liver function and/or portal hypertension (increased

pressure in the liver circulation) may appear. The signs indicating portal hypertension include ascites (accumulation of fluid in the abdomen), varices (enlarged veins, especially in the stomach and esophagus), and hepatic encephalopathy (a syndrome of cognitive impairment due to the accumulation of ammonia and other substances normally cleared by a healthy liver) [57,63].

HCV infection may also lead to extrahepatic manifestations, which include mixed cryoglobulinemia, Sjogren's syndrome, B-cell non-Hodgkin's lymphoma and presence of serum auto-antibodies [61]. Individuals with chronic Hepatitis C infection are also likely to develop type II diabetes [64-67]. The mechanisms underlying these extrahepatic manifestations are not fully understood.

Management of a Patient with Chronic HCV Infection

The management of HCV infection includes serological and virological tests for HCV, liver biopsy and ultrasound-based tests [68,69].

Anti-HCV antibodies indicate that a patient has been infected with HCV [68,69]. Specific virological testing for HCV RNA further differentiates the patients who have been infected and then cleared the virus from those who become chronic carriers. The presence of HCV RNA in the absence of anti-HCV antibodies strongly indicates acute HCV infection. Molecular determination of the HCV genotype is important to help in the IFN-based treatment of chronic Hepatitis C patients [68,69].

In addition to laboratory tests, liver biopsy and ultrasound-based tests are often used to evaluate the severity of HCV-associated liver disease. The results of serum enzymatic assays, such as elevated ALAT levels, indicate the indirect effects of HCV on infected hepatocytes, which reflect immune injury and altered liver function since infected hepatocytes are the targets of host cellular and humoral immunity. Liver biopsy and ultrasound-based tests have been used to assess HCV-associated liver disease, including liver inflammation, steatosis, fibrosis and hepatocellular carcinoma [68,69].

Factors that Promote Progression of Chronic Hepatitis C

Multiple factors, including host factors, the transmission route of HCV and viral factors, can affect progression of chronic Hepatitis C.

Host factors include race, age, gender, co-infection with HIV or HBV, obesity, alcohol, HLA and Interleukin-28 (IL-28) genotyping, etc. For example, HCV clearance is less common in black persons [66]. Young patients have a higher rate of disease progression than older patients. The average time from HCV infection to development of cirrhosis and hepatocellular carcinoma in older patients (average age, 58 years) is more than twice as long as in the younger patients (average age, 29 years) [70,71]. Women tend to eliminate HCV more rapidly, have a lower rate of disease progression and a lower mortality rate from HCV-associated liver disease than men [72]. Viral factors include genotype and viral mutations.

Human genetic variation in the IL-28 locus

A genetic polymorphism near the IL-28B gene, which encodes IFN-lambda, is associated with the variable outcome of HCV infection. For example, the minor G allele at the reference sequence (rs) 8099917 in the IL-28B locus is associated with HCV chronicity [73]. The association of the IL-28B locus with HCV indicates the importance of innate immunity, especially IFN-lambda, in the pathogenesis of HCV [74]. The IL-28A/B and IL-29 genes encode IFN-lambda proteins. Genetic polymorphism has been found near the IL-28B gene on chromosome 19, 3 kilobases (kb) upstream of the IL-28B gene [75].

Furthermore, human genetic variation at the rs12979860 polymorphism site affects the progress of steatosis during the treatment of chronic Hepatitis C patients. The human CC genotype is associated with a lower rate of liver steatosis, which indicates less disturbance of lipid metabolism by HCV infection [76]. Furthermore, the human CC genotype is associated with spontaneous clearance of acute HCV infection [77] as well as response to IFN-based treatment [78,79].

Human Leukocyte Antigen (HLA) haplotypes

Both HLA-I and HLA-II haplotypes can affect the clinical course of HCV-infected patients. For example, HLA-I B54 is closely associated with the progression of liver injury and HLA-C*04 are associated with HCV persistence in Hepatitis C patients [80]. This may be attributed to the fact that certain HLA-I molecules may not be able of presenting HCV epitopes to cytotoxic T cells, which are required for clearance of HCV infection. The association of HLA-C*04 with HCV persistence suggests that Natural Killer (NK) cells may play an important role in HCV clearance, since HLA-C*04 is a ligand for receptors on NK cells [81].

HIV co-infection

HIV co-infection accelerates liver disease progression in chronic Hepatitis C patients. HCV reactivation and persistence have been reported in patients coinfected with HIV, even though some of these patients had previously cleared HCV infection when they were HIV-negative. Liver cirrhosis progresses more quickly in HCV/HIV co-infected patients than in HCV mono-infected patients [82].

Studies on the possible mechanism of accelerated liver disease progression in HCV/HIV co-infected patients yielded controversial results. Daar et al. suggest that it is due to the deteriorated immune status of HIV co-infected patients, based on the fact that as the decrease of $CD4^+$ T lymphocyte counts correlates with an increase in HCV RNA levels [83]. However, Strasfeld et al. failed to retrieve this correlation [84].

Lifestyle risk factors

The incidence of liver cirrhosis is higher in HCV-infected patients who consume alcohol daily [66]. The mechanism of increased liver injury in these patients is multi-factorial, which includes a combination of diminished immune clearance of HCV, oxidative stress, hepatic steatosis, increased iron stores, and increased apoptosis of hepatocytes [85]. Tobacco consumption and obesity are also lifestyle risk factors associated with hepatocellular carcinoma [86].

HCV genotype

In comparison to other HCV genotypes, genotype 1 is related to a higher rate of HCV persistence and a lower rate of successful treatment with IFN-based therapy. Genotype 1 patients also demonstrate a higher level of insulin resistance [87,88]. HCV genotype 3 is associated with the development of liver steatosis and fibrosis. This could possibly be in relation to the genotype-3a core protein, which is stronger than the genotype-1b core protein in up-regulating the fatty acid synthase gene promoter [89].

Occult HCV Persistence

Patients recovered from HCV infection are clinically free of Hepatitis symptoms and negative for serum tests of HCV RNA as shown by standard viral assays. However, HCV may remain in their serum and cells at very low levels. Recently, several studies have revealed that patients continue to harbor HCV in their serum at very low levels and the replicative intermediates of HCV were detected in Peripheral Blood Mononuclear Cells (PBMCs), monocyte-derived Dendritic Cells (moDCs), and hepatic tissues as long as 8 years after the resolution of Hepatitis C [90-92]. Such persistent replication of residual HCV after spontaneous or therapy–induced recovery from Hepatitis C is referred to as occult HCV persistence.

As mentioned above, HCV reactivation and persistence have been reported in patients coinfected with HIV, who had previously cleared HCV infection when they were HIV-negative [82]. The correlation of occult HCV infection with disease progression has not been documented. The significance of occult HCV is to be further determined.

Therapy of HCV Infection

Combination therapy and its efficiency

Until recently, the standard therapy for HCV infection has been a combination of pegylated IFN-α (pegIFN-α) and ribavirin for 6 to 12 months, depending on the viral genotype [93]. The antiviral effect is monitored by measuring HCV RNA in patient serum before and during treatment. At least a 2-log10 decrease from baseline in HCV RNA is required to be seen by 12 weeks, if therapy is to continue for the full course of 48 weeks [93,94].

Only patients with detectable HCV RNA should be considered for the combination therapy of pegIFN-α and ribavirin. HCV genotype must be identified before treatment since this dictates the duration of treatment, the dosage of ribavirin and the virological monitoring procedure. For HCV genotype 1, patients require 48 weeks of treatment, with HCV RNA quantification performed at weeks 4, 12, 24 and 48 to monitor treatment result. For genotypes 2 and 3, therapy result is monitored at 4 and 12 weeks and a normal course of therapy requires 24 weeks of treatment. Genotype 4 requires therapy for 48 weeks, as for patients with genotype 1 [93].

Sustained Virological Response (SVR) is defined as the absence of detectable HCV RNA in the serum 6 months after the end of therapy. Most sustained virological responders remain negative in HCV-RNA assays for the rest of their lives. HCV genotype is an important predictor of the outcome of antiviral therapy. For genotype 1, 48 weeks therapy produces a SVR in 40-50% of patients. For genotype 2, the SVR percentage increases to near 90% with 6 months of standard therapy. Patients with genotype 3 have a SVR percentage of approximately 70% with 4 months of therapy [93].

A human genetic polymorphism near the IL-28B gene on chromosome 19 is highly predictive of HCV clearance with standard therapy in adults infected by genotype 1 virus [74]. This study [74] focused on SVR in chronic HCV patients receiving a 48-week course of pegIFN-α -2b or pegIFN-α-2a combined with ribavirin therapy. Because the polymorphism which associates with better treatment response occurs with substantially greater frequency in European than in African populations, this genetic polymorphism could also explain the different response levels between African-Americans and patients of European ancestry [75]. The frequencies of human genotypes TT, GT and GG for rs8099917 were 0.42, 0.51, and 0.07 among patients with treatment failure versus 0.68, 0.29, and 0.03 among those with SVR, respectively. Minor G allele carriers had a higher risk of treatment failure than patients with the TT allele ($P = 3.11\times10^{-8}$) and thus the G allele was identified as the risk allele. Besides rs8099917, other alleles in the IL-28B locus have been reported to be associated with the outcome of HCV infection, including rs8105790, rs11881122, rs7248668 [73] and rs12979860 [77].

Evaluation of therapy adherence for predicting SVR

Combination therapy of pegIFN and ribavirin is recommended as first-line treatment approach for chronic Hepatitis C patients. However, IFN therapy is often associated with adverse effects including psychological disturbances, poor appetite, skin rash, anemia and leukopenia [95]. These adverse effects are more common in patients receiving combination therapy than in those receiving IFN monotherapy [96].

The severe adverse effects of HCV therapy frequently lead to dose reduction and/or treatment discontinuation. Treatment discontinuation owing to severe adverse effects was reported in 6-13% of HCV-infected patients who receive combination therapy [97]. Furthermore, dose reduction of pegIFN and/or ribavirin owing to adverse effects was reported in 25-40% of patients who receive such therapy [96,97].

Good adherence to combination treatment enhances SVR in chronic Hepatitis C patients [98,99]. Discontinuing ribavirin as part of Hepatitis C therapy reduced SVR [98], and reducing the ribavirin dose within the first 12-20 weeks of treatment in patients with genotype 1 led to a significant decline in SVR [99]. These studies indicate that adherence to full dose combination therapy is crucial for obtaining a SVR.

Specifically Targeted Anti-Viral Therapy for HCV (STAT-C)

New HCV therapies have been expected to increase the efficiency of antiviral activity. HCV is a highly mutable RNA virus and can easily develop mutations to resist therapies. Enzyme inhibitors targeting viral polymerase, protease and helicase are emerging as new therapies. Many of the STAT-C antiviral therapies are in preclinical and clinical development.

The protease inhibitor BILN 2061 targets HCV NS3. It was the first protease inhibitor shown to have a high efficacy against HCV both *in vitro* and *in vivo* [100]. However, due to cardiac toxicity, its clinical development was stopped. Another new HCV protease inhibitor, VX-950 (telaprevir), was developed into the first STAT-C therapy to be licensed in 2011. However, when it was used as a monotherapy, some patients quickly developed viral breakthrough, which was related to the selection of viral variants with decreased sensitivity to the drug [101]. Boceprevir was the second protease inhibitor of NS3 to be licensed. It reduces the viral load when administered as a monotherapy, and further reduces the viral load when combined with IFN-α and ribavirin [102]. Telaprevir along with bocepravir have been licensed as protease inhibitors to be used with pegIFN-α and ribavirin. The combination therapy can prevent the emergence of mutant HCV strains [103]. The addition of either of these protease inhibitors to pegIFN and ribavirin increases the SVR to near 75% from 45-50% for genotype 1 HCV infected patients. Both of these protease inhibitors are specific to genotype 1 [104,105].

Serious adverse effects of the standard HCV therapies have restricted their use. The new antiviral therapies, telaprevir and bocepravir, have been shown to be highly specific with tolerable adverse effects. While these new STAT-C antivirals are being used with pegIFN and ribavirin, the ultimate goal would be to develop combinations of highly efficient and tolerable antivirals [104,105].

HCV Quasispecies and Host Selective Pressure

In persistently infected hosts, the RNA virus genome is described as a dynamic population of heterogeneous closely related variants. The fundamental cause of the generation of HCV quasispecies is replication enzymes. RNA viruses, including HCV, influenza virus and HIV, replicate by viral polymerases. Their enzymes, RdRp, are error-prone and lack 3' exonuclease proof-reading activity [6].

Host selective pressure however plays a dominant role in driving HCV mutations. Without selective pressure from host immune responses, HCV does not undergo high rates of mutation [106]. The selective pressures on mutants include specific anti-HCV antibodies, HCV-specific helper T lymphocytes (Th) and HCV-specific Cytotoxic T Lymphocytes (Tc, or CTL). These specific immune responses neutralize HCV infection of target cells, mediate the killing of HCV-infected cells and prevent virus dissemination. However, since HCV is highly mutable, the HCV genome can persist as quasispecies and evade these host immune responses. If the host immune responses are strong and sustained in the first few months of acute HCV infection, HCV infection will be cleared. Otherwise, the inadequate host immune responses provide an environment that drives HCV evolution, and thus HCV infection will persist [106].

Hypervariable Region 1 (HVR1) of the HCV E2 protein is an example of a highly mutable site subject to immune pressure. The genes which encode the envelope glycoproteins E1 and E2 are the most heterogeneous genes of HCV, especially the 81 nucleotides encoding HVR1. The HVR1 region is a 27-amino-acid sequence located in the N-terminal portion of the HCV envelope protein from positions 384 to 410. HVR1 is under selective pressure since it is important for virus infection and a target for host immune responses. HVR1 can be used to identify individual HCV strains and study HCV quasispecies. It contains linear B-cell epitopes and is believed to be the major immunogenic domain of E2. HVR1 mutation occurs during the natural course of HCV infection. Amino acid substitutions of HVR1, which occur rapidly during the acute phase of infection, help HCV escape from the host immune response [7].

HCV Quasispecies and Viral Fitness Cost

The balance between immune evasion and virus fitness cost determines the outcome of HCV infection [107,108].

HCV actively evade the host response by using mulitiple mechanisms, one of which is the highly genetic variability that leads to accumulated mutations within CTL epitopes to impair recognition by CTL. Although HCV is highly mutable, variability is not unlimited.

For example, the NS3 protease of HCV does not mutate extensively. This might be an indication that the NS3 gene is indispensable for HCV fitness, and its mutation would be lethal for HCV. A recent study has examined whether the variability of the NS3 protease could be limited by viral fitness [107]. In this study, the conserved nature of NS3 was confirmed by sequence analysis of HCV RNA from blood samples of HCV-infected patients. Artificial mutations were introduced at five positions in the epitope of the NS3 protease by site-directed mutagenesis. Introduction of the five mutations prevented CTL recognition, implying that the mutations facilitate immune evasion of HCV. However, three of the five mutations reduced NS3 protease activity and RNA replication, suggesting there was a significant viral fitness cost for the immune evasion of HCV. The reduction of viral fitness might explain why some of these mutations are not likely to appear or be sustained in nature.

In another study, resistant HCV replicon variants that contained mutations in the NS5B polymerase gene were obtained by using polymerase inhibitors [108]. It was found that certain mutations in the HCV replicons showed reduced replication capacity, which again indicates a balance between viral mutation to evade immune response and a viral fitness cost. This study also supports the concept that the balance between immune evasion and virus fitness cost may determine the outcome of immune escape mutations. Furthermore, although a single mutation may impair HCV fitness, virus escape mutations may bring improved viral fitness [107,108].

All in all, the balance between immune evasion and virus fitness indeed determines the outcome of immune escape mutations. If immune evasion does not seriously reduce HCV fitness, the mutants are likely to exist. However, with immune evasion at the cost of HCV fitness, the mutations are not likely to occur naturally, or will not persist, which may finally lead to HCV immune clearance [107-111].

Experimental Animal Models for HCV

Currently, the two most important animal models available for HCV research are chimpanzees and mice with albumin-urokinase plasminogen activator/severe combined immunodeficiency disease (Alb-uPA/SCID) transplanted with human hepatocytes.

Chimpanzees are the only natural host of HCV besides humans. Importantly, the clinical courses of HCV infection observed in chimpanzees and humans are similar, which is a major prerequisite for the use of chimpanzees as an HCV infection model. For example, a study performed in chimpanzees has contributed to the concept that specific cellular immune responses are essential for the resolution of HCV infection [112]. HCV-infected chimpanzees demonstrate genomic response to IFN-α therapy [113]. Further studies in chimpanzees have shown that HCV could evade host immune responses by altering the population of regulatory T cells (Treg) [114]. Although chimpanzees will continue to be an indispensable model for HCV research, their utilization has been severely restricted due to their limited availability, cost and related maintenance and ethical concerns [115,116].

The Alb-uPA/SCID mice can be used as an alternative animal model to chimpanzees for HCV infection. These mice are special in that they support engraftment and proliferation of transplanted human hepatocytes. The human hepatocyte population in mouse liver rapidly expands and replaces much of the diseased mouse parenchyma. The mice with chimeric human livers can then be inoculated with human serum or cell culture supernatant containing infectious HCV particles, and they support durable HCV replication at levels comparable to those seen in HCV-infected humans [117,118]. Recently, a second mouse model has been reported. They are immunodeficient mice lacking Fumaryl acetoacetate hydrolase (Fah), Recombination activating gene 2 (Rag2) and the gamma chain of the receptor for IL-2 (IL-2rgamma). This mouse model supports the transplantation of human hepatocytes at any age of the mouse, which might improve the robustness of engraftment and mortality [21,119].

The mouse models are easier to handle than chimpanzees and they are becoming more readily produced. They allow to study HCV infection of human hepatocytes in vivo, and support the assessment of antivirals targeting the viral and host factors present in transplanted human hepatocytes. For example, gene expression profiles from HCV-infected chimeric mice have shown the role of innate immune

responses during HCV infection [120]. However, the mouse models have disadvantages including significant mortality due to bleeding issues associated with high expression of urokinase in liver cells, and the variable robustness of infection. Being deficient in adaptive immune responses, the use of mouse models does not allow studying the role of the immune system in the pathogenesis of liver disease, nor the potential efficacy of immune-based therapies or vaccines.

In summary, the chimpanzee model has improved our understanding of HCV pathogenesis, the interaction of HCV with host immune system, and the potential to develop new vaccines. The mouse model transplanted with human hepatocytes has been useful for the testing of new antiviral agents *in vivo* [21] and the study of innate immune responses [120].

HCV in Cell Culture

HCV replicons

Because no cell culture model of HCV infection was available, it has been difficult for many years to study the HCV life cycle and host-virus interactions that determine the outcome of infection. Starting from 1999, HCV replicons have been used to study HCV in cell cultures [21,121].

An HCV replicon is an RNA construct that contains viral elements necessary for virus replication and a selection marker. It can replicate efficiently in certain cultured cells in vitro. The selection marker used is the neomycin resistance gene, which facilitates the identification of cell clones in which replicon replication occurs. The electroporation of Huh7 hepatoma cells with HCV replicon and then cell culture with neomycin allow for such a selection of clones in which HCV replicon replication occurs [21,121].

HCV replicons contain NS3-NS5 genes or the full-length coding region that is enough to drive HCV replication. Therefore, they have also been used to develop drugs targeting HCV protease, helicase or polymerase in cell culture. HCV replicons do not replicate efficiently without adaptive mutations. Furthermore, they release infectious HCV particles into culture medium [21,121].

Studies based on HCV replicons have revealed some of the host-virus interactions that regulate the processes of HCV RNA replication and protein translation, and have been used to discover and develop drugs for the treatment of HCV infection.

Retroviral or lentiviral HCV pseudoparticles (HCVpp)

HCVpp consist of HCV envelope glycoproteins assembled onto core particles of a retrovirus or lentivirus. The presence of green fluorescence protein or luciferase marker genes packaged within these HCVpp facilitates the reliable and fast identification of virus entry as well as antibody-mediated neutralization of infection [21,122,123].

The HCVpp system is widely used because it is highly robust and amenable to high-throughput assays for quantification in virus neutralization studies. However, differences in the export pathway of HCVpp and native HCV could affect the properties of the viral particles [21].

JFH-1 strain of HCV

In 2005, the first cell culture system for HCV was reported by three laboratories [32,124,125]. The newly isolated virus was obtained from a Japanese patient with fulminant Hepatitis C (JFH-1). Infected Huh7-derived cells produced infectious HCV particles. JFH-1 can infect human hepatoma-derived Huh7 cells, human non-hepatic cells (Hela and 293 cells), chimpanzees and the chimeric mouse model (Alb-uPA SCID mouse) [32,124,125]. This JFH-1 culture system has facilitated both *in vitro* and *in vivo* studies of HCV.

The JFH-1 strain of HCV contains the full-length HCV genome, can replicate efficiently in Huh7 cells, releases infectious HCV particles into culture medium and can be passed effectively in cell cultures [124]. It provides a tool for studying detailed aspects of the HCV life cycle, including viral entry, trafficking, viral assembly and egress, which had not been previously approachable using HCV replicons or HCVpp [21].

The JFH-1 strain is HCV genotype 2a. However, it can be used for the creation of chimeric HCV strains of different genotypes. The chimeric viruses can be produced using the structural proteins of other isolates, but robust HCV infection requires the presence of the NS proteins from the JFH-1 isolate [126]. This limitation precludes the analysis of full-length patient-derived strains. Another limitation is the dependency of transformed hepatoma cell lines to grow the virus. The transformed hepatoma cell lines, however, do not reflect the cell physiology of primary human hepatocytes, the natural target of HCV infection [21]. Moreover, JFH-1 does not infect immune cells, a characteristic different from HCV in patient serum [127]. Nonetheless, the JFH-1 strain has laid a foundation to examine new aspects of the HCV life cycle and to develop new drugs both *in vitro* and *in vivo* for combating HCV.

Hepatitis C Vaccine

HCV is able to establish persistent infections in up to 85% of infected patients with severe clinical consequences. Although it is a heavy burden on public health, there is currently no vaccine available for Hepatitis C. Since vaccination is one of the most cost-effective approaches for the management of infectious diseases, development of an effective HCV vaccine is an important objective to prevent HCV infection and reduce HCV-related mortality and morbidity. Evaluating different vaccination strategies that can induce HCV-specific immunity is critical for the development of an effective vaccine [61].

Induction of HCV-specific antibodies as well as multi-specific, functional CD4 and CD8 T cells in humans is the immunological hallmark of a successful HCV vaccine [128-131]. However, HCV quasispecies and multiple genotypes continue to challenge the development of an efficient vaccine [109]. Recent research has shown that a single strain of HCV could elicit broad cross-neutralizing antibodies against all known major genotypes of HCV [132]. This provides considerable encouragement for HCV vaccine developement.

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Hepatitis C Symptoms

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Abstract

Patients infected with the hepatitis C virus (HCV) can present with all the symptoms of acute phase; however, up to half of these patients will progress onto chronic hepatitis [1]. These chronically infected patients are at high risk for developing liver cirrhosis and potentially hepatocellular carcinoma (HCC). Currently, there is limited data indicating that antiviral therapies can reverse or improve these diseases. Due to these therapies, it has been difficult to determine the long-term course of the disease in the absence of treatment [2]. Approximately a fourth of all chronic hepatitis C patients can be expected to develop cirrhosis. Of these, a fifth will decompensate to end-stage liver disease (ESLD) and an additional 3-4% will develop hepatocellular carcinoma (HCC) [3]. This chapter provides definitions for both acute and chronic infection as well as the symptoms most commonly associated with this disease.

Acute Infection

Acute hepatitis C virus (HCV) infection is best defined as the presence of either clinical signs or symptoms of hepatitis below 6-month duration; whereas chronic HCV is beyond a 6-month duration. Patients can acquire HCV by blood or semen with blood contact causing the majority of transmissions. The main risk groups are intravenous drug users (IVDA's), recipients of blood transfusions before 1992, health care workers and HIV infected MSM [4-5]. Detectable levels of HCV RNA can be seen after 2 weeks following exposure, with rapid increases followed by increases of serum alanine aminotransferases (ALT) 3-4 months after exposure. This rise classically signifies the initial stages of hepatic injury [2,6]. Deterding et al. showed in their study that the mean alanine aminotransferase (ALT) for those with acute infection was 1174 unit/L and 51 percent of their 254 person study had a bilirubin level above 3 mg/dL [7]. Their study concluded that patients infected with acute hepatitis C can develop nonspecific symptoms, but many remain asymptomatic and are unaware that they have acquired the disease.

In general, 75% of all patients infected with acute HCV are asymptomatic with the remaining fourth developing symptomatic acute hepatitis [8]. These symptoms classically present as jaundice, fatigue, myalgia, abdominal pain, anorexia, nausea, vomiting, low-grade fever, malaise, dark urine and clay colored stools. If symptoms do occur, they typically present 2-3 months after HCV exposure and are mild, nonspecific or intermittent. Some studies have shown that more than half of the patients, who become symptomatic, present with jaundice [9,10]. These same studies have also shown that with appropriate therapy, a decrease trend in AST serum levels signifies improvement in disease progression. This improvement typically occurs within several weeks [11].

A third of all patients exposed to hepatitis C tend to have complete resolution of their symptoms [8]. Some of the reasons for clearance of this viral infection may include female gender, younger age and those presenting with overt clinical symptoms during their infectivity period [8]. Other studies have also shown that females are able to clear the virus more than men (40% vs. 19%); however an exact explanation was not fully developed [12]. Another study that analyzed the same data hypothesized that elevated estrogen levels could be the reason [13]. Data has shown that patients younger than 40 years of age tend to clear the virus sooner than those older than 40 years of age [14]. The presence of clinical symptoms, particularly jaundice, during the acute illness has been shown to coincide with higher rates of self-limited disease. Patients who present with symptoms are referred to as having stronger immune responses than those who are asymptomatic; therefore, having the ability to clear the virus sooner [12]. Extensive data related to co-infection with HIV, an immunocompromising disease, has been shown to decrease clearance in those patients with acute HCV [12-14]. Due to the fact that the majority of those with acute hepatitis C infections are asymptomatic, it is important to screen for the disease in populations most at risk. Different pharmacotherapies have been established to treat those infected with acute hepatitis C. Data has shown great success with pharmacotherapy, which is why it is necessary to identify these patients during the early phase of the disease course [8]. Figure 1 clearly exemplifies that natural history of disease progression from acute HCV infection to death without pharmacotherapy.

Chronic Infection

As defined in section 2.3, chronic hepatitis C is defined by the persistence of HCV RNA beyond 6-month duration after viral transmission [15]. Once chronic infectivity period has begun, it is uncommon for these patients to have spontaneous resolution of their disease. A fifth of patients with chronic hepatitis C ultimately progress to liver cirrhosis and hepatocellular carcinoma (HCC) as liver destruction progresses.

Like acute HCV, a majority of patients with chronic HCV typically present without symptoms. The disease course varies among patients, and they complain of a variety of symptoms such as right abdominal discomfort, nausea, fatigue, myalgia, arthralgia, and/or weight loss [2]. One study has shown that 70% of patients with chronic hepatitis C most commonly present with musculoskeletal pain; whereas half of these patients also complain of feeling fatigued [16]. These symptoms tend to be very variable; therefore, they do not reflect disease activity and cannot reflect the severity of liver injury.

As mentioned in section 2.3, age is an important risk factor in determining which patients are at high risk for developing advanced liver disease in those with chronic hepatitis C [17]. Patients older than 40 years of age are at higher risk for disease progression with fibrotic changes noted on liver biopsies [17]. As the patient ages, the annual fibrosis progression rate accelerates exponentially. Data

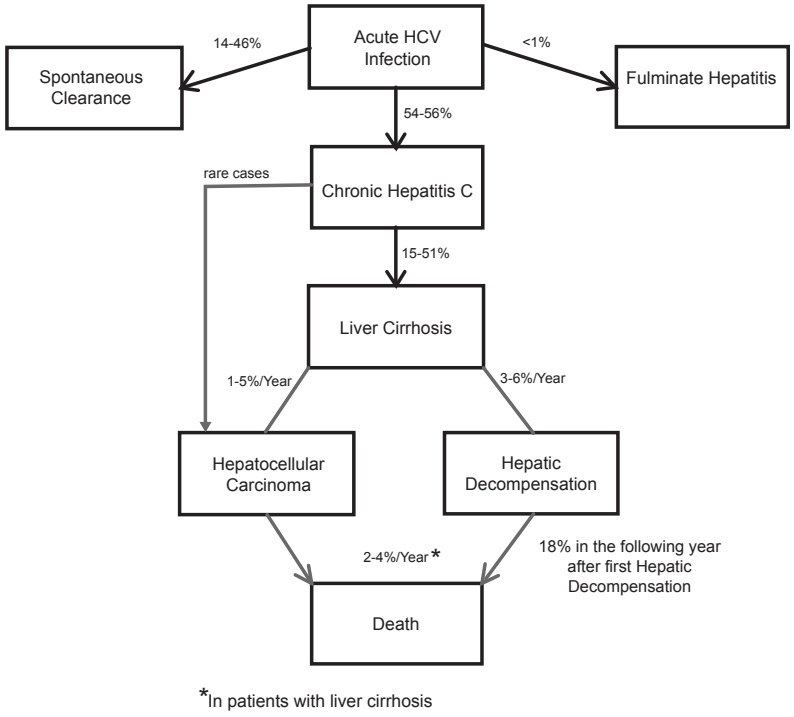

Figure 1: Natural history of the Hepatitis C infection.

has shown that liver regenerating capability, immune system changes and telomere-shortening are responsible for the increased rate of fibrosis [17]. Figure 2 exemplifies the risk factors for progression to cirrhosis.

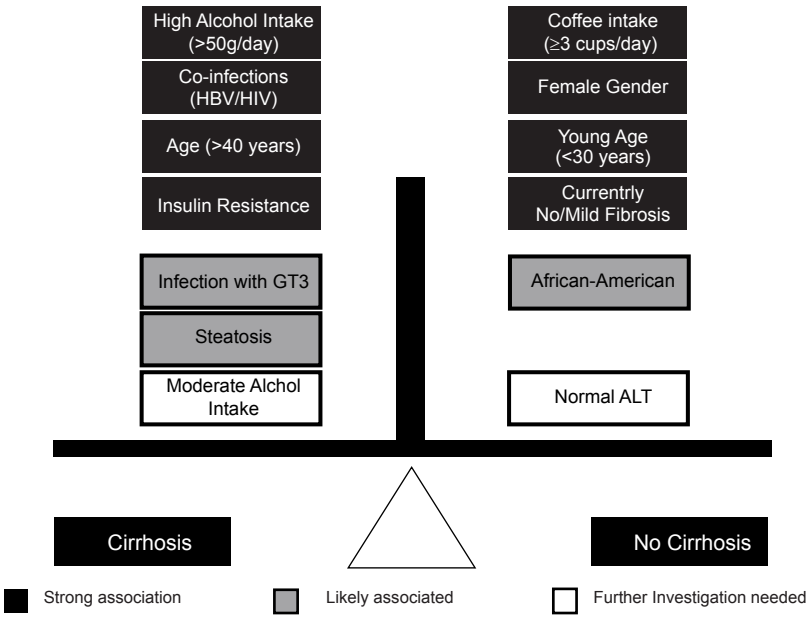

Figure 2: Risk factors for development of cirrhosis in chronic hepatitis C.

Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC), a result of end-stage liver disease from progressive hepatitis C infection, is a primary tumor of the liver. A third of all causes of hepatocellular carcinoma cases in the United States are due to hepatitis C progression [15]. Lok et al. found a linear relationship between the number of years after diagnosis of chronic hepatitis C and the risk of developing HCC [18]. He clearly demonstrated in his work that after a3-year history of chronic hepatitis C infection, approximately 2% will have HCC. After a 5-year history, approximately 6-7% will show evidence of HCC, and after one year, the incidence is approximately 10%. Figure 3 demonstrates similar results by other authors showing the yearly percent risk of HCC after diagnosis of HCC following chronic HCC [19]. Risk factors for the development of HCC after chronic HCV infection are similar to those associated with the development of liver cirrhosis. As with acute hepatitis C, the risk factors for infectivity may include older age, male gender, co-infection with HBV or HIV, and alcohol consumption. Patients with type-2 diabetes mellitus have also been shown to be at higher risk for developing HCC, regardless of sex, age or follow-up period [20].

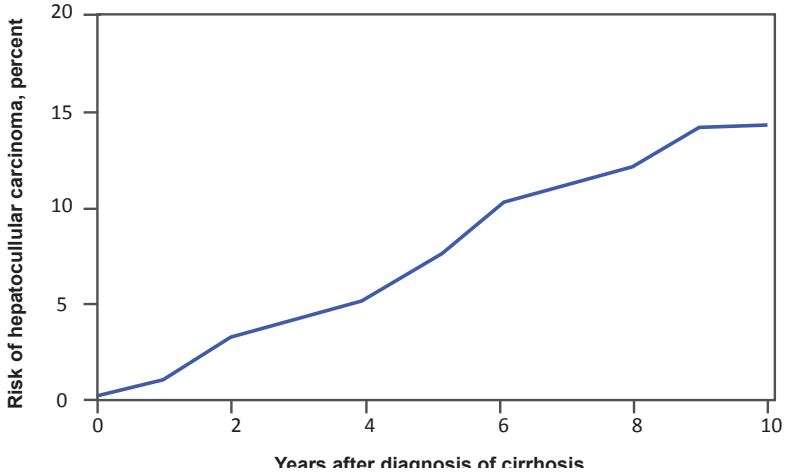

Figure 3: Risk of HCC after diagnosis of cirrhosis.

Conclusion

Acute hepatitis C is a viral infection of the liver that lasts up to 6 months after exposure, whereas, chronic hepatitis C progresses beyond 6 months. As clearly illustrated in this chapter, the symptoms of both acute and chronic hepatitis are similar. Males are at higher risk of developing the disease, as well as progressing onto end-stage liver disease and hepatocellular carcinoma. Females tend to clear the virus more readily than males do, likely secondary to a hormonal attribution. Patients older than 40 years of age are more susceptible for progressing from an acute infectivity state to a chronic state, likely due to weaker immune systems. Patients co-infected with HIV are also at higher risk for progressing to chronic hepatitis and end-stage liver disease. Physicians should be aware that a majority of patients with both acute and chronic hepatitis C are asymptomatic; however when symptoms do arise, the tests available to diagnose and screen these patients should be addressed. Unfortunately, no vaccinations are available against hepatitis C; however, as medicine progresses, it won't be a surprise that one day there will be a vaccine. Early initiations of pharmacotherapy in patients who are acutely infected and are compliant tend to do much better than those who delay therapy.

Acknowledgment

The authors thank Dr. Bassam Husam Rimawi for assistance in preparing this chapter.

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Hepatitis C Virus: Molecular Pathways and Treatments

Chapter: Transmission of Hepatitis C Virus

Edited by: Oumaima Stambouli

Published by **OMICS Group eBooks**

731 Gull Ave, Foster City. CA 94404, USA

Cover OMICS Group Design team

First published February, 2014

A free online edition of this book is available at www.esciencecentral.org/ebooks

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Transmission of Hepatitis C Virus

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Introduction

Background

Hepatitis C has been considered to be the most commonly emerging viral Hepatitis worldwide with major universal devastating consequences. Every year, a round four million people are infected with the Hepatitis C Virus and over 150 million people are chronically infected and at risk of developing liver cirrhosis and/or liver cancer. More than 350 000 people die from Hepatitis C-related liver diseases every year. Hepatitis C is an infectious disease affecting primarily the liver caused by the Hepatitis C Virus (HCV). The existence of Hepatitis C (originally "non-A non-B Hepatitis") was postulated in the 1970s and proven in 1989.Hepatitis C infects only humans and chimpanzees [1].

Transmission of HCV has been changed immensely since its discovery, Blood and blood products was the primary mood of transmission particularly prior to 1992, though nowadays has been replaced by IUDs. Further it has been changed according the clinical status of infected individuals as the viral particles were confined to the serum and lymphocytes in acute and chronic cases of HCV, though in occult cases, the virus is not detectable with conventional testing but can be found with ultra-sensitive tests. Methods of transmission has been reflected considerably on the geo-epidemiology of HCV worldwide, in the developing countries where the prevalence of HCV is IUD is accounted for up to 90% of reported cases though in developing countries as they have the highest prevalence rates, blood, nosocomial and occupational transmission methods still taking the lead [2,3].

This chapter aims to high light the method of transmission of HCV and outlining the preventive measure to be taken to minimize the spread of Hepatitis C Virus.

Nature of Hepatitis C Virus particle

Hepatitis C Virus belongs to the genus *Hepacivirus* a member of the family *Flaviviridae*. Until recently it was considered to be the only member of this genus. However a member of this genus has been discovered in dogs-canine *Hepacivirus*. There is also at least one virus in this genus that infects horses. Several additional viruses in the genus have been described in bats and rodents.

As illustrated in (Figure 1), Hepatitis C Virus particle consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of protein, and further encased in a lipid (fatty) envelope of cellular origin. Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope.

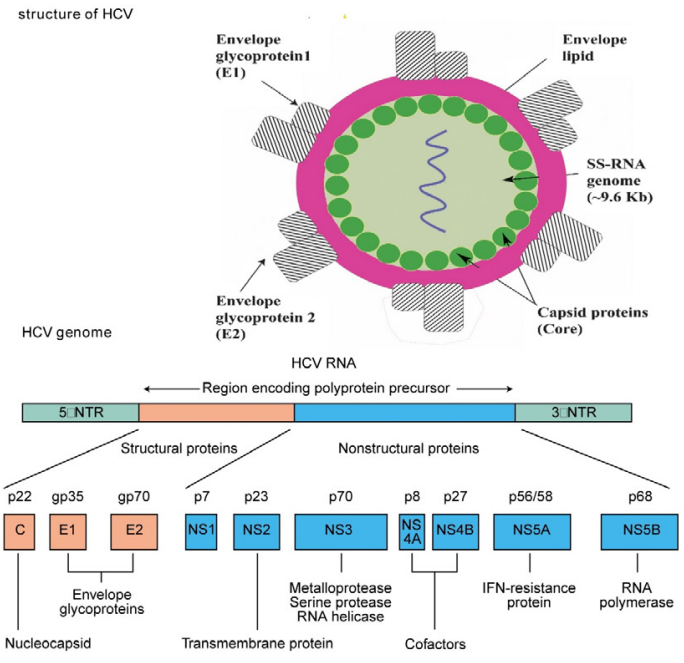

Figure 1: Diagrammatic illustration of Hepatitis C Viral particles.

Hepatitis C Virus has a positive sense single-stranded RNA genome. The genome consists of a single open reading frame that is 9600 nucleotide bases long. This single open reading frame is translated to produce a single protein product, which is then further processed to produce smaller active proteins. At the 5' and 3' ends of the RNA are the UTR that are not translated into proteins but are important to translation and replication of the viral RNA. The 5' UTR has a ribosome binding site (IRES - Internal Ribosome Entry Site) that starts the translation of a very long protein containing about 3,000 amino acids. The core domain of the Hepatitis C Virus (HCV) IRES contains a four-way helical junction that is integrated within a predicted pseudo knot. The conformation of this core domain constrains the open reading frame's orientation for positioning on the 40S ribosomal subunit. The large pre-protein is later cut by cellular and viral proteases into the 10 smaller proteins that allow viral replication within the host cell, or assemble into the mature viral particles. Structural proteins made by the Hepatitis C Virus include Core protein, E1 and E2; nonstructural proteins includeNS2, NS3,NS4, NS4A, NS4B, NS5, NS5A, and NS5B.

Environment and survival conditions of Hepatitis C Virus

Environmental and survival conditions vary greatly from on pathogen to another and it was found to play an important role in Transmission of viruses. Compared to other viruses, HCV is a relatively hardy pathogen. The half-life of the virus particles in the serum is around 3 hours and may be as short as 45 minutes. In an infected person about 10^{12} virus particles are produced each day. In addition to replicating in the liver the virus can multiply in lymphocytes. Like many viruses, the Hepatitis C Virus is gradually inactivated outside the body of a host. The presence of heat can have a drastic impact on the virus's lifespan outside the body. The virus can remain infectious outside a host for about sixteen days at 25°C and two days at 37°C, while it can remain active for more than six weeks at temperatures less than or equal to 4°C. When heated to temperatures of 60°C and 65°C, however, the Hepatitis C Virus can be inactivated in eight and four minutes, respectively [4,5].

Several variables have a direct impact on how long HCV can survive outside the body. Testing blood on exposed surfaces while considering the surface's texture, room temperature, amount of blood exposed, viral load (low/high) and various contaminants in the environment makes the determination of how long Hepatitis C survives outside the body very complex. Infectivity studies in a chimpanzee suggest that HCV may survive on environmental surfaces at room temperature at least 16 hours but not longer than 4 days. The potential for HCV to survive in the environment re-emphasizes the importance of cleaning and disinfection procedures, safe therapeutic injection practices, and harm reduction counseling and services for injection drug users [6-8].

HCV Known to survive outside the body for days in dried blood on surfaces, Hepatitis C can persist for months in a liquid medium under favorable conditions. According to the U.S. Centers for Disease Control and Prevention, HCV can survive on environmental surfaces at room temperature for at least 16 hours but no longer than four days. In contrast, the HIV virus can only live on surfaces for several hours. Recent advances in on the survival of HCV have shown;

- HCV survives longer in liquids than it does when dried on surfaces. They found that in a liquid environment, HCV was detectable for up to five months at lower temperatures.

- HCV could survive in a liquid medium for two days at 37°C (body temperature), 16 days at 25°C and at least six weeks at 4.4°C (average refrigerator temperature).

- HCV remained viable in a syringe for up to 63 days. Circumstances that increased HCV infectivity include syringes with detachable needles, lower temperature and larger volume syringes.

HCV's ability to live for a prolonged period of time outside the body under the right conditions has extraordinary implications for its transmission. Some of the carriers known to transmit the virus include straws used for nasal drug use, needles used for administering drugs, tattooing, sharing personal care equipment like razors or toothbrushes, certain sexual devices and reuse of medical equipment in healthcare settings. Although we know that it spreads between blood sources, inanimate objects often act as the intermediary to transmit infection. Thus, understanding how long the Hepatitis C Virus can survive outside the body - in all situations - can help guide us toward failsafe practices for reducing the risk of HCV transmission [9].

Concepts of Transmission of Hepatitis C Virus

Laboratory diagnosis of Hepatitis C Virus

Diagnosis of Hepatitis C Virus involves detection and confirmation of the diagnosis of Hepatitis C Virus (HCV) infection and assessment of the severity of liver disease. In addition, evaluation of patients with Hepatitis C should include determination of the patients' suitability for treatment.

Diagnostic tests for Hepatitis C as shown in (Table 1) can be divided into the following two general categories:

1-Serological assays that detect antibody to Hepatitis C Virus (anti-HCV)

2-Molecular assays that detect, quantify, and/or characterize HCV RNA genotypes and subtypes within an infected patient.

Serological assays have been subdivided into screening tests for anti-HCV, such as the Enzyme Immunoassay (EIA), and supplemental tests such as the Recombinant Immuno Blot Assay (RIBA). Three generations of anti-HCV tests have been developed, and each generation has resulted in an improvement in the sensitivity of detecting anti-HCV Third-generation anti-HCV tests (EIA-3 and RIBA-3, respectively) contain antigens from the HCV core, non- structural 3, nonstructural 4, and nonstructural 5 genes. Detection of HCV RNA in patient specimens by polymerase chain reaction (PCR) provides evidence of active HCV infection and is potentially useful for confirming the diagnosis and monitoring the antiviral response to therapy.

Currently, the second-generation enzyme immunoassay (EIA-2) for antibodies to HCV (anti-HCV) is the most commonly screening test for HCV infection. The diagnosis of HCV infection can be supported or confirmed by the Recombinant Immunoblot Assay (RIBA) or tests for HCV RNA. Nowadays, the second-generation enzyme immunoassay (EIA-2) for antibodies to HCV (anti-HCV) is the most favorite practical screening test for HCV infection. The diagnosis of HCV by EIA can be supported or confirmed by the Recombinant Immunoblot Assay (RIBA) or tests for HCV RNA. RIBA detects antibodies to individual HCV antigens and confers increased specificity compared to EIA-2. Qualitative Reverse Transcription- Polymerase Chain Reaction (RT-PCR) assays for HCV RNA are simpler than quantitative tests and sufficient for confirmation of the diagnosis of HCV infection.

Type of the Test	
1.Anti-HCV (antibody)	
EIA (Enzyme Immunoassay)	**Uses:**
Recombinant Immunoblot Assay (e.g. RIBA™)	Verify if necessary positive EIA with HCV RNA detection Indicates past or present infection, but does not differentiate between acute, chronic or past infection.
2.HCV RNA (virus)	
Qualitative tests	**Quantitative tests**
PCR – Amplicor HCV™	PCR - Amplicor HCV Monitor™
Transcription-Mediated Amplification (TMA) - Versant HCV	Branched DNA signal amplification – a) Quantiplex™ HCV RNA (bDNA) b) Versant HCV RNA Quantitative Assay
	Other tests - Super Quant, LCx, real-time PCR…
TO; Detect presence or absence of virus. Detects virus 1-3 weeks after exposure. Detection of HCV RNA during course of infection may be intermittent. A single negative PCR is not conclusive.	**TO;** Determines **titre* of HCV. Used to monitor patients on antiviral therapy.
3.HCV core antigen EIA	
Trak-C	
TO; Detects presence or absence of virus. Detects virus 1-3 weeks after exposure. Under evaluation for the monitoring of patients on antiviral therapy	
4.Genotype	
TO; Groups isolates of HCV into 6 genotypes based on genetic differences.	
With new therapies, length of treatment varies based on genotype.	

Table 1: Diagnostic tests for Hepatitis C Virus.

While the vast majority of anti-HCV-positive patients who present with chronic liver disease have ongoing HCV infection as confirmed by the presence of HCV RNA in serum, only 35 percent and 25 percent of anti-HCV-positive blood donors are RIBA-and HCV RNA-positive, respectively. The proportion of anti-HCV-positive blood donors who are confirmed to be HCV RNA-positive varies from 70 percent for those who are RIBA-positive to 2-25 percent for those who are RIBA-indeterminate and none for those who are RIBA-negative. Thus, supplementary and confirmatory tests for HCV infection should always be performed in asymptomatic low-risk subjects who are found to be anti-HCV-positive, particularly if they have normal aminotransferase (ALT) levels; but these tests may not be necessary in all anti-HCV-positive patients who present with chronic liver disease [10].

Severity of liver disease is best assessed by liver biopsy. There is in general a poor correlation between serum ALT level and activity of liver disease. More importantly, several recent studies found that significant liver disease can be found in anti-HCV-positive patients despite normal ALT levels. These studies reported that 70 percent of RIBA-positive blood donors who had persistently normal ALT levels have chronic Hepatitis or cirrhosis on biopsy. Although most donors (77 percent) who had abnormal liver histology were HCV RNA-positive, significant liver disease was also found in 30 percent of RIBA- positive donors who were HCV RNA- negative and had normal ALT levels on three separate occasions. This may be related to the fluctuating course of chronic HCV infection with intermittently normal ALT levels and undetectable levels of viremia. It may also reflect variations in sensitivities of "home-made" RT-PCR assays for HCV RNA. Liver biopsy should be always recommended except in elderly patients, patients with severe concomitant medical problems, and those with coagulopathy, since neither serum HCV RNA nor ALT level can reliably predict activity or degree of fibrosis. HCV genotyping could be used to monitor the clinical response of HCV therapy, it could only be recommended as a research tool rather than a routine diagnostic test [11].

Epidemiology and risk factors of Hepatitis C Virus

Hepatitis C Virus is one of the most important viral Hepatitis that appears to be endemic in many parts of the world. There are, however, substantial geographic and temporal variations in the incidence and prevalence of HCV infection, largely due to differences in regional risk factors for the transmission of HCV. The overall world prevalence of HCV was estimated to be 3% as an over 170 million person were infected. Such prevalence varies greatly from one country to another and even among the province within the same country. Most of the studies however were based on testing of selected populations such as blood donors [12]. However, population-based surveys are rarely available for most parts of the world. Low rate area (0.1-0.9%) such as Northern Europe to 0.1–0.5% in Western Europe, North America, parts of Central and South America,, intermediate area (1-5%), have been reported from Brazil, Eastern Europe, the Mediterranean area, the Indian subcontinent, and parts of Africa and Asia., High rate area >5% such Arabian peninsula, Africa and the highest prevalence of HCV has been found in Egypt (17–26%) [13,14].

The major risk factors for HCV infection are blood transfusions from unscreened donors and intravenous drug use as shown in (Figure 2). However, exposure to HCV-infected blood from other health-care-related procedures and regional cultural practices are increasingly recognized as having an important function in HCV transmission in some parts of the world. Since the introduction and improvement in the 1990s of the screening of blood donors, HCV transmission by blood transfusions is now exceedingly rare (around or less than one per million) in developed countries. Unfortunately, the screening of blood donors for HCV is not yet routinely performed by some blood banks in developing countries. Most new cases in developed countries are related to intravenous drug use. Health-care-related procedures leading to nosocomial HCV transmission are not restricted to hemodialysis facilities. Several reports from Western countries have clearly documented nosocomial transmission of HCV through inadvertent sharing of multi-dose vials or unsterilized instruments, among others [15]. Similar nosocomial transmission of HCV outside dialysis units is certainly not less likely to occur in developing countries but has not been reported until now. Additional risk factors for HCV transmission include occupational exposure, especially by accidental needle stick, as well as perinatal transmission (about 6%), whereas the transmission of HCV by sexual activity appears relatively inefficient.

New social groups are highly prone to HCV these include individual incarceration centers and those with Mental disorders. Different studies found that the sero-prevalence of Hepatitis C Virus among mentally ill patients, approximately 11 times that of the general adult population. Unique risk factors were associated with spread of HCV among these groups include, homelessness , alcohol, drug abuse and risky sexual activities hence then special attention should be directed to such important social groups in order to prevent spread of HCV and comply with the consequences associated with it [16].

Patients infected with HCV usually confected with Hepatitis B and HIV viruses. The exact number of patients co-infected with HCV

and HBV is unknown. In patients with chronic Hepatitis B, estimates of the rates of HCV co-infection vary from 9% to 30%. The primary concern with HBV/HCV co-infection is that it can lead to more severe liver disease and an increased risk for progression to liver cancer (HCC) [2,17]. This is also evident in patients who are HIV-positive are commonly co-infected with HCV. These viruses have shared routes of transmission: percutaneous exposure to blood, sexual intercourse, and from a mother to her infant. Infection with HCV can be asymptomatic, self-limiting, or progress to cirrhosis or cancer.

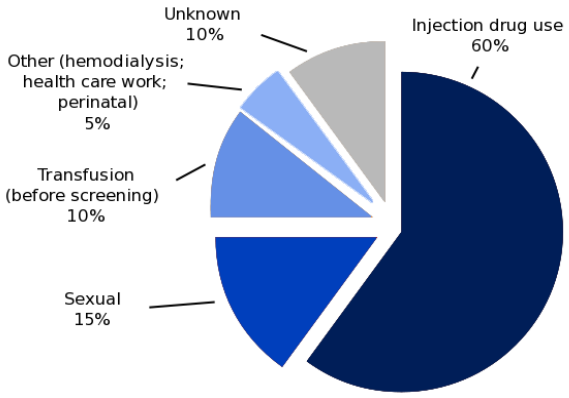

Figure 2: Risk factors associated with Transmission of HCV.

The clinical history of HCV is a unique, usually its mild and rarely patients need a clinical consultation and thus it is usually discovered at the chronic stage. Chronicity rates range from 50 to 90%, with somewhat lower rates in children and young healthy women (50-60%) and higher rates in older individuals and African descents. This however more complicated when the patients co-infected with HIV or HBV.

Transmission of HCV

HCV is generally transmitted by the parenteral route. Well known and common modes of transmission involve blood transfusions and/or other parenteral contact with blood products. In the developed world, the primary route of transmission is Intravenous Drug Use (IDU), while in the developing countries the main methods are blood transfusions and unsafe medical procedures. However ever, up to 50% of individuals deny exposure to any of these known risk factors, and infection is often designated as "community acquired," though strong believe suggest these could be accounted for by IDU.

Blood transfusion-transmission

Before the initiation of HCV antibody screening, approximately 10% to 20% of individuals, who had received multiple blood transfusions or plasma products sero-converted to anti-HCV positive. Thus, the relative risk was 0.45% per unit transfused. However, the introduction of routine HCV antibody screening of blood products has led to a sharp decrease in the transmission rate of HCV. For example, the risk of acquiring antibodies to HCV by blood transfusions using current screening procedures was calculated as 1/100,000 units transfused. This remaining low risk is believed to be principally caused by blood's susceptibility to infection just after acquisition of HCV infection and before the appearance of anti-HCV antibodies. Therefore, the detection of HCV RNA by RT-PCR would potentially allow for the identification of such infectious units, although this technique is not in routine use for screening blood donor population [18].

Retrospective studies of blood donor and recipient repositories from the mid 1960's and early 1970's demonstrated that almost 25% of recipients were infected with HCV several events in the 70's and 80's, such as moving to volunteer blood donation, implementation of screening and exclusion of Hepatitis B surface antigen (HBsAg) positive blood, allowed for a significant reduction in Transfusion-Transmission of HCV (TT HCV). In addition, in the early 1980's the recognition of TT HIV led to more stringent exclusion criteria for blood donations and a reduction in TT HCV.

Nowadays the current risk of TT HCV in developed countries is 1 in a million (0.0001%) per unit transfused. In developing countries such Indian-subcontinent, China, South-East Asia and Africa the situation is still ambitious, and transfusion transmission of HCV is still a leading mode of transmission among these countries as illustrated in (Figure 3). This is particularly due lack of adapting the safety standard regulations among blood banks. These may include; policies and systems that encourage t blood donation by volunteers have not been successfully implemented; lack basic blood banking practices, lack of standardization and adapting modern blood testing and adding newly needed tests such as NAT testing. Therefore, policies are urged to prevent TT HCV among these countries and the experiences of the developed countries have to be mirrored At the time of identification and deferral, blood donors may be notified of their HCV status, including RNA and antibody confirmatory test results that may help HCV infected individuals to receive timely therapy to deter disease progression [20]. In the era of new and potent antiviral agents against HCV, timely intervention can help prevent future public health burden associated with HCV-related liver diseases.

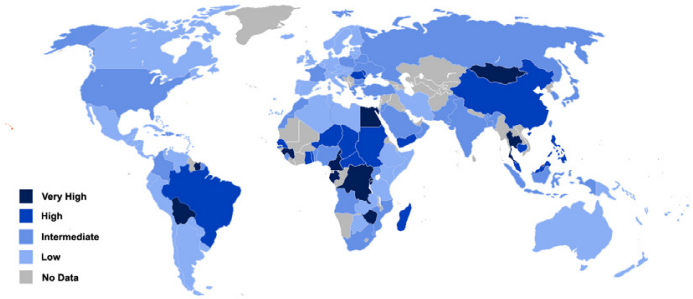

Figure 3: Geo-epidemiology of HCV transfusion-transmission worldwide.

Intravenous Drug Users (IDUs)

Injection drug use is known to play a major role in HCV transmission and there has been an emerging epidemic of Hepatitis C Virus (HCV) infection among Injection Drug Users (IDU) - particularly among developed countries. Injection Drug Users (IDUs) account for a disproportionately large burden of Hepatitis C Infection. Ninety percent of new infections worldwide (90% in Australia, 72% in Canada, and 54% in the United States) are contracted through injection drug use, and the majority of chronic infections, particularly in developed countries, are attributed to injection drug use In Injecting Drug Users (IDUs) population there has been increased shifts among addicts from inhalatory to injectable drugs due decrease in quality and availability of heroin. Further, the effect of injecting drugs is more intense and satisfying as HCV is found in a high concentration in spoons and rinsing liquids that could be used in association with needle drug use [21].

A variety of variables has to be considered when differentiating risk for these infections within injecting drug users. These include the duration of injection drug use as the prevalence of infection increases with longer duration of injection drug use which reflects the effect of cumulative exposure and thus the higher rate of HCV transmission among IUDs. More recent initiates into injection drug use have higher rates of new infection compared with more experienced drug users. This highlights the importance of targeting preventive interventions early for them to be most effective as there is increased risk among newer injectors

Despite advancements in the management of chronic Hepatitis C and suggestions that treatment of recently acquired Hepatitis C can lead to Sustained Virological Response (SVR) rates of up to 98%,there continues to be a low rate of treatment uptake among current IDUs [22]. Studies conducted in IDU populations in developed countries suggest that very few IDUs infected with Hepatitis C Virus have received antiviral therapy. The continuing reluctance to treat IDUs is driven by concerns about the risk of reinfection, high rates of concomitant alcohol abuse, and high rates of concomitant mental health issues, all potentially impacting treatment compliance and effectiveness

A variety of measures which should be taken may include;

- Create community-led education and messaging strategies on Hepatitis C risks, injection transmission risks (e.g., sharing drug preparation equipment in addition to sharing drug injection equipment), and HCV testing resources.

-Improve and increase infrastructure for HCV surveillance and data collection

- Create age-appropriate (e.g., young adult) substance use and Hepatitis C interventions and prevention strategies that are evidence based and effective

- Expand both community-based and basic science research activities to better understand how to effectively address the emerging crisis of Hepatitis C Infection among young IDUs

Contaminated needles

HCV is transmitted by contaminated needles. The rate of transmission probably depends, in part, on the quantity of blood transferred to the recipient by the needle stick, the titer of virus, and the depth of inoculation. Approximately 2% of exposed individuals will develop viremia and/or anti-HCV antibodies after needle stick exposure. The rate of HCV infection after sticks with solid needles appears to be lower as compared with accidents with hollow cannula devices. The risk of acquiring Hepatitis C by needle-stick injury ranging from 3 to 10.3%, comparable to HBV risk= 5 - 40%and HIV risk = 02 - 0.5 %.The prevalence of anti-HCV in health workers does not differ significantly from blood donors. However, viremia can be detected very rapidly (within an hour) in exposed personnel without this leading to persistent infection while sero conversion to anti-HCV can occur very late. Epidemiologic studies of HCV have indicated that transmission among patients in health care settings is associated with contaminated vehicles such as multi-dose medication vials and re-used needles and syringes and among injecting drug users is associated with contaminated drug paraphernalia such as cookers and cotton [23,7].

Sexual transmission

Sexual transmission of HCV infection has been demonstrated but is less frequent compared with Hepatitis B. Studies in the United States and Europe have revealed low rates of exposure (0%-6.3%) in heterosexual partners of individuals with chronic HCV infection. Interestingly, recent reports from Asian countries have found higher positivity rates of 7.3% to 27.5%.It is noteworthy that HCV RNA has not generally been found in semen, vaginal fluid, urine, stools and Saliva. Antibodies to HCV have been found in 1% to 12% of prostitutes, an increase of the HCV antibody positivity rate from 4% to 9% has been described in heterosexual partners if there is co-infection with HIV [24]. It appears however, that the prevalence of HCV antibodies in sexual partners of heterosexual and homosexual relationships is similar.

While Hepatitis C is not classified as an (Sexual Transmitted Infection) STI, there is a risk of Hepatitis C transmission if the blood of one person enters the bloodstream of another person during sexual intercourse. Men who have sex with men and who also have HIV have a higher proportion of Hepatitis C transmission through sexual exposure compared to all people with the Hepatitis C infection.

Nosocomial acquisition of HCV

Hepatitis C Virus has been known to be an important nosocomial pathogen, and several outbreaks have been linked to breaches in standard precautions for blood-borne infections during nursing procedures or interventions such as colonoscopy surgery and dialysis. Hepatitis C Infection is commonly associated with patients with hemodialysis particularly among developing countries health care settings. HCV prevalence in HD varies geographically, both within and between countries. The prevalence of HCV antibodies ranged between 8% to 80%.A number of risk factors have been identified for HCV infection among dialysis patients, including the number of blood transfusions, the duration of ESRD, the mode of dialysis, and the prevalence of HCV infection in the dialysis unit. The risk of HCV infection appears to correlate with the duration and frequency of hemodialysis [25]. A variety of other nosocomial factors may affect the risk of transmission of HCV to patients and staff in dialysis units.

-Transmission of HCV from infected patient to dialysis staff by needle-stick injury

-Breakdown in standard infection control practices

-Physical proximity to an infected patient

-Dialysis machines

-Dialyzer membranes, hemodialysis ultra-filtrate, and peritoneal fluid

-Reprocessing of dialyzers

A variety prophylactic measures have been suggested to avoid infection by HCV in the hemodialysis environment, and range from isolating patients with HCV infection to adopting a series of biosafety measures specific for HD, such as preparing medications in a separate area, cleaning and disinfecting dialysis station surfaces, washing hands and changing gloves between patient contacts, and items dedicated for use only with a single patient. Strict adherence to universal infection control precautions seems to be enough to control the spread of disease in HD units.

Habitual and community-Associated factors

Community has been considered to be an important source for Hepatitis C, and up to 50% of individuals deny exposure to any of these known risk factors where, infection is often designated as community. Persons incarcerated in correctional systems comprise a certain proportion among population and have a disproportionately greater burden of infectious diseases, including infections with Hepatitis Viruses and other infections of public health importance. As most of inmates of prisons and jails were released and returned to the community [1,24].

Within the community incarcerated populations are particularly at a higher risk of HCV infection. Among adult prison inmates, 16%-41% have serologic evidence of HCV infection, and 12%-35% have chronic HCV infection; rates vary by geographic region. Though prevalence of HCV antibody among detained or incarcerated juveniles is estimated at 2%-3.5%, approximately 12% of persons aged 16 years reported at least one arrest in their lifetimes. Upon incarceration, all adults and the majority of juveniles lose access to the usual public and private health-care and disease-prevention services. Their health care becomes the sole responsibility of either the correctional system or less frequently, the public health system. In some countries entry into the correctional system provides an opportunity to access health care.

Vertical transmission of Hepatitis C Virus

Compared with adults, infection in children is much less well understood. Worldwide the prevalence of Hepatitis C Virus infection in pregnant women and children has been estimated to 1-8% and 0.05-5% respectively. The vertical transmission rate has been estimated to be 3-5% and there is a high rate of spontaneous clearance (25-50%) in the children. Higher rates have been reported for both vertical transmission (18%, 6-36% and 41%) and prevalence in children (15%).

In developed countries perinatal transmission is now the leading mechanism of HCV transmission. In the absence of virus in the mother's blood transmission seems to be rare. Factors associated with an increased rate of perinatal infection include membrane rupture of longer than 6 hours before delivery and procedures exposing the infant to maternal blood. Cesarean sections are not recommended. Breast feeding is considered safe if the nipples are not damaged. Perinatal infection of a child does not increase the risk in a subsequent pregnancy. All genotypes appear to have the same risk of transmission.HCV infection is frequently found in children who have previously been presumed to have non-A, non-B Hepatitis and cryptogenic liver disease. The presentation in childhood may be asymptomatic or with elevated liver function tests. While infection is commonly asymptomatic both cirrhosis with liver failure and hepatocellular carcinoma may occur in childhood.

Prevention of Hepatitis C Virus Transmission

Understanding the modes of Transmission of HCV and the ways it spreads among communities and heath care settings, plays important steps in formulating the strategies of prevention of such infectious virus. Hepatitis C transmission is preventable [26]. Effective prevention interventions reduce transmission and the subsequent impact of infection on individuals and the community. Reducing HCV transmission minimizes greatly morbidity and mortality caused by, Hepatitis C and to minimize the personal and social impact of the disease. Although the priorities of such strategies may vary from one country to another and even among health care centers themselves. Immediate and long run strategies should be implemented targeting both the community and health care settings. (Table 2) illustrates the most of these strategies [15]. However, special emerging programs should be highlighted these include.

1. Immediate continuous prevention strategies
a) Universal prevention planning
(i) Well-planned educational programs regarding the risk of HCV both at the community and health institutions levels.
(ii) Implementation of international and national guidelines regarding the prevention of HCV particularly at special hospital settings as blood banks and hemodialysis units and high risk groups at the community.
(iii) Strict adherence to such guidelines and regular assessment to its applications.
(iv) Introducing specific patient-care practices.
b) Special settings prevention programs
(i) Blood and blood products, HCV screening program and using thioproprin, haemovigilance.
(ii) Hemodialysis; strict adherence to nosocomial prevention program; review practices to ensure they are consistent with recommendations and applied routinely,
(iii) Laboratory and health care; improving laboratory testing, better sterilization, safer injection, and less exposure to blood products.
2. Long-run preventive strategies
a) Universal preventive planning
(i) Vigilance and health alert programs which should report any problem and allow to interfere at any time.
(ii) Elucidation is needed for better prevention, screening, and updating HCV treatment
(iii) Prevention of HCV infection progress
(iv) Eradicate the massive use of unsafe medical procedures
b) Special settings preventive planning
(i) Injecting drug users
(ii) HIV-HCV co-infected patients

(iii) Prisoners inmates
(IV) Patients with Mental Disorders
3. Research planning and priorities
Well-designed research programs should be established both at country level and regional levels which may include.
(i) Population-based surveillance studies.
(ii) Evaluation of safety and efficacy of antiviral therapy for HCV alone and with other co-infected viruses particularly HIV.
(iii) Further evaluation of iatrogenic causes of HCV.

Table 2: Preventive and combat strategy programs for Hepatitis C Virus.

Blood banking services

Despite the great scientific progress that has been achieved in preventing transmission of HCV among blood banking services as blood has been considered to be very safe nowadays such burden still exist. Improved blood banking practices and the development and implementation of increasingly sensitive serological and nucleic acid amplification technology assays for screening donors for HCV over the past few decades have helped minimize the residual risk from transfusion transmitted HCV in the developed world [25,27].

Transfusion-transmission of HCV continues to be a problem particularly among developing nation. This however, may be related to many factors among these countries such as infrastructure, cultural and behavioral circumstances, human resources, political structure and economy. These countries should introduce key preventive mechanisms to ensure safe blood include elimination of paid donors and development of national donor pools comprising volunteer repeat blood donors, combined with implementation of standardized and maximally sensitive screening assays for HCV.

Hospital care settings

Nosocomial transmission of HCV particularly among hemodialysis still poses the main burden on health services worldwide. The nosocomial type of transmission was probably the dominant means of HCV spread in the dialysis unit. We would advocate strict enforcement of the universal measures for infection control, and assignment of patients to different dialysis machines, depending on their viral marker positivity. The incidence and prevalence of HCV infection among dialysis patients is steadily declining. The decline was initially due to the reduction in post-transfusion HCV infections, subsequently, it has reflected the implementation of infection-control measures to prevent nosocomial transmission within dialysis units. In developing countries medical-related transmission of HCV, such as hospitalization and/or surgical and dental procedure still accounted for a higher risk among hospital care setting, hence then adhering to universal precautions within healthcare facilities becomes priority. Further, more lack of insufficient supply of sterile syringes, dictates that medications should be given orally rather than via injection (when possible).

Community prevention programs

HCV is considered to a dynamic disease particularly among young people within the community. Hence then education and social programs should be introduced and special care centers has to be established particularly for IUDs and individuals with Mental disorders. These groups are particularly at a higher risk of HCV, due to poverty, literacy, homelessness, ignorance, multiple sex partners and despair. Furthermore, they have less access to health care services within the societies. Strengthening the capacity of education providers and the providers of services to young people to ensure they have access to harm. Enhance training and support for community based Hepatitis C educators, including injecting drug user peer educators' reduction knowledge and skills.

Post exposure prophylaxis for Hepatitis C Virus infection

At the moment there is no proved effective post exposure prophylaxis to HCV and no clear evidence for using immunoglobulin and ant-viral agents despite the ongoing clinical trials for using such antiviral agents. This however adds a further burden on the prevention programs for HCV particularly among health care workers, hence then particular attention should be taken regarding education and preventive methods to HCV. Therefore, implementing primary preventive strategies should be the corner stone for any program this include behavior modification both in community and health care settings. Awareness of the existing risk of acquiring HCV, education and training the involved personnel to implement the universal standard precautions avoid exposure to HCV [27]. However, (Figure4) highlights the management of exposure to Hepatitis C infection this includes performing the testing for anti-HCV antibodies and liver enzymes as shown in (Table 1).

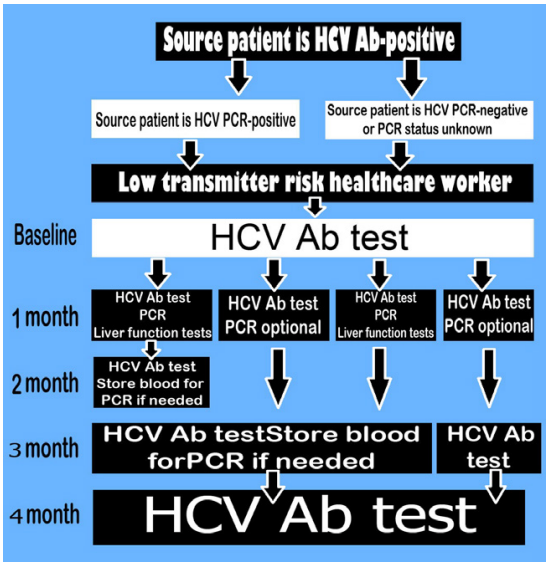

Figure 4: Diagnosis and management of post exposure to Hepatitis C infection.

Conclusions and Recommendations

There is an urgent need to better understand HCV prevalence and incidence rates in different countries around the world to help curb transmission from preventable causes, and especially through blood transfusion. Blood transfusion and other parenteral exposures in health care settings are still major routes of HCV transmission among individuals in developing countries compared to the rest of the world where the major route of transmission is currently IDU. Further tight global cooperation is particularly needed to make the blood more safe among developing countries. As studies of transfusion transmitted infections and of donors identified as infected by routine screening have provided significant insights into HCV transmission, epidemiology and pathogenesis. There is also a need to develop up-to-date data on HCV disease burden on a global scale, in part, derived from systematic screening of donors for HCV infection. Furthermore, a creation of blood donor databases and specimen repositories, both at national and international levels, to facilitate epidemiological surveillance and pathogenesis and treatment studies in the future may be needed. Strict adherence to a nosocomial prevention programs among health care center, screening and isolation of patients at risk if needed. A combination of harm reduction strategies, such as the provision of new needles and syringes and treatment of substance use, decrease the risk of Hepatitis C in intravenous drug users by about 75% among communities. Hepatitis C surveillance systems need to be developed and enhanced to provide accurate data to inform the planning and delivery of prevention and disease management options.

Workforce development is critical for minimizing the impact of Hepatitis Con individuals and the community as a whole. Easy access to information about transmission, testing, treatment and referral through a broad range of professionals underpins the majority of the priority action areas. Clinical, prevention and community services and organizations which support people with Hepatitis C need to be adequately resourced and informed to deliver appropriate services to people with Hepatitis C. Specific support should also be provided to peer-education and support services. Improving research activities that could solely contribute to reducing the impact of Hepatitis C on the community and understanding of Hepatitis C, including the economic impact of actions and inactions. These should be clearly linked to the needs of affected communities and identify methods of overcoming the barriers to prevention, testing, diagnosis, treatment and management of Hepatitis C, including identifying preferred models of care. Future concepts may include vaccination though, no vaccine protects against contracting Hepatitis C yet. However, a number are under development and some have shown encouraging results.

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Hepatitis C Virus Genome

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Introduction

Hepatitis C Virus (HCV) is a major cause of worldwide morbidity and mortality. The World Health Organization estimates around 150 million of the world's population being chronically infected [1]. An estimated 2.7-3.9 million of the United States population is infected with chronic HCV.Approximately, two thirds of those infected do not know their diagnosis [2,3]. A hallmark of HCV is itspropensity to establish persistence, with 75-85 % of the infected individuals unable to eliminate the virus without therapy [4]. HCV has also been implicated with HCV-associated metabolic syndrome, hypobetalipoproteinemia and insulin resistance [5]. As newer molecular targets for HCV treatment are being researched, a comprehensive review of the HCV genome and its lifecycle may serve as an essential tool in its study.

HCV Genotype and Epidemiology

The Flaviviridae families of viruses are divided into three genera, namely the Flavivirus, Pestivirus, and the Hepacivirus. The HCV belongs to Hepacivirus genus. There are six major genotypes and each differs by at least 30% of their nucleotide sequence. Each genotype in turn contains many subtypes and those are alphabetically designated as a, b, c, etc. Each subtype can have as much as 20% nucleotide sequence diversity. The lack of proof reading capacity in the viral RNA polymerase, the short half-life of the virus (few hours) and an estimated 1012viron production per day are the basis for the genetic diversity of the virus. The genotypes 1,2 and 3 have the biggest geographical distribution. HCV genotypes 1a and 1b are the most common genotypes, accounting for approximately 60% of worldwide HCV infections [6]. HCV type 1a infections predominate Northern Europe and Americas, and type 1b predominate Southern and Eastern Europe and Japan [7]. HCV type 4 is found mostly in the Middle East and Central African countries [7]. HCV type 5 has beenprimarily isolated from South Africa, and genotypes 3 and 6-11 are widely distributed across Asia [6,7].

Thedetermination of specific HCV genotype may determine the type and duration of therapy, thereby assisting in predicting a response to antiviral treatment and outcomes [8].

Genome Structure

HCV is about 9.6 kb positive strands RNA virus with a radius of about 25 nm.The density of the HCV virion is around 1.24 g/cm3 in Cesium Chloride (CsCl), with a sedimentation coefficient of 200 S in sucrose gradients. A lighter fraction of the virion (density of 1.04 to 1.06 g/cm3) is due to its association with serum beta-lipoprotein. The denser fraction of the virion (density of around 1.17 g/ml in sucrose) is involved in the formation of noninfectious immune complexes [9]. The nucleocapsid of the virus has a density of 1.25 g/cm3in sucrose [9]. HCV lacks efficient proofreading ability during replication, making the virion highly mutable. This behavior of the virus brings up the notionthat HCV probably persists as a collection of viral quasi-species [10].

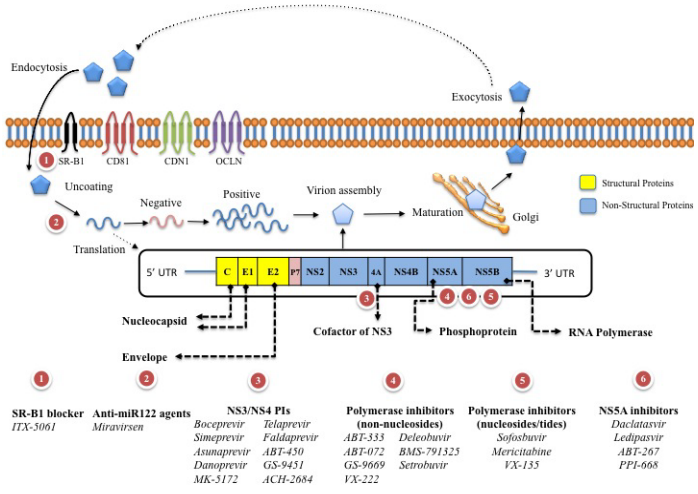

Figure 1: HCV Genome and Life Cycle.

The HCV genome stretches from 3' to 5'and each end is an Untranslated Region (UTR) connected by the Open Reading Frame

(ORF).It codes for a polypeptide with about 3000 amino acids depending on the HCV genotype. The 5' UTR is not capped, and it folds into a complex secondary RNA structure. This structure is formed with a portion of the core-coding domain, an internal ribosome entry site (IRES - that mediates direct binding of ribosomal subunits), and cellular factors which lead to translation.Recent research showed thatabundant liver-specific microRNA (miRNA), miR-122, bound to the HCV 5'-UTR and enhanced viral RNAreplication. This finding uncovered a newangle for possible antiviral intervention by antagonizing the function of miR-122. This 5' UTR contains 341 Nucleotide (nt) located upstream of the ORF translation initiation codon, the most conserved region of the genome[11]. The3' UTR is shorter and less structured than the 5' UTR. The 3' UTR contains approximately 225 nt. It is organized in three sections from 5' to 3'.These three sections include a variable region of 30-40 nucleotide, a long poly(U)-poly(U/UC) tract and a highly conserved 3'-terminal stretch [12]. This end is essential for viral replication (Figure 1).

HCV Proteins

The HCV ORF contains 9024 to 9111 nucleotides.However, the number of nucleotides is dependent on the genotype.The ORF encodes at least eleven proteins, including three structural proteins, a small protein p7 ion channel, six Non-Structural (NS) proteins and the Frameshift 'F' protein.The structural proteins consist of core 'C' proteins and envelop 'E' proteins.The function of P7 protein is undetermined. The non-structural proteins consist of NS2, NS3, NS4A, NS4B, NS5A and NS5B types.

Structural proteins

a) Core 'C' Protein: The HCV core protein 'C' is a basic RNA-binding protein.This core forms the viral capsid. This C protein consists of three domains: an N-terminal hydrophilic domain of 120 amino acids, a C-terminal hydrophobic domain of about 50 amino acid, and a single peptide of 20 amino acids [13]. These core proteins are either membrane-bound or membrane-free, and exist as either a dimeric or multimeric form [14]. They interact with cellular proteins and are important in the viral lifecycle [15]. The HCV core proteins regulate the activity of cellular genes (*c-myc* and *c-fos*), and have apoptotic and anti-apoptotic functions. These cellular regulatory activities control the transcription of viral promoters [16].

b) Envelope 'E' Glycoproteins: The envelope glycoproteins consist of E1 and E2, an essential component of the HCV virion that is vital for viral entry and fusion. Two areas of hydrophobic amino acids, which are separated by a short polar regions of fully conserved, charged residues, make up the transmembrane domains of E1 and E2. These envelope glycoproteins are localized in endoplasmic reticulum compartment, and have multiple functions. These functions include membrane anchoring, endoplasmic reticulum localization and heterodimer assembly [17].

c) Frameshift'F' Protein: The F protein or Alternate Reading Frame Protein (ARFP) is created due to a ribosomal frame-shift at the N-terminal core-encoding region of the viral polypeptide. The clinical significance of this protein is evident in recent studies, which have identified antibodies to F protein in chronically HCV infected patients. This may suggest the production of the F protein during the infective phase of the virus [18].

Non-structural proteins

a) p7: p7 ion channel is a membrane polypeptide made up of 63 amino acids. p7 transmembrane domains are arranged in α-helices that are connected by a cytoplasmic loop. Though p7 is not required for RNA replication in vitro, it seems to determine the productivity of infection in vivo. Mutations of the cytoplasmic loop in chimpanzees have shown to suppress infectivity by affecting intra-liver transfection of HCV cDNA [19].

b) NS2: NS2 is a non-glycosylated transmembrane protein.It is a catalytic short-lived protein that loses its protease activity after self-cleavage from NS3.It is essential for the complete replication of the virus. A phosphorylationdependent degradation of NS2 occurs by means of protein kinase casein kinase 2 [20].

c) NS3-NS4A: NS3 protein contains a serine protease domain at its N-terminal (1/3rd region) and a helicase/NTPase domain at its C-terminal (2/3rd region).NS4A is a cofactor for the NS3's protease activity[21]. NS3–4A serine protease cleaves and thereby inactivates two crucial adaptor proteins in innate immune sensing which might have a crucial implication on the pathogenesis and persistence of HCV infection. Just as in HIV treatment, the NS3-4A serine protease has emerged as a prime target for the design of specific inhibitors as antiviral agents76Two NS3 protease inhibitors - Boceprevir and Teleprevir have been approved for the treatment of genotype 1 HCV infections[22]. Other PI in development includes ABT-450 and TMC 435. The NS3 helicase works on unwinding of the double stranded RNA for replication.

d) NS4B: NS4B is an integral membrane protein. Its functions have been elucidated as membrane anchoring for the replication complexes;Endoplasmic Reticulum (ER)derived membrane localization. It serves as a scaffold for the HCV replication complex. It has a possible role in HCV carcinogenesis, impairment of ER function, and regulation of both viral and host translation [23]. The membrane functions of NS4B are carried out byatleast four trans-membrane domains and an N-terminal amphipathic helix. A small molecule drug ACH-806 is in phase 1b/2 clinical trial, as an NS4B antagonist for HCV treatment [24].

e) NS5A: NS5A is a phosphorylated zinc-metalloprotein. It plays an important role in regulation of cellular pathways, membrane localization, transcriptional activation, and assembly of the replication complex [25]. Although NS5A's role in replication is not entirely clear, it seems associated with lipid rafts.Lipid rafts are derived from intracellular membranes that bind to the C-terminal region of a vesicle-associated membrane-associated protein, crucial for the formation of the HCV replication complex [25]. It is hypothesized that it forms a two-dimensional array on intracellular membranes; thereby creating a 'basic railway' that would allow the sliding of RNA. It also might function as a molecular switch between replication and assembly of the virus. NS5A also plays a role in interferon resistance by inhibiting RNA-activated Protein Kinase. NS5A inhibitorsare currently in phase II and III stages of clinical trial [26].

f) NS5B: NS5B is an RNA dependent RNA polymerase (RdRNAp) that is responsible for the replication of the virus. The first step is the formation of the negative strand complementary RNA which will be the template for the subsequent positive strand HCV RNA replication. The conformational structure is such that it creates a completely enclosed replication site. Nucleoside and non-nucleoside NS5B inhibitors are in various stages of clinical trials [27].

HCV Lifecycle

The precise mechanisms of HCV replication are still poorly understood, however studies predict its replication processes to be

similarto other positive-strand RNA viruses. The HCV virus interacts with a number of cell surface receptor molecules such as CD81 and LDL receptors to enter the cell [9]. The initial attachment and penetrationprocesses lead to local pH changes. These pH changes in effect lead to cellular and viral endosomes fusion and conformational changes to the envelope proteins [9]. The NS5B RNA-dependent RNA polymerase now catalyzes a two-step process of HCV replication. The initial stepinvolves intermediate complementary negative-strand synthesis carried out by positive-strand genomic RNA template. A negative-strand RNA now serves as a template to produce numerous progeny strands. These progeny strands are positivelycharged, and will eventually be used for polyprotein translation, synthesis of replication intermediates, and new virus particle packaging. These processes occur in the endoplasmic reticulum membranes [28]. Once formed, they are extruded into the ER and eventually the virusreleases via the vesicular secretory pathway.Studies and observations that show the involvement of other host factors on viral replication and the contribution of other HCV related proteins, such as cyclophilin B, are emerging and in earlier stages. Once elucidated, those could also lead to new and improved therapies.

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Hepatitis C Life Cycle

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Abstract

Hepatitis C virus (HCV), a positive sense, single-stranded RNA virus of the Flaviviridae family, is a significant cause of acute hepatitis that displays considerable propensity for progressing to chronic hepatitis. Although previously occult, in the past decade much of the HCV life cycle and replication has been revealed through studies involving HCV cell culture and replication. Elucidation of the life cycle has had many important implications in providing potential targets for novel therapeutics, as each step of the viral life cycle may be utilized as a treatment target. This chapter illustrates the complex process of viral entry, including a discussion of its receptors, co-receptors and host factors. This chapter also will discuss the formation and release of viral particles, a key process in the pathogenesis of this viral disease.

Viral Entry

Viral entry of HCV is a complex process involving the coordination of many receptors, co-receptors, and host factors. HCV is composed of several proteins that are necessary for attachment and entry into the host cells. The structural proteins of HCV include core, E1, and E2 [1-3]. Core, the main structural component of the virus, is critical in gene modulation and expression, signaling pathways, and lipid metabolism [2]. E1 and E2 are envelope glycoproteins required for receptor-mediated cell entry that surround the viral particles and provide a site for receptor binding and fusion with the hepatocyte apical domain [1].

The virus is also made up of seven non-structural proteins: p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B [1,2]. NS2 is a viral autoprotease that is important during processing of the viral polyprotein, discussed later, by mediating cleavage between NS2 and NS3. NS3 functions to encode N- and C-terminal proteases as well as cleavage of downstream sites NS3/4A, NS4A/4B, NS4B/NS5A, and NS5A/NS5B. Although the function of NS4B is largely unknown, it is believed to induce membranous web formation, which is a specialized site on the endoplasmic reticulum (ER) membrane that is necessary for virion release from the cell. NS5A binds the viral RNA and other host factors close to the HCV core and lipid droplets (LDs) and NS5B is the RNA-dependent RNA polymerase (RdRp). All of these proteins are essential in the HCV lifecycle, including attachment, entry, fusion, translation, posttranslational processing, HCV replication, viral assembly, and release (Table 1). As such their complete functions will become clearer as the chapter progresses [1,2,4].

Viral Attachment

Initial viral attachment is believed to involve the hypervariable region (HVR1), which is located within the E2 envelope glycoprotein, along with various lipoproteins, such as apoE, apoB, apoC1, apoC2, and apoC3. This combination of proteins forms a structure called

Receptor	*Description*	*Action*
Structural proteins		
E1/E2	Type 1 transmembranegylcoproteins	Surround the viral particles; key role in viral entry via receptor binding and fusion
Non-Structural proteins		
p7	Small ion channel protein	Required for viral assembly and release
NS2/NS3	Viral autoprotease	Key target for HCV antiviral drug development
NS4A	Cofactor for NS3 protease	Forms a stable complex with NS3
NS4B		Induces membranous web formation
NS5A	Dimeric zinc-binding metalloprotein	Binds the viral RNA and various host factors in close proximity to the HCV core and lipid droplets
NS5B	RNA-dependent RNA polymerase	Synthesis of new viral RNA genomes
Viral attachment		
HVR1	Hypervariable regions in the E2 envelope glycoprotein	Possible role as HCV neutralizing epitope; initial viral attachment to its receptor/co-receptor
LVP	Complex of lipoprteins (apoE, apoB, apoC1, apoC2, and apoC3)	Inlfuence HCV entry
LDLR	LDL receptor	Bind HCV and promote its cellular entry
Viral entry		
SRB1	Glycoprotein receptor; known major receptor for HDLs	Along with CD81, it is a binding partner of HCV E2
CD81	Includes 4 transmembrane domains	Involved in adhesion, morphology, proliferation, and differentiation; binds HCV through E2
CLDN1	Tight junction protein	May interact with CD81 as a part of HCV receptor complex; does not directly interact with HCV
OCLN	Tight junction protein	Involved in a later phase of HCV entry after SRB1 and CD81
EGFR	Receptor tyrosine kinase (RTK)	Regulates CD81 and CLDN1 interactions and membrane fusion; cell-cell transmission of HCV
EphA2	Receptor tyrosine kinase (RTK)	Regulates CD81 and CLDN1 interactions and membrane fusion; cell-cell transmission of HCV
NPC1L1	Cholesterol sensing receptor expressed on hepatocytes	HCV entry factor

Table 1: Essential Proteins Involvedin the HCV Lifecycle.

the lipoviroparticle (LVP), which may influence viral entry [2]. Heparan sulfate proteoglycans, located on the surface of hepatocytes, binds to HVR1 in HCV E2 to initiate viral attachment. Several other membrane co-receptors have been identified as aides to HCV attachment and entry including: scavenger receptor class B type 1 (SRB1), CD81, claudin-1 (CLDN1), and occludin (OCLN).

SRB1 and CD81 function in binding of HCV E2 via the HVR1 region. SRB1, a receptor for high density lipoproteins (HDLs), is highly expressed in the liver [5]. It was found that HCV entry is enhanced in the presence of HDL through positive modulation of the HCV/SRB1 interaction. One study even proved that the level of HCV entry and infectivity was in direct correlation to the level of SRB1 on the hepatocytes [1,3,5,6].

CD81, normally expressed on the hepatocyte surface, is believed to join the entry process after SRB1 by binding to HCV E2 and promoting a conformational change in the E1/E2 envelope proteins [1,3,5]. This change facilitates the pH-dependent fusion of the virus with the host cell and subsequent clathrin mediated endocytosis. CLDN1 and OCLN are tight junction proteins whose role in the process of viral entry is not fully elucidated. Although neither appears to interact with HCV directly, they are believed to interact with CD81 and aid in viral endocytosis [1,6].

Receptor tyrosine kinases, epidermal growth factor receptor (EGFR) and ephrin receptor A2 (EphA2), and Niemenn-Pick C1-like 1 (NPC1L1) were recently identified as participants in HCV host cell entry. These receptors are believed to regulate the activity of CD81 and CLDN1 in membrane fusion as well as contributing to the cell-to-cell transmission of HCV. NPC1L1, a cholesterol sensing receptor found on the apical surface of hepatocytes, is also believed to play a role in HCV entry [1].Zeisel et. Al [6] illustrated how molecular mechanisms and targets for antiviral therapiesrelated to entry ofthe hepatitis C virus into hepatocytes (Figure 1):

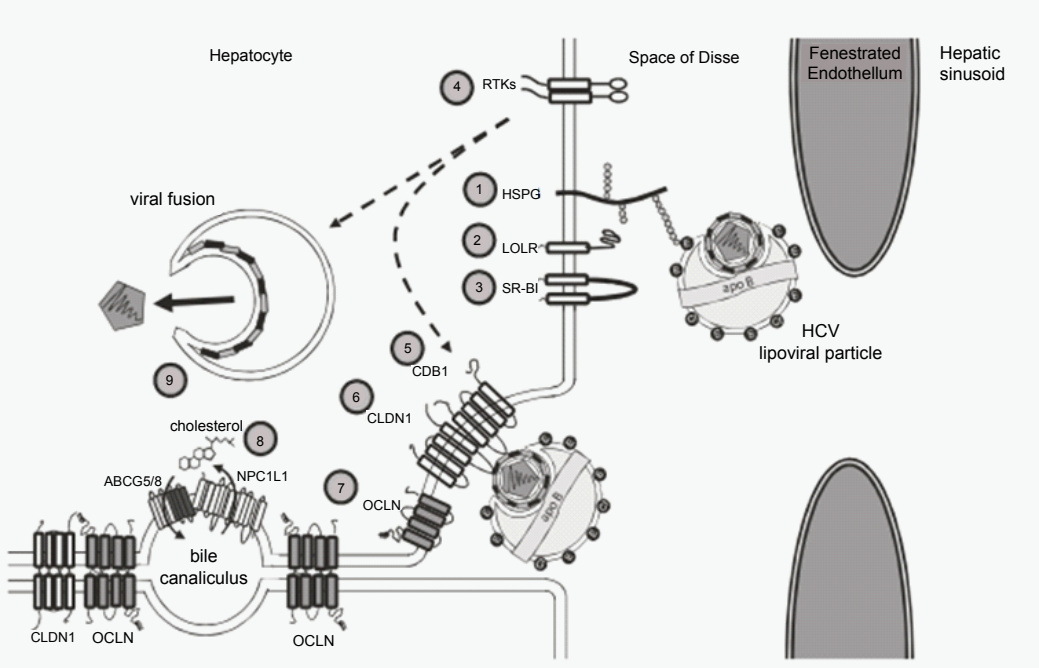

Figure 1: Summary of the Receptors Required for HCV fusion and Entry into Host Hepatocytes.

Translation

Subsequent to HCV internalization by host hepatocytes, the HCV virus proceeds to undergo a process called cap-independent, IRES mediated translation. HCV, like many viruses, lacks an inherent translational apparatus and is, therefore, reliant on host cell machinery for translation. This lack engenders a competition between host mRNA and viral RNA for use of the host cell machinery. In response to this competition, viruses have evolved to generate a translational advantage over the host cell by adopting a process of internal ribosome entry site (IRES) mediated initiation [7].

IRES, located in the 5'-untranslated region (5'-UTR), is composed of structural domains that direct binding of the 40S ribosomal subunit to the viral RNA [7,8]. The open reading frame of the large viral RNA genome is flanked by 5' and 3' untranslated (UTR) regions [8]. 5'-UTR, which replaces the eukaryotic 5'-cap found in host cells, consists of four domains and is highly conserved among strains of HCV. IRES is a large region of 5'-UTR that encompasses domains II-IV of the four domains. It is believed that the IRES structure allows for the virus to function without the translation initiation factors that are required in cap-dependent processes [1,2,7].

IRES initiates translation by recruiting the 40S ribosomal subunit to domain III in the absence of any host translation initiation factors [10]. Binding of 40S is complex and it involves multiple sites of connection with IRES, mostly involving domain III. The 40S subunit binds IRES with the ribosomal P-site directly adjacent to the AUG start codon and thus renders additional scanning of the sequence unnecessary [2,7]. As a consequence of how the virus is bound, binding positions the AUG codon in the mRNA binding cleft of the 40S subunit.

Following recruitment of the 40S ribosomal subunit, the 48S complex is constructed by the addition of the eIF2-GTP-Met-tRNA ternary complex and eIF3 to the 40S ribosomal subunit-IRES complex. The ternary complex functions in the formation of codon: anticodon base pairing with the AUG start codon. The role of eIF3 is, primarily, to stabilize the ternary complex for improved translation efficiency, but it has a secondary function in the initiation complex development. Subsequently to binding of the ternary complex and eIF3, eIF5 mediates release of eIF2-GDP via GTP hydrolysis. Additional GTP hydrolysis by eIF5B is required for the release of eIF3 during the joining of the 60S ribosomal subunit. This leads to the formation of the 80S complex, a translational competent ribosome that may proceed with elongation and termination of the viral protein.

Cap-independence is able to generate an additional selective advantage for viral cell translation by continuing to function after selective repression of cap-dependent initiation by viral proteases. These viral proteases shut off host cell translation by cleaving the eukaryotic initiation factor (eIF), which is essential for cap-dependent initiation of translation [7,8]. IRES is a region that exists in other viruses, but unlike in other viruses where it turns off host cap-dependent translation, in HCV it works in conjunction with the host processes.

Polyprotein Processing

Regardless of final replication strategy, it is necessary for all viruses to initially express their genomes as functional mRNAs in order to effectively utilize host cell translational machinery to produce viral proteins. RNA viruses have thus evolved to derive many separate proteins from a single precursor genome. The HCV virus genome encodes a large single polyprotein precursor that is over 3000 amino acid residues in size [11,12]. This precursor is subsequently co- and post-translationally processed by host and viral proteases into the structural and non-structural proteins, C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B, that make up the mature hepatitis C virus [1,11,13,14].

HCV polypeptide processing is unique in that all of the polypeptides are associated with the endoplasmic reticulum (ER) or ER-derived membranes [11,12]. This suggests that assembly and replication of the genome occurs within the ER. It is, therefore, the ER signal peptidases that cleave the structural proteins from its polyprotein precursor as well as mediating further processing at the C terminus of the capsid protein (C). The non-structural proteins are cleaved from the polyprotein by HCV proteases NS2, NS3, and NS4A [11,12,14].

Research has shown that the N- and C-terminal sequences on either side of a cleavage site work cooperatively to modulate effective and efficient cleavage of the precursor polyprotein. The constraints that the N- and C- terminals place on cleavage are thought to be essential to regulate kinetics and quantitative expression of final protein products post-translation. Coordinated cleavage of the polyprotein precursor is essential for the lifecycle of HCV. Each cleavage product likely controls the cleavage of other sites on the precursor molecule thus having a very fine level of control over the process [11].

Although complete cleavage of most of the polyprotein occurs during or immediately after translation, there are only partial cleavages at the E2/p7 and p7/NS2 sites. This failure of completion leaves an uncleaved E2p7NS2 molecule. Over time, NS2 is progressively cleaved from the precursor molecule, but E2 and p7 remain bound in most strains of HCV. Although the importance of this finding is largely unknown, it is hypothesized that it places conformational constraints at the level of these cleavage sites and thus controls quantitative expression of the final protein products [11].

Formation and Release of Viral Particles

Until recently, viral assembly and release was not very well studied due to difficulties in production of infectious viral particles in cell culture [14,15]. Several studies were done to predict HCV assembly by creating virus-like particles (VLPs) to mimic its activity, but the process was still largely obscure [14,16,17]. This roadblock was overcome when one study proved that a specific strain, JFH1, of genotype 2a HCV was able to efficiently produce infectious virions in culture. Prior to this discovery, it was initially postulated that there were distinct established roles for structural proteins versus non-structural proteins [15].

Structural proteins were believed to be purely involved with physical virion components, whereas the non-structural components (with the exception of NS2) were essential for replication of HCV. This hypothesis was eventually debunked after investigations were able to be performed at a much more detailed level subsequent to the breakthrough of viral growth in cell culture using JFH1. Ensuing evidence lead researchers to believe that HCV encoded proteins could no longer be separated by roles because some proteins were found to play a role in both processes [14,15].

With the ability to grow viral cells in cell culture, much of viral assembly and release of viral particles has been revealed. Initiation of assembly requires release of replicated genomes from specialized sites on the ER membrane termed the membranous web. These genomes must then come in contact with the viral core protein to form the virion capsids. There is believed to be three main interconnected processes that occur on separate sides of the ER membrane, the cytosolic side and the lumenal side. The first stage involves the initial assembly at lipid droplets (LDs), which are cytosolic storage organelles that are believed to be a major contributor from the host cell to virion assembly. The second stage includes the viral factors that contribute after initial assembly. The final stage occurs on the lumenal side of the ER membrane where the partially assembled virion engages with a very low density lipoprotein (VLDL) to facilitate completion and maturation of the virus [9,15].

Assembly of the virus is initiated on the cytosolic side of the ER membrane, but maturation occurs in the ER lumen prior to being released from the cell. Initially, core protein binds to LDs and proceeds to coat the surface of the organelle. The viral replication complex is then recruited to the LD surface using the core and NS5A proteins. This interaction enables transfer of replicated RNA from the replication complex to associate with the core for encapsidation of the genome. NS3 joins the process at this step to produce fast-sedimenting core-containing particles, which are presumed to be non-infectious until further maturation. Late in the assembly process, E1 and E2 envelope glycoproteinsare incorporated into the amino-terminal region of the polyprotein followed by mediation with NS2 to confer infectivity to the virion [16]. Jones et al. [15] (Figure 2) and Bartenschlager et al. [9] (Figure 3) clearly demonstrated the assembly process of the hepatitis C virus particles.

Viral Replication

Although the live is the major site of HCV replication, there has been strong evidence pointing to replication in the peripheral blood mononuclear cells as well. HCV requires most of the previously mentioned non-structural (NS) proteins in order to undergo replication. In particular, NS3-NS5B are essential in the formation of the intracellular membrane associated replicase complex, which allows for the production of viral proteins and RNA in a distinct compartment. Although the individual steps of viral replication are largely unknown, it is clear that NS5B RdRp is a key player in synthesis of plus- and minus-strand RNA.The NS5B protein is able to synthesize the RNA primer independently of the polymeric templates in the presence of high ATP or GTP. This suggests a possibly de novo initiation of viral replication in vivo that is dependent on ATP/GTP concentration [4].

The first step of viral replication involves penetration of the host cell to release viral RNA from the virus particle into the cytoplasm.

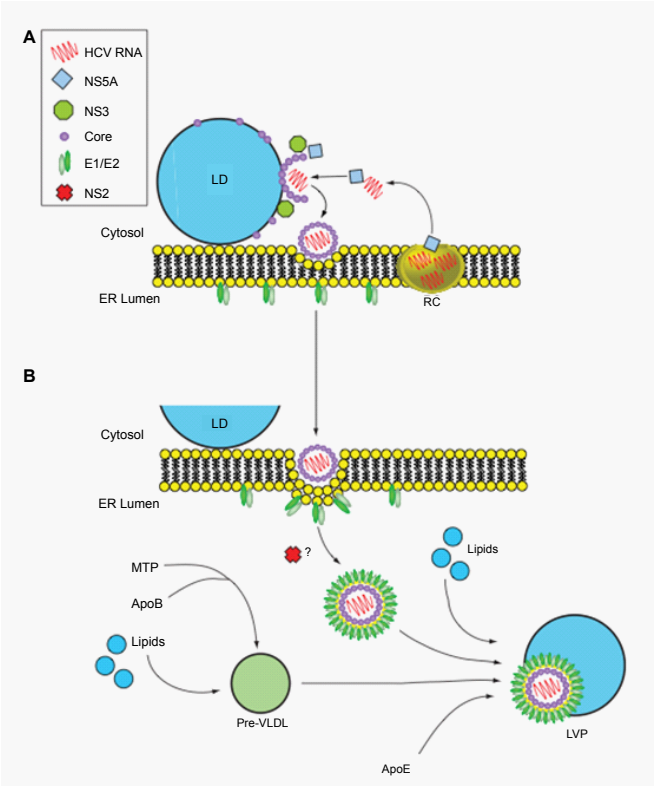

Figure 2: Assembly Process of the Hepatitis C Virus Particles.

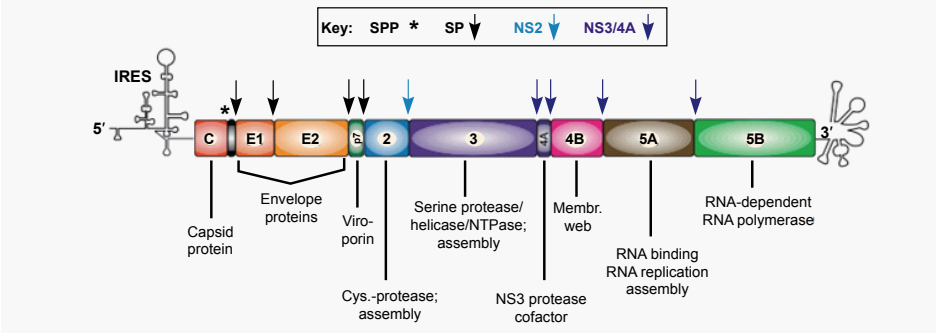

Figure 3: Hepatitis C virus: Assembly and Release of Virus Particles.

This is followed by translation of the genomic RNA, polyprotein processing, and formation of the replicase complex. Formation of plus-strand input RNA molecules are used to synthesize new minus-strand RNA intermediates for polyprotein expression and packaging into progeny virus. The final step of replication involve release of virus from the infected cell [4].

Acknowledgment

The authors thank Dr. Bassam Husam Rimawi for assistance in writing this article.

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HCV Immune Evasion

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Introduction

Infection with Hepatitis C Virus (HCV) results in chronic infection in the majority of infected individuals. Primary (acute) infection is usually asymptomatic, although some patients present with jaundice. Following acute infection approximately 20% of patients will spontaneously clear the virus, while 80% will develop persistent HCV infection. The mechanisms by which some individuals clear the virus and others develop chronic infection are not yet fully understood, but likely reflect a complex interplay between the host and the virus at the level of the immune response [1].

Persistent HCV infection is a significant cause of morbidity and mortality due to progressive liver fibrosis. Eventually 20-50% of patients will develop cirrhosis. Individuals with chronic hepatitis C and cirrhosis are at increased risk of liver failure, liver transplantion and hepatocellular carcinoma development [2].

Following initial infection, HCV RNA can be detected in plasma within days of exposure, often weeks before liver enzyme levels rise. Peak viraemia occurs in the first 8 to 12 weeks of infection, then drops to lower levels and persists. A number of situations may occur at this time:

– Plasma HCV RNA becomes undetectable within the first few months of infection and remains undetectable indefinitely (viral clearance)

– Plasma HCV RNA is inconsistently detected early in infection and a stable pattern of recovery or persistence is not evident for more than 6 months. Intermittent viraemia may represent re-infection (which is observed in intravenous drug users) or escape from an initially successful immune response.

The mechanisms of persistence of HCV remain incompletely understood, but recent studies have revealed important insights into the mechanisms of HCV immune evasion.

The human immune system represents an extraordinary and complex barrier against environmental threats, such as bacteria and viruses, and comprises many effector systems, cells and molecules, which are present in all the organs of the body and defend it from infection and disease. The innate and the adaptive immune systems, are effective providers of anti-viral immunity. Innate immunity represents the first, non-specific, arm of defence against pathogenic entry, and it is capable of repeatedly eliminating many potential infections through activation of Pattern Recognition Receptors (PRRs). Shortly after the innate immune response begins, the more specific adaptive immune response develops and acts in a more targeted manner, retaining immunological memory. Like other viruses, HCV has evolved immune evasion mechanisms which allow it to overcome the body's natural anti-viral defences, with major consequences, not only for spontaneous viral clearance, but also for response to conventional therapies.

Pattern Recognition Receptors (PRRs)

Dendritic Cells (DCs) are an important cell population involved in innate immunity and represent key mediators of anti-viral immunity. They are classified into conventional DCs (cDCs), which target a wide range of pathogens, and plasmacytoid DCs (pDCs), which, in response to viral infection, produce large amounts of Type I IFNs, and have been implicated in restraining HCV infection [3].

Upon viral infection, PRRs of the innate immune system sense the virus as "non-self", via the identification of defined conserved patterns within viral RNA, DNA and/or protein called Pathogens Associated Molecular Patterns (PAMPs). PRRs trigger signalling cascades (see Figure 1), leading to transcription of numerous anti-viral genes, including IL-1β and Type I and III Interferons (IFNs), which are then secreted by the infected cells and activate cytokine-specific signalling pathways [4].

The innate immune response utilises three main classes of PRRs to sense HCV: Toll-Like Receptors (TLRs), the Retinoic Acid-Inducible Gene I (RIG-I)-like Receptors (RLRs), and the Nucleotide oligomerisation domain-Like Receptors (NLRs) [4,5]. PRRs are highly expressed in bone marrow-derived immune cells, such as the liver-based macrophages (Kupffer cells) and hepatic dendritc cells, but also on resident liver cells [hepatocytes, Liver Sinusoidal Endothelial Cells (LSECs), and hepatic stellated cells] [6].

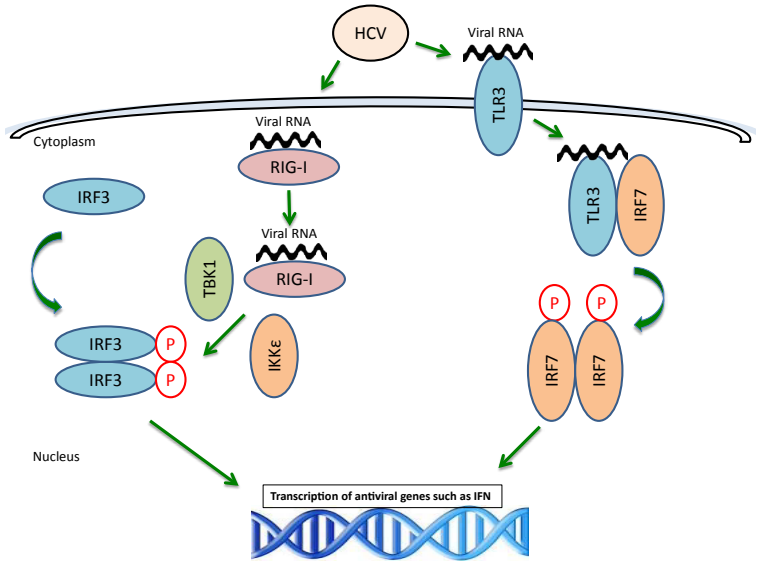

Figure 1: DUpon HCV entry and uncoating, viral RNA is detected through pattern recognition receptors (PRRs) such as RIG-I or Toll-like receptors. Upon activation and binding to its adaptor molecule MAVS, RIG-I initiates a signaling cascade leading to phosphorylation and nuclear accumulation of IRF3. In the nucleus IRF3 promotes transcription of antiviral and immunomodulatory genes including IFNs. Similarly, binding of HCV RNA to TLRs results in a signaling cascade leading to production of IFNs and inflammatory cytokines. HCV has been reported to subvert these innate immune defense mechanisms and others at several levels explained in detail in the text.

Toll-Like Receptors (TLRs)

TLRs are highly expressed by innate immune cells, such as DCs, but also by hepatocytes and Kupffer cells. This family of receptors include TLR1, 2 4, 5 and 6, which are found on the cell membrane, and TLR3, 7, 8 and 9, which reside on endosomal membranes. However, only a few members of the family are currently known to be involved in HCV detection. Cell membrane TLRs signal through five main adaptor molecules containing a Toll/IL-1 Receptor (TIR) domain: the adaptor molecule Myeloid Differentiation primary response gene 88 (MyD88), MyD88-Adapter-Like (MAL), TIR-domain-containing adapter protein inducing IFN-β (TRIF), TRIF-Related Adapter Molecule (TRAM) and the negative signalling regulator Sterile α and Armadillo-Motif-containing protein (SARM). These TLRs activate MyD88 via MAL, and initiate a signalling cascade involving IL-1 Receptor-Associated Kinase (IRAK) and TNF Receptor Associated Factor (TRAF) family members, resulting in activation of the transcription factor NF-κB, thus promoting induction of pro-inflammatory cytokines. Conversely, endosomal TLRs and TLR4 signal through TRAM, TRIF and the TANK-binding kinase 1/IκB kinase ε (TBK1/IKKε) complex, which in turn triggers Interferon Regulatory Factors 3 (IRF3) translocation to the nucleus and consequently induces Type I IFN production [7-11].

Traditionally, TLR2 and 4 are activated in response to non-viral PAMPs. However, a new role of these receptors in virus recognition has recently emerged: indeed, TLR2 can recognise HCV core and NS3 proteins, and HCV-dependent activation of TLR4 can stimulate Type I IFNs and IL-6 expression [12-14]. Conversely, TLR3 induces IFN-α, β and other inflammatory cytokine production in response to the presence of replicating HCV double-stranded RNA intermediates during active infection in liver cells [15,16], whereas TLR7 and 9 expressed in pDCs and Kupffer cells produce high amounts of IFN-α in response to HCV infection [3,17].

The HCV non-structural proteins NS3 and NS4A play a major role in immune evasion, as they oligomerise to form a NS3/4A complex with protease activity. Indeed, NS3/4A cleaves and inactivates various innate immune signalling adaptor proteins, including members of the TLR signalling pathway, in particular TRIF, thereby blocking TLR3-dependent and independent signalling [18]. Another HCV protein, NS5A, can also bind to MyD88, inhibiting recruitment of IRAK, resulting in interference with TLR signal transduction [19].

Retinoic Acid-Inducible Gene I (RIG-I)-Like Receptors (RLRs)

Among the RLRs family of cytosolic receptors, RIG-I recognises short 5' triphosphate dsRNA, whereas Melanoma Differentiation-Associated (MDA) protein 5 interacts with longer dsRNA (over 2000 nucleotides). RLRs are characterised by a C-terminal domain, a DExD/H-box RNA helicase domain, and an N-terminal Caspase Activation and Recruitment Domain (CARD). The third member of the family, Laboratory of Genetics and Physiology-2 (LGP2), also known as Probable ATP-dependent RNA helicase DHX58, cannot induce a cellular response alone, as it lacks the CARD domain, but it is essential for effective anti-viral responses mediated by RIG-I and MDA5 [20]. When activated, these receptors signal through the mitochondrial-associated adapter molecule, IFN Promoter Stimulator-1 (IPS-1) (also known as MAVS/VISA/Cardif), and TBK1/IKKε complex, leading to activation of AP-1, IRF3/7 and NF-κB to induce pro-inflammatory and anti-viral cytokines, including type I interferon expression [21].

RIG-I detection of HCV is well characterised and involves interaction between the RIG-I helicase domain, a 5'-triphosphate (5'-ppp) and the poly U/UC region of the HCV genome in the 3' non-translated region [22-24]. This is a highly conserved sequence among all HCV genotypes and also essential for viral replication [25-27]. HCV binding to RIG-I promotes a conformational change which triggers TRIM25-dependent ubiquitination of the CARD domains. This step is essential for RIG-I binding to the adaptor protein 14-3-3ε, which facilitates its translocation to the Mitochondrial-Associated ER Membrane (MAM), interaction with IPS-1 and ultimately activation of IRF3 and NF-κB [28-30].

HCV NS3/4A protein not only blocks RLR signalling by targeting and cleaving the mitochondrial associated IPS-1, thus rendering it incapable of activating the RIG-I signalling pathway [29,31], but it can also block the phosphorylation and activity of IRF-3 [32].

To date, there is no evidence of an involvement of MDA5 in sensing HCV PAMPs. However, as this PRR recognises long dsRNA, the possibility of an interaction with HCV dsRNA replicative intermediates during late infection stages cannot be excluded.

Nucleotide oligomerisation domain-Like Receptors (NLRs)

NLRs are intracellular sensors of PAMPs and Damage-Associated Molecular Pattern Molecules (DAMPs), and contribute to the formation of the inflammasome. This complex comprises a sensor protein, the adaptor protein Apoptosis-associated Speck-like protein Containing a CARD (ASC) and caspase 1 [33]. Once activated by RNA viruses, NALP3 triggers expression of the pro-forms of IL-1β and IL-18; this is followed by recruitment and activation of caspase 1, which in turn triggers secretion of the active forms of IL-1β and IL-18 [34]. During chronic HCV infection, this signalling pathway is activated in Kupffer cells, which phagocytose HCV, triggering TLR7 signalling. Caspase-1-dependent IL-1β mature form production is, in this case, driven by potassium efflux, although the exact mechanism is yet to be fully understood [35]. Conversely, IL-18 secretion has been linked to acute HCV infection [36]. IL-1β could also play a direct role in viral clearance during acute HCV infection as it has been reported to affect HCV RNA replication through activation of the Extracellular Regulatory Kinase (ERK) [37].

The Interferon Anti-Viral System

IFN was first identified in 1957, when chick embryos infected with an inactivated influenza virus produced and released an unknown protein into the surrounding fluid that was able to protect non-infected cells against viral infection. This factor was simply named "interferon" as it "interferes" with viral infection [38]. This discovery represented a significant milestone in understanding how the immune system responds to pathogens and, in particular, viruses. Nearly four decades later, the IFN, Janus Kinase (JAK)/Signal Transducers and Activators of Transcription (STAT) signalling pathway has been well characterised and has provided new insights into the anti-viral immune response and the mechanism of many diseases.

Interferon Family of Cytokines

IFNs are a multi-gene family of inducible cytokines that are best known for their potent anti-viral properties [39-41], although they also play a critical role in cell growth [42] and have immunomodulatory effects [43]. There are three main classes of IFNs: Type I, Type II and Type III. Type I IFNs include IFN-α (subdivided into IFN-α1, -α2, -α4, -α5, -α6, -α7, –α8, –α10, –α13, –α14, –α16, –α17, and –α21), IFN-β, IFN-ε, IFN-κ, and IFN-ω (which are expressed in humans), IFN-δ (which is found in pigs and cattle), IFN-ζ (only expressed in mice), and IFN-τ (only found in cattle) [44-46]. Type II IFN is represented only by IFN-γ and it is mainly involved in bacterial infections and is expressed by activated T cells, macrophages and NK cells. Type III IFNs are classified into four family members: IFN-λ1, [Interleukin-29 (IL-29)], IFN-λ2 (IL-28A), IFN-λ3 (IL-28B), and the newly discovered IFN-λ4 [47-49]. Until recently, the role of type III IFNs in anti-viral immunity remianed largely unknown. However, the discovery of a Single Gene Polymorphism (SNP) upstream of the IFNL3 (IL-28B) gene, which predicts both viral clearance in acute Hepatitis C infection and response to exogenous IFN treatment, has highlighted the importance of this family of cytokines in HCV infection [50].

Each type of IFN activates a specific cognate receptor, and this interaction dictates how a cell responds to infection. All type I IFNs bind to the IFN-α receptor (IFNAR), a two-chained heterodimer, composed of IFNAR1 and IFNAR2 subunits. As opposed to IFNAR1, IFNAR2 can have 3 different spliced variants: a long transmembrane IFNAR2c chain and two truncated forms, a transmembrane IFNAR2b and a soluble sIFNAR2a, which can modulate IFN-α signalling [51,52]. Type II IFN binds to IFN-GR (IFN-GR1 and IFN-GR2 subunits). Type III IFN receptor complexes consist of two chains, IFNLR1 [IL-28 Receptor-α, (IL-28Rα)] and IL10R2 [51]. Upregulation of IFNAR1 and IFNAR2 often occurs on circulating cells upon HCV infection and this expression of IFNAR in the liver of chronic HCV-infected patients is linked to a positive response to IFN-α therapy [53-55]. Type I IFN receptors are ubiquitously expressed in the body, while type III IFN receptors have a more restricted cellular distribution, and, as for IFNARs, have been strongly implicated in response to HCV infection [56-58].

Interferon Signalling Pathway

Once released by infected cells, IFNs establish an anti-viral state via signalling through the Janus kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway, resulting in upregulation of over 500 IFN-Stimulated Genes (ISGs). An overview of this process is shown in (Figure 2). Binding of IFNs to their corresponding receptor results in auto-phosphorylation and activation of the receptor-associated JAK kinases, which subsequently phosphorylate the receptor chains and consequently recruit STAT transcription factors, which form homo- or heterodimers and translocate into the nucleus where they bind to specific promoter elements. IFN-α-induced JAK/STAT signalling involves activation of the receptor-associated Tyrosine Kinase (TYK)2 and JAK1 and the STAT1-STAT2 heterodimer, which ultimately binds to a third transcription factor, Interferon Regulatory Factor (IRF9) to form the IFN-Stimulated Gene Factor 3 complex (ISGF3). Following translocation to the nucleus, ISGF3 binds to a specific element known as the IFN-Stimulated Response Element (ISRE), which is present in the promoter region of certain ISGs, thereby inducing their transcription. Some ISGs possess only ISRE or, Gamma-Activated Site (GAS) elements in their promoter regions while others contain both elements [48,59].

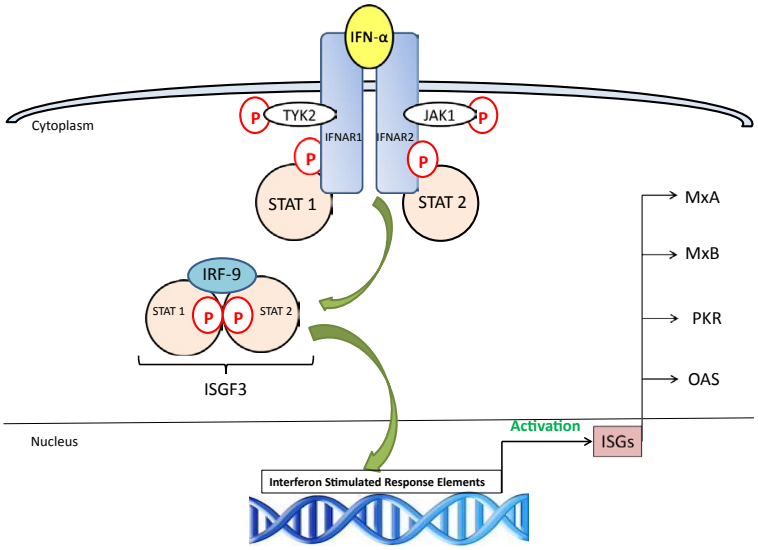

Figure 2: Secreted Interferon binds to and activates its cognate receptor, which in turn recruits kinases of the Janus kinase family. These kinases phosphorylate STAT1 and STAT2 leading to their heterodimerization and association with IRF9. This complex, termed ISGF3, can translocate to the nucleus where it stimulates transcription of ISGs. ISG mRNA is then translated in the cytoplasm giving rise to potent anti-viral effectot proteins. HCV has been reported to subvert these innate immune defense mechanisms at several levels explained in detail in the text.

(Figure – Courtesy of Paul Kelly, Trinity College, Dublin).

JAKs and STATs Proteins

In mammals, there are four main JAK proteins [JAK1-3 and TYK2)]. These tyrosine kinase proteins become activated after interaction between the IFN ligand and its receptor. JAK proteins contain seven JAK homology domains (JH1-7) which are widely expressed in all cell types, except for JAK3, which is found exclusively in cells of myeloid and lymphoid origin. The JH1 domain is important for the JAK enzymatic activity. Phosphorylation of JH1 tyrosine residues leads to JAK conformational changes in the protein to facilitate binding of IFN receptors. JH2 is a pseudokinase domain as it lacks a functional enzymatic activity, but is most likely involved in regulating the activity of JH1. JH3 and the C-terminal portion of JH4 are Src-Homology-2 (SH2)-like domains. The N-terminal end of JH4 up to the JH7 of JAKs form the FERM domain, which is also involved in cytokine receptor binding [60].

In mammals, 7 STAT proteins have been identified (STAT1-4, 5a, 5b and 6). All members of the STAT family share 6 structurally conserved domains: N-terminal (NH2), coiled-coil, DNA Binding (DBD), linker (link), SH2 and the Transcriptional Activation Domains (TAD). The NH2 domain stabilises inactivated STAT proteins, whereas the coiled-coil is involved in nuclear import. The DBD mediates STAT binding to ISRE or GAS elements, while the linker domain acts as a bridge between the DBD and the SH2 domain. The SH2 domain recognises phosphorylated proteins on cell membrane receptors and promotes homo- and/or hetero-dimer formation. Finally, the TAD domain contains the STAT phosphorylation sites (tyrosine and serine residues) activated during IFN signalling [60,61].

To date, STAT1 and 2 are the best characterised members of the family in relation to IFN-α signalling and are primarily involved in the canonical pathway. STAT3 may also play an important role in IFN-α-mediated anti-viral immunity: recent evidence has shown that HCV evades IFN-α mediated antiviral activity by directly promoting STAT1 and STAT3 protein degradation both in vitro and in immune cells from HCV infected patients, suggesting a fundamental role of the JAK/STAT pathway in mediating viral infection [62].

Once in the nucleus, STAT proteins interact with several transcriptional co-activator proteins, whose recruitment depends on STAT phosphorylation state. These include p300 and cAMP-responsive-element-binding protein Binding Protein (CBP), which possess histone acetyltransferase activity and are important for the regulation of chromatic remodelling that increases IFN-α or IFN-γ-dependent transcription, and Minichromosome Maintenance deficient 5 (MCM5), which is essential for STAT1 target gene transcription [63-67]. HCV NS3/4A and core proteins have been shown to target the JAK/STAT pathway by inhibiting STAT1 phosphorylation at serine 727 and by interacting with the STAT1 SH2 domain, respectively [68,69]. Protein Phosphatase 2A (PP2A)-dependent STAT1 hypomethylation is also induced by HCV, with consequent decreased STAT1 DNA-binding and increased STAT1 association with the negative regulator Protein Inhibitors of Activated STATs (PIAS) [70].

Negative Feedback Controls of IFN Signalling

Negative feedback of inflammatory signalling pathways plays a pivotal role in controlling immune responses and preventing autoimmunity. The JAK/STAT pathway is negatively regulated by PIAS, Suppressor of Cytokine Signalling (SOCS), the Ubiquitin Specific Peptidase 18 (USP18), and the T cell Protein Tyrosine Phosphatase (TcPTP).

SOCS proteins are induced in response to JAK-STAT activation, and prevent further STAT activation by a variety of mechanisms, including binding to the receptors and/or JAKs, thus inhibiting receptor phosphorylation and subsequent STAT recruitment. SOCS proteins also promote proteasomal degradation of JAKs [71,72]. In particular, SOCS1 and SOCS3 are induced in response to Type I IFNs, and are important regulators of the anti-viral response [73]. Interestingly, SOCS3 overexpression has been shown to suppress HCV replication in vitro in an mTOR-dependent manner, and, most recently, SOCS1 have been found to promote HCV replication by blocking the anti-viral activity of IFN [74,75], suggesting that both proteins act via alternative mechanisms regarding regulation of IFN activity. However, HCV has been shown to reduce SOCS1 gene expression and upregulate SOCS3 [76-78]. This mechanism employed by HCV might represent a crucial step in HCV infection pathogenesis, including suppressed inflammation and even hepatocellular cancer progression.

PIAS1 and PIAS3 proteins specifically inhibit STAT protein transcriptional activity by binding to activated STAT1 and STAT3, respectively, preventing STAT/DNA binding, thus preventing ISGs expression [79]. Upregulation of PIAS3 has been reported in HCV-infected heroin users [80]. USP18 (also known as UBP43) was initially identified as the specific protease for ISG15 cleavage, and specifically binds to the IFNAR2 receptor subunit of IFN-α, thus inhibiting JAK1/IFNAR2 receptor interaction [81]. Finally, TcPTP dephosphorylates activated STAT1 and STAT3, but a role in JAK inactivation has been also documented [82-84].

Anti-viral Effector Genes of HCV

Activation of IFN signalling can promote the expression of over 500 genes. Type I IFNs upregulate hundreds of ISGs involved in direct anti-viral effects, innate immune signalling, metabolism and apoptosis [85]. Type III IFNs stimulate expression of a similar set of ISGs, despite signalling through distinct receptor types [86]. To date, several ISGs targeting each step of the HCV life cycle have been identified, either preventing or promoting virus replication [87-89]. This was achieved using in vitro hepatic cell lines, due the lack of a suitable small animal model with full HCV permissiveness. The exact role of the majority of these genes in modulating HCV replication and vice versa is still unknown, although some of these ISGs with potent anti-viral activity have been characterised, such as Protein Kinase R (PKR), 2'-5' Oligoadenylate Synthetase (OAS), Myxovirus resistance gene A (MxA), and Interferon Stimulated Gene 15 (ISG15).

PKR

Upon binding to dsRNA, PKR becomes activated by autophosphorylation. Fully functional PKR subsequently deactivates the eukaryotic initiation factor-2-α (eIF2-α) by phosphorylation of the α subunit, blocking protein translation of host mRNA, including ISGs. However, HCV uses this mechanism for its own advantage; as RNA translation occurs via an Internal Ribosomal Entry (IRES), viral protein production is not affected by eIF2-α blockage [90]. HCV E2 and NS5A protein can also bind to PKR protein kinase catalytic domain, rendering this kinase unable to block protein translation and therefore viral replication [91,92].

2'-5'- OAS

As with PKR, the three OAS proteins (OAS1, OAS3, and OASL) are activated by dsRNA and trigger oligoadenylate synthetase/ribonuclease L (RNase L)-dependent cleavage of viral RNA and, interestingly, host RNA in single stranded regions, preferably after UU or UA dinucleotides. These cleaved RNA products can further activate cytosolic PRRs, such as RIG-I and MDA5, thus enhancing Type I IFN signalling response [93-95]. Similar to PKR, the HCV NS5A protein can inhibit OAS anti-viral activity by binding to the N-terminal region of the protein [96].

MxA

MxA is involved in vesicle trafficking and is known to prevent replication of many types of viruses by binding and wrapping around viral nucleocapsids, rendering them incapable of replication [97]. The exact mechanism involved in HCV replication is not fully understood; however, recent evidence showed that the HCV core protein specifically colocalises with MxA in a granular pattern in the cell cytoplasm, and this is enhanced by treating cells with Ribavirin and IFN-α [98].

ISG15

ISG15 is an ubiquitin-like molecule that targets protein in a mechanism called "ISGylation", which involves sequential activation of an E1-activating, an E2-conjugating, and an E3 ligation enzyme. ISGylation alters protein property directly by addition of ISG15 and reduces target protein degradation by competing with ubiquitin conjugation. Although ISG15 promotes protection against most viruses, HCV seems to represent the exception to this rule. ISG15 can ISGylate a wide variety of proteins, including PKR and RIG-I, inducing a proviral state within the host cell during very early stages of HCV infection [99,100], and it can also promote HCV virus production in in vitro infection models [101].

Natural Killer Cells

Another component of the innate immune system, Natural Killer (NK) are very important in the primary immune response against viral pathogens [102], eliminating virally infected cells both directly via cytolytic mechanisms and indirectly by secreting cytokines such as IFN-γ [103]. NK cells also demonstrate regulatory and reciprocal interactions with T and B cells, DCs, macrophages and endothelial cells, thus functioning to amplify or attenuate immune responses [104].

In HCV infection NK cells are thought to be necessary for optimal priming and cytolytic function of virus specific T cells due to production of IFN-γ [105]. Cytokine-stimulated NK cell lines and primary NK cells isolated from healthy donors have the ability to lyse HCV replicating cells [106,107].

It has been reported that NK inhibiton can occur through binding of HCV viral envelope protein E2 [108]. This binding results in decreased cytotoxicity and IFN-γ production, and can be regarded as a strategy to establish HCV as a chronic infection. It has also been shown that HCV NS5A protein, through monocyte-derived Transforming Growth Factor-β (TGF-β) production, down-regulates expression of NKG2D on NK cells, thus reducing their cytotoxic potential and IFN-γ production [109]. Another mechanism by which HCV avoids recognition by NK cells is through HCV core protein inducing p53-dependent gene expression of TAP1 (Transporter associated with Antigen Processing 1) and consecutive Major Histocompatibility Complex (MHC) class I up-regulation. Elevated MHC class I levels following HCV core protein expression induce negative signals that lead to inhibition of NK cytotoxicity [110].

Adaptive Immune Responses

The adaptive immune system plays a central role in pathogenesis and outcome of disease in patients with HCV infection. The mechanisms involved in this specific immune response, as well as counter measurements taken by the virus to evade this response will be described.

B Cells and Humoral Immmunity

The humoral component of the adaptive immune response makes use of viral specific antibodies produced by B cells. Typically anti-HCV antibodies may be detected 50-60 days after HCV infection [111]. However, these antibodies only signify a humoral response to

HCV proteins and do not evaluate the ability of these antibodies to neutralise HCV infection. Only a small fraction of antibodies are able to inhibit virus binding, entry or uncoating. These antibodies may potentially block HCV infection and are termed 'neutralising antibodies'. The HCV envelope glycoproteins E1 and E2 are important targets for virus neutralisation as their interaction with host cell factors is required to initiate productive infection [112].

Neutralising antibodies are generally considered an important mechanism for the control of initial viraemia and protection from reinfection in viral infections. In HCV infection, the role of neutralising antibodies in mediating viral clearance remains controversial. The recognition that hypogammaglobulinemic humans can spontaneously eradicate HCV suggests that antibody responses may be dispensible [113].

Studies investigating immune responses in chimpanzees and humans suggest that HCV clearance can occur in the absence of neutralising antibodies or those antibody responses alone are insufficient to eradicate HCV in the majority of cases [114-116]. Additionally HCV infection does not elicit protective immunity against reinfection [117]. However chimpanzees and humans who have cleared HCV spontaneously appear to be less likely to develop chronic infection after re-exposure [118,119]. A study of healthy young women infected with an identical, single source viral inoculum demonstrated that early induction of neutralizing antibodies was seen in individuals with viral clearance. By contrast, in individuals with viral persistence, absent or low-titer neutralizing antibodies were observed in the early phase of infection. Persistence of infection occurred in these individuals despite the induction of cross-neutralizing antibodies in the late phase of infection [120].

In chronic infection, HCV-specific neutralizing antibodies may be detected in most patients. Multiple mechanisms for the failure of the humoral immune response have been suggested. In infected individuals, HCV exists as a quasispecies i.e. a pool of constantly changing, distinct, but related genomic variants. Evolution of the quasispecies within targeted epitopes, such as the Hypervariable Region 1 (HVR1) of the Envelope 2 (E2) protein, may lead to escape from neutralizing antibodies [121]. It has been shown that the interplay of HVR1, High-Density Lipoproteins (HDL) and the Scavenger Receptor (SR-B1) can prevent the effect of neutralizing antibodies, enhancing cell entry of HCV and infection [122]. HVR1 may play a more general role in mutational escape by serving as a decoy for neutralizing antibodies. In this way it may protect less mutable, but functionally important epitopes [123]. HVR1 influences the biophysical properties of released viruses and this domain is particularly important for infectivity of low-density particles. HVR1 obstructs the viral CD81 binding site and protects conserved neutralizing epitopes [124].

The interaction between non-neutralizing and neutralizing antibodies may play a key role in determining the outcomes of HCV infection. Binding of non-neutralizing antibodies to specific epitopes results in protection against neutralizing antibodies [125]. Specific glycans have been identified on E2 responsible for modulating cell entry and protection against neutralizing antibodies [126]. Finally, recent work has demonstrated the ability of HCV to escape neutralization via direct cell-to-cell transfer of the virus [127,128].

T Cell Responses

The majority of acute infections with HCV are asymptomatic and often unrecognised. Therefore, in vivo studies of T cell immune responses during acute infection have only been possible in experimental chimpanzee models or indiviudals with an identifiable exposure for which the time of infection may be documented.

The hallmark of HCV viral clearance is a robust and broad specific T cell response. By contrast a weak and narrowly focussed T cell response is observed in chronically infected HCV individuals [129,130]. An efficient and vigourous T cell response during the acute phase is typically seen in individuals with spontaneous clearance of infection [131]. In resolved HCV infection, circulating HCV specific antibodies decline over a period of decades and may often become undetectable, while functional CD4+ and CD8+ T cells are maintained for decades [132]. These T cells substantially decrease the risk of persistent infection upon re-exposure to HCV [118]. In individuals who go on to develop chronic infection, a transient or absent primary T cell response is observed [129].

The exact mechanisms underlying the cellular immune evasion capacities of HCV remain incompletely understood. Impairment of the interactions between antigen-presenting DCs and T cells may result in an impaired cellular response. It has been shown that a decrease and dysfunction of DCs correlates with impaired HCV-specific CD4+ T-cell proliferation in patients with HCV infection [133]. Individual HCV proteins, core, NS3, NS4, NS5 as well as fused Polyprotein (Core-NS3-NS4) were found to impair functions of both immature DCs and mature DCs on multiple levels including reduced IL-12 secretion, induction of Fas ligand expression to mediate apoptosis and inhibition of TLR signalling [134]. Also, HCV interactions with DC-specific receptors DC-SIGN and DC-SIGNR may contribute to the establishment or persistence of infection both by the capture and delivery of virus to the liver and by modulating DC function [135].

CD8+ T cell dysfunction

It has been suggested that dysfunction of CD8+ T cells, the primary effector cells that mediate viral clearance through the secretion of antiviral cytokines, is a major determinant of viral persistence [136,137]. T cell exhaustion results in the inability to proliferate in response to antigen and the failure to produce cytokines such as IFN-γ. T cell exhaustion is recognized in other viral infections such as HIV and is characterized by high levels of expression of inhibitory receptors such as protein-Programmed Death 1 (PD-1). It has been shown that large proportions of HCV-specific CD8+ T cells express high levels of PD-1 [137] and that these cells are prone to apoptosis [138]. Not all dysfunctional T cells express PD-1, nor are they all rescued by blockade of the PD-1/PD-1 ligand pathway and as a result a number of emerging molecules and pathways have been implicated in mediating the T cell exhaustion characteristic of chronic viral infection, including T-cell immunoglobulin and mucin domain-containing protein 3 (Tim-3) [139] and the receptor 2B4 (CD244) [140].

Factors potenitally leading to T cell exhaustion include chronic antigen stimulation, dysfunction of CD4+ T cells or the action of regulatory T cells or cytokines.

CD4+ T cells

While CD8+ T cells are the major effector cells against viral pathogens, the presence of CD8+ T cells that can proliferate, exhibit cytotoxicity, and produce IFN-γ does not ensure recovery. Mouse models have shown that CD4+ T cells play an important role in sustaining virus-specific CD8+ T cells [141]. A critical determinant of outcome in HCV is whether these CD8+ T cells were primed in the presence or absence of CD4+ T-cells [142]. In patients with chronic infection, failure of IL-2 secretion, as opposed to physical deletion or complete functional unresponsiveness, appears to be an important determinant of the status of CD4+ T cell populations in chronic

HCV infection [143].

Regulatory T Cells

Different T cell subsets with suppressive functions have been described. Among these are regulatory T cells (Tregs), a subpopulation often identied during chronic HCV infection. They are identified by the constant expression of factor Forkhead box P3 (FoxP), CD4+ and CD25+. The signature of the Tregs is their potent ability to suppress the effector cells. In chronic HCV infection Tregs have been found at a higher frequency when compared with resolved HCV infection [144]. Research into protein-derived peptides from HCV and the antigen specificity of Tregs showed that only a few peptides, with overlapping regions, were able to stimulate Tregs [145]. This indicates that there could be a dominant region of the core of HCV capable of activating Tregs, thereby suppressing the immune response. Evidence against Tregs in promoting the development of chronic infection was recently reported in a prospective study of 27 acutely infected patients. In fact, no significant difference was seen in the proportion of CD4+CD25+high T cells in the peripheral blood at baseline between the individuals who cleared infection and those who developed chronic infection [142].

Viral Mutation

One method used by many viruses to evade the immune system is that of viral mutation. The estimated replication rate of HCV is 1012 virions per day [146]. The error-prone RNA polymerase that HCV uses for replication results in minor virus variants with the potential for immune evasion. As the replication rate for HCV is so high, this gives rise to quasispecies of HCV in each infected individual [147]. The most conserved region of the HCV genome is the 5' non-coding region demonstrating 90% homology with other viral strains [148]. The most variable part of the genome encodes the envelope proteins, E1 and E2 [149]. Within these proteins lie the hypervariable regions, HVR1 and HVR2, regions that are very 'mutation prone'. Two kinds of mutation can occur during viral replication. Silent or synonymous mutations do not result in a change to the amino acid sequence of a protein, or result in the insertion of an alternative amino acid with similar properties to that of the original amino acid, and in either case there is no significant change in phenotype. Non-synonymous mutations lead to amino acid changes in the virus that may leave it less fit or may even be lethal to the virus.

It has been shown that during chronic infection HCV is subjected to selection pressures from both humoral and cellular immunity, resulting in the continuous generation of escape variants [123]. If a mutation is benefical to the virus, it will adopt this mutation allowing it to escape the immune system [150]. The altered peptide ligands that arise may even down-regulate the T cell response against original ligands [151].

HCV is in a constant search to optimise its genome, without imparing function, and with a mutation rate of approximately $1.92x10^{-3}$ base substitutions per genome site per year [152], it makes use of a very capable system to evade the immune response.

Conclusion

To summarise, it appears many factors contribute towards HCV persistence and HCV has developed a variety of overlapping immune evasion mechanisms, targetting both the innate and adaptive immune response, that contribute to preventing viral clearance in the majority of individuals. Ongoing research will likely identify new mechanisms of immune evasion in the future that will be fundamental to our understanding and development of novel strategies to prevent and control HCV infection.

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Innovations in Hepatitis C Therapy

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Abstract

Until 2011, there was a standstill in the development of new, more efficient therapies for hepatitis C virus (HCV). For over 20 years, clinicians were using interferon or peginterferon alfa with ribavirin with moderate to poor responses. The elucidation of the lifecycle of HCV within hepatocytes is providing us with much more effective antiviral treatments. In this chapter, we provide an overview of HCV therapies, background of traditional HCV therapies and insight into the newly approved HCV protease inhibitors and the ongoing studies on polymerase and cyclophilin inhibitors.

Keywords: Antiviral therapy; Hepatitis C virus; Hepatology; Infectious diseases

Introduction

Since the discovery of hepatitis C virus (HCV) in 1989, approximately 170 million people are now affected worldwide, with the majority of affected individuals unaware of their condition [1,2]. The Centers for Disease Control and Prevention (CDC) recommends one-time HCV testing for all those born between 1945 and 1965because of the increased prevalence of HCV in this demographic group and high incidence of HCV-associated decompensated liver cirrhosis, hepatocellular carcinoma (HCC), and HCV-related deaths [3].

Until 2012, the recommended therapy for all HCV genotypeswith detectable HCV RNA viral loads >50 copies/mL, a liver biopsy with portal or bridging fibrosis, and at least moderate inflammation and necrosis was pegylated interferon alfa (p-IFN) and ribavirin (RBV) [4].Treatment was expensive, poorly tolerated, and had variable sustained virologic responses (SVR) amongst the different viral genotypes (80% in genotypes 2 and 3, 45% in genotype 1). The approval of direct-acting antiviral protease inhibitors (PIs) improved SVRin genotype 1 HCV (HCV-1) and gave us a promising future and potential cure of HCV.

Early virologic response infers a reduction of 2-$\log_{10}$ IU/mL or greater at week 12 of therapy. Even sooner virologic responses, including undetectable viral load at week 2 (very rapid virologic response) and week 4 (rapid virologic response) of therapy are positive predictors of SVR. Therapy is futile and therefore discontinued in patients with a null response, defined as lower than a 2-$\log_{10}$ IU/mL reduction in HCV RNA at week 12 of therapy. Futility rules also recommend stopping therapy in cases of a partial response, which is defined as a 2-$\log_{10}$ IU/mL reduction at 12 weeks yet persistently detectable HCV RNA at 24 weeks of therapy. Relapse is defined as an undetectable HCV viral load throughout therapy, yet detectable viral load upon post-therapy follow-up testing. Breakthrough response is defined as an undetectable viral load followed by increase to 100 IU/mL or 1-$\log_{10}$ IU/mL during therapy.

Interferon Alfa

Interferon alfa is a synthetic production of the natural cytokine, interferon, produced by the immune system in response to viral infections. Interferon alfa has several mechanisms of action, including immune system enhancement, activity against antiviralreplication, and protection of uninfected cells. In 1991, the Food and Drug Administration (FDA) approved interferon alfa for the treatment of chronic hepatitis C [5]. The typical protocol of 3 million units three times a week for 48 weeks achieved an SVR of approximately 9% for genotype-1 HCV (HCV-1) and 30% in genotypes 2 (HCV-2)and 3 (HCV-3). Though interferon alfa-2a (Roferon A®) and consensus interferon (Infergen®) were later approved in 1996 and 1997, respectively, SVR still only ranged from 7-20% [6]. The trice daily frequency of interferon led to a major barrier in compliance.

Polyethylene glycol (PEG), an inert molecule with no independent antiviral activity, was attached to the recombinant forms of interferon to form pegylated interferon (p-IFN). Pegylation increased the half-life of interferon and allowed for once weekly dosing. This positively impacted patients with poor compliance to the trice daily interferon. The weekly subcutaneous injection forms, p-IFN alpha-2a (PEG-intron)and p-IFNalfa-2b (Pegasys), improved tolerance of interferon and reduced hepatic inflammation, thereby enhancing the efficacy and SVR rates of the regimen [5,7]. PEG-intron was FDA approved in 2001 [8]. It improved SVR to 14% in HCV-1 and 47% in HCV-2 and HCV-3 [8,9]. Pegasys attained FDA approval in 2002 and studies showed an SVR of 28% for HCV-1 and 56% for HCV-2 and HCV-3 [10]. Subsequently, studies demonstrated a higher response rate with the combination of p-IFN with RBV, which improved SVR to 41-51% in HCV-1 and 70-82% in genotypes 2 through 6 [11-13]. Despite all the incremental improvementsin SVR, patients with HCV-1co-infectionwith human immunodeficiency virus (HIV), elevated HCV viral load, and/orAfrican American race still had lower rates of SVR ranging from 7-29% [11-16].

The most commonly reported side effect associated with p-IFN is fatigue [17]. Other adverse effects include headache (42%), nausea (36%), insomnia (41%), bone marrow suppression (31-34%), fever (23%), rash (28%), and depression (21%) [8,17,18]. Serum thyroid

stimulating hormone levels should be check before, during and post-therapy as both hypothyroidism and hyperthyroidismcan occur. Clinicians should note particular attention to the potential neutropenia, thrombocytopenia and depression. Interferon dosage should be reduced when the absolute neutrophil count falls below 750/uL or the platelet count falls below 80,000/uL. Interferon therapy should be discontinued if the absolute neutrophils count falls below 500/uL or the platelet count falls below 50,000/uL. Figastrim may be used to treat the neutropenia caused by interferon. Prior to IFN initation, patients should be screened for depression and initiated on antidepressant therapy (usually selective serotonin reuptake inhibitor) if necessary [19]. Due to IFN's toxicity and poor tolerability, 24.5% patients fail to complete therapy [20]. This has increased the need for IFN-free regimens, which are currently under investigation.

Ribavirin

Ribavirin is a nucleoside inhibitor used to halt HCV viral RNA synthesis and viral mRNA capping [21]. Due to its' limited effect on HCV viral load and relapse elevation in transaminase levels within weeks of therapy cessation, RBVshould not be used as monotherapy. Therefore, ribavirin was combined with p-IFN to improve SVR [11,12,22-25].

The limitations to p-IFN/RBV include a poor SVR, tolerability, lengthy treatment duration, and toxic profiles. The major adverse effect to be noted with RBV is a 9-36% excess risk of dose-dependent hemolytic anemia, requiring a dose-reduction or withdrawal of ribavirin [26,27]. A hemoglobin reduction below 10g/dl is seen in up to 20% of patients and 8.5 g/dl in 5% of patients [19]. A routine laboratory test, especially in patients with coronary artery disease, chronic obstructive pulmonary disease or HIV, is recommended every 2-4 weeks while on RBV [11]. Epoetin alfa may improve hemoglobin levels and enable adequate dosages of ribavirin to be maintained [27]. A negative pregnancy test must be confirmed prior to initiation of therapy due ribavirin's teratogenicity and embryocidal effects. Two modes of contraceptive therapy should be implemented and continued for 6 weeks post-therapy. Ribavirin should also be avoided in patients with advanced liver or kidney disease, unstable heart disease, autoimmune hepatitis and women actively breastfeeding. Drug-drug interactions must be noted prior to initiation of ribavirin.

Protease Inhibitors

In May 2011, the FDA approved highly potent first-generation NS3/4A DAA protease inhibitors, telaprevir and boceprevir, for treatment-naïve and treatment-experienced HCV-1patients [28]. This included patients with HCV-related compensated liver disease, including liver cirrhosis [29].These agents inhibit HCV NS3/4A serine protease, necessary for the proteolytic cleavage of the HCV encoded polyprotein into mature forms (NS4A, NS4B, NS5A and NS5B) essential for viral replication. Second-wave PIs, includingsimeprevir (formerly TMC-435), and faldaprevir (formerly BI-201335) are presently in phase III trials [25,28-30].

Due to HCV's high genetic variability, variants with lower susceptibility to DAAs can occur naturally prior to even starting therapy, thus giving DAAs a low barrier for resistance when given as monotherapy [31]. Co-administration with p-IFN/RBV helps to prevent resistance and virologic breakthrough and hence, current guidelines recommend the use of DAAs in combination with p-IFN and RBV. In HCV-1 monoinfected patients, the addition of these agents in conjunction with p-IFN/RBV has significantly improved SVRfrom 40% to 70% [14,15,32-34]. Ongoing studies using PI are also showing improved SVR rates in patients co-infected with HIV [35]. Telaprevir and boceprevir are primarily active against HCV-1 with some activity against HCV-2 and HCV-4. The new PIs under investigation are showing promising activity against all HCV genotypes. Ongoing studies using shorter courses (6 and 12 weeks) of DAA therapy are showing promising SVR rates. Shorter course regimens, if approved, will be particularly useful in patients with compliance issues [36,37].

Patients with prior null or partial virologic response are unlikely to achieve SVR even with DA-based therapy. Furthermore, developments of resistance substitutions while on therapy are possible and therapy should be discontinued unless adequate response is achieved.If p-IFN/RBV is discontinued for any reasons, PIs should be discontinued as well. Telaprevir has not been studied in patients with end-stage renal disease. It is not recommended in patients with moderate-severe hepatic impairment (Child-Pugh B or C, score ≥7) or decompensated liver disease.

Adverse effects associated with telaprevir include rash (56%), fatigue (56%), anemia (36%), pruritis (47%), nausea (39%), and diarrhea (26%) [30]. Adverse effects associated with boceprevir include anemia (45-50%), neutropenia (14-25%), nausea (43-46%), dysgeusia (35-44%), and diarrhea (35%) [38]. Black box warnings are present when using telaprevir or boceprevir with p-IFN/RBV in patients who develop severe exfoliative skin diseases (i.e. Stevens Johnson syndrome, drug reaction with eosinophia and systemic symptoms (DRESS), or toxic epidermal necrolysis) Drug-drug interactions are also potential barriers for HCV PIs due to their potent induction of CYP3A [30,39].

NS5A Inhibitors

NS5A is a protein that forms the HCV replication complex with NS3-NS5B, responsible for mediating interferon responses and inhibiting apoptosis [40]. Several NS5A inhibitors are currently undergoing clinical trials, including daclatasvir (formerly BMS-790052), which has demonstrated potent inhibition of NS5A and HCV replication. Clinical trial with daclatasvir and p-IFN/RBV combination therapy improved SVR from 25% to 92% of patients with HCV-1 [41]. Adverse events noted with daclatasvir were similar to those seen in the pIFN/RBV background group. Other NS5A inhibitors include BMS824393 (used as monotherapy [42]), ACH-2928, PPI-461, and AZD-7295. Studies involving second-generation NS5A inhibitors show more potent inhibition against HCV-1 and HCV-2 than first-generation NS5A inhibitors with higher barriers for resistance and better safety profiles, further illustrating a promising clinical utility in HCV management [43].

Polymerase Inhibitors

Given the essential role of RNA-Dependent RNA polymerase NS5B in HCV replication, developments have been made to target these allosteric and active binding sites [44]. Two classes of NS5B polymerase inhibitors are available: nucleos(t)ide analogues (i.e. sofosbuvir) and non-nucleoside polymerase inhibitors (i.e. BI 207127, filibuvir, VX-222). These agents have a broader genotypic coverage with a higher genetic barrier to resistance than first-generation DAA protease inhibitors. Unlike first-generation HCV NS5A inhibitors in which preexisting resistant variants have been identified in about 16% of patients, there are no preexisting resistant variants to nucleos(t)ide NS5B polymerase inhibitors to date [45]. Furthermore, NS5B inhibitors have improved adverse effect profiles and are potent enough to be given once daily, allowing for potential p-IFN-sparing regimens. Although trials are still currently being undertaken, >90% of patients with

HCV-1 and HCV-3 have achieved SVR with sofosbuvir combination therapy [46,47]. Studies involving nucleotide polymerase inhibitors were halted due to development of series adverse events, including cardiac, renal toxicity (BMS-986094), hepatotoxicity (GS-938) [48].

Cyclophilin Inhibitors

In addition to DAA's, cyclophilin inhibitors are providing a novel advance to HCV therapy. Cyclophilin (Cyp) inhibits HCV polyprotein processing, prevents viral replication, interferes with NS5B polymerase activity and neutralizes HCV activity [49]. Unlike DAAs, oral-based Cyp-inhibitors including alisporivir, NIM-811 and SCY-635, target the host protein and provide support when treatment resistance or virologic breakthrough occurs [49,50]. Although these agents are still undergoing clinical trials and experience with these agents is scarce, they offer a new strategy for augmenting the antiviral activity of HCV.

Resistance to HCV Antiviral Therapy

Hepatitis C virus has a rapid replication and virion clearance rate. NS5B polymerase, which is essential in HCV replication, lacks proofreading capabilities [51]. This causes a high genetic diversity amongst HCV virions, which is a prerequisite for selection of resistant strains and viral breakthrough during DAA-based therapy [32,52]. This rapid emergence of resistant wild-type mutant variants was seen when HCV PIs telaprevir and boceprevir were used as monotherapy for 2 or more weeks [45]. *A*) and consensus* and *In-vivo* variants with various resistance levels have been described with DAA monotherapy, including the R155K/T and V36A/M substitutions seen in HCV-1a and A1566V/T mutations seen in HCV-1b. Resistant variants were detected in 50-75% of phase III telaprevir and boceprevir treatment-naïve patients who did not achieve SVR.

While HIV and hepatitis B generate intermediate DNA during their replication cycle, HCV replicates in the cytoplasm directly via production of negative RNA strands. Therefore, archived resistance-associated variants cannot occur, as DNA and long-term survival of resistant variants are unlikely to occur. Unlike HIV, there is no biologic basis for archiving of PI-resistant variants in the body. In fact, telaprevir resistant variants converted to wild-type virus in 96% of patients' in less than 16 months after stopping therapy [53]. Detectable DAA resistance mutations occur at very low frequencies (<5%) and become undetectable ata median of 1-2 years [54].

The two FDA-approved DAAs are completely cross-resistant, resulting in limited clinical utility as an alternate agent when one fails. There are currently no evidence-based recommendations for routine baseline resistance testing in patients being treated with DAA-therapy.In general, the most ideal way to prevent the emergence of resistance-associated mutations is a rapid, intense viral suppression via potent antiviral combination therapy of drugs that possess a high barrier to resistance without cross-resistance and diverse mechanisms of action.

HIV and HCV Co-Infection

The prevalence of chronic HCV patients co-infected with HIV is about 25-30%, likely secondary to the shared modes of transmission (i.e. intravenous drug use, men who have sex with men) [55,56]. The pathogenesis of fibrosis, end-stage liver disease, HCC and HCV-related death is also accelerated in patients with HIV/HCV co-infection [57,58]. Other than issues with tolerability and toxic profiles, limitations in HIV/HCV co-infection therapy include poor rates of SVR (especially in HCV-1 and/or African American patients) and low rates of treatment initiation. Since both first-generation FDA-approved DAAs are substrates and inhibitors of cytochrome p-450 isoenzyme 3A4, there are significant druginteractions with HIV PIs and non-nucleoside reverse transcriptase inhibitors. The addition of HCV PIs to p-IFN/RBV in HIV/HCV co-infected patients improves SVR from 40-70% in patients with HCV monoinfection and 29-70% in patients with HIV/HCV-1 co-infection [14,15,33,34,55]. Though data is still limited,studies are still being undertaken, and current use of DAAs in co-infected patients are off-labeled, the limited experiences with DAA-based therapy for HIV/HCV co-infection, including patients with HCV-1, shows a promising rise in SVR without increased drug-drug interactions or unmanageable toxicities.

Summary

Unlike other chronic viruses such as HIV and hepatitis B, HCV can be eradicated with effective therapy. Patients who achieve early virologic response have a better chance of cure [59]. The addition of PIs has added a very promising paradigm to HCV therapy. With new, interferon-sparing, completely oral, shortened duration combination therapiesin the horizon, SVR will likely increase with concomitant reductions in the chances of developing liver cirrhosis, hepatic decompensation, HCC and need for liver transplantation. Clinicians should acquire training and become involved in these exciting and promising discoveries in medical therapy.

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HCV and Immune Molecules

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Abstract

Strong and sustained T-cell responses are essential for HCV clearance. T-cell responses are regulated by antigen-presenting cells among which dendritic cells (DCs) are the most potent professional antigen-presenting cells. In this chapter, the crucial immune molecules expressed on myeloid DCs (mDCs) and T-cells are discussed. T-cells and mDCs can express activating molecules, which facilitate T-cell proliferation and function and are beneficial for virus clearance. While the outcome of HCV infection depends on the interaction of HCV and host immune response, HCV has evolved multiple mechanisms to evade host immune responses by inducing changes in immune mechanisms. Thus, T-cells and mDCs of HCV infected patients can express up-regulated levels of inhibitory molecules and down-regulated levels of activating molecules, which facilitate T-cell apoptosis and anergy and finally lead to virus persistence. T-cells in patients with chronic HCV infection express higher level of programmed death receptor-1 (PD-1) compared to T-cells in healthy people. mDCs in these patients express higher levels of inhibitory molecules, the ligand 2 of PD-1 (PD-L2) and Fas ligand (FasL), and lower levels of activating molecules, human leukocyte antigen (HLA)-DR and CD86. The altered expression of these immune molecules during HCV infection plays a crucial role in determining the infection outcome.

T-cells and Dendritic Cells

This chapter is focused on immune molecules involved in the T cell and dendritic cell (DC) interaction, which plays a crucial role in HCV clearance.

Efficient activation of an immune response in humans depends on the interaction of antigen presenting cells (APCs) and T-cells. T-cells cannot directly recognize antigens. Instead, T-cells can only recognize and then respond to APCs in the context of the epitope-carrier molecules of the major histocompatibility complex (MHC) represented in man by human leukocyte antigen (HLA) class II molecules. Dendritic cells (DCs) are the most potent professional APCs [1].

In 1973, DCs were identified as a novel type of immune cells by Ralph Steinman and Zanvil Cohn at Rockefeller University. By observing cells from mouse spleen that adhered to glass and plastic surfaces, they found that there was a cell population with special microscopic properties. This cell population was distinct from mononuclear phagocytes, granulocytes and lymphocytes. The cell nucleus was large, refractile, contorted in shape, and contained small nucleoli. The abundant cytoplasm was arranged in varying length and width, and contained many large spherical mitochondria. These cells comprised about 1.0-1.5% of the total nucleated cell population observed. This cell population was subsequently named DCs [2]. For the discovery of the DCs and of their key role in controlling specific immunity, Ralph Steinman was awarded the 2011 Nobel Prize in Medicine [3].

There are two distinct subpopulations of DCs in human: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). The primary function of mDCs is the uptake, processing and presentation of antigens, while pDCs are the major producers of type I interferons [4]. Due to their important role in antigen presentation, mDCs are also referred to as conventional DCs.

DCs play a crucial role in regulating immune responses. This is because of their optimal positioning as sentinels in the periphery, their rapid migration to the draining lymph nodes, their ability to acquire and present antigens in MHC molecules and their high expression of the costimulatory molecules CD80 and CD86 [1,5,6]. In draining lymph nodes, antigen-bearing DCs interact with CD4 T-cells. The T-cell receptor (TCR) is triggered by HLA class II carrying the antigen peptide ("signal 1") and CD28 is engaged by CD80/CD86 ("signal 2") expressed on DCs. These interactions lead to clonal expansion of antigen-specific T-cells [7]. DCs, in a lesser fashion, can also express HLA class I and antigen peptide, which enable them to interact with CD8 T-cells. Although DCs can also cross present antigens to CD8 T-cells, the interaction between DCs and CD4 T-cells has been given more attention. This is because that strong and long-lasting CD4 –cell response is associated with HCV clearance.

T-cell responses determine the outcome of acute HCV infection. Strong and sustained T-cell responses are essential for HCV clearance. Acute HCV-infected patients with strong HCV-specific T-cell responses spontaneously clear HCV infection, while patients with impaired T-cell responses develop chronic hepatitis [8-13]. An important characteristic of HCV infection is that 70% to 80% of HCV-infected patients develop chronic hepatitis C [14-17].

Among human viruses, HCV is the most successful in establishing chronic infections. HCV has evolved unique mechanisms to block critical steps in the innate immune response [18]. Understanding the mechanism of HCV immune evasion is crucial for the design of effective strategies to control HCV.

Activating Molecules whose Expression are Altered during HCV Infection

Decreased expression of HLA-DR and CD86 on mDCs during chronic HCV infection

Clonal expansion of antigen-specific CD4 T-cells requires at least two signals sent from APCs by their expression of HLA class II ("signal 1") and CD80/CD86 ("signal 2") [7]. The major function of HLA class II molecules is to present processed antigens, which are derived mostly from exogenous sources, to CD4 T-cells and HLA class II molecules (DR, DP and DQ) are therefore important to

initiate an antigen-specific response [19]. If signal 2 from CD80/CD86 is lacking, antigen presentation leads to T-cell anergy, which is an important mechanism of immunologic tolerance [7].

Patients with chronic HCV infection have impaired T-cell responses against HCV [8-13]. Concomitantly, mDCs from such patients are impaired in their ability to stimulate T-cell proliferation [20]. This could be attributed, at least partly, to their decreased expression of HLA-DR and CD86, compared to the mDCs from healthy donors, potentially induced by the viral infection.

The decreased expression of HLA-DR and CD86 of mDCs from chronic hepatitis C patients will be responsible for decreased levels of signal 1 (HLA-DR) and signal 2 (CD86) from mDCs, and to activate CD4 T-cell responses [20], ultimately rather leading them to anergy as mentioned above.

NF-κB activity is diminished in mDCs from chronic hepatitis C patients

NF-κB is a transcription factor crucial for immune responses. NF-κB controls the expression of HLA-DR and CD86, and acts as an anti-apoptotic factor for mDCs [21-24].

NF-κB activity in mDCs from chronically infected HCV patients is diminished compared to that in mDCs from healthy donors. Furthermore, the decreased NF-κB activity is responsible for functional changes and increased apoptosis of mDCs in such patients [20]. Given the importance of mDCs in immune responses, NF-κB activity in mDCs might downregulate protective immune responses during HCV infection.

Serum level of B lymphocyte stimulator (BLyS) is increased in hepatitis C patients

BLyS, a molecule which is also termed B cell-activating factor belonging to the TNF family (BAFF), is a member of the tumor necrosis factor (TNF) superfamily [25-27]. BLyS was first reported to be a cytokine crucial for B-cell maturation, function, and survival [25-27]. BLyS-knockout mice demonstrate significantly reduced spleen weight, markedly reduced numbers of peripheral blood B cells, and a profound reduction in total serum immunoglobulins [27].

BLyS is predominantly produced by myeloid cells, including monocytes, macrophages, DCs and neutrophils [28,29]. Full-length BLyS molecule is expressed on immune cells as membrane-bound BLyS (mBLyS). mBLyS expressed on DCs has a co-stimulatory activity on both CD4+ and CD8+ T-cells at multiple stages, including naïve T-cells, recently antigen-primed T-cells, and memory T-cells [30,31]. The full-length mBLyS expressed on APCs provides a complete and potent second signal for T-cell activation, which leads to T-cell division and cytokine secretion [32,33].

After being cleaved by polyprotein convertases, the extracellular C-terminal fragment of the BLyS molecule, containing amino acids 134-285, is released as soluble BLyS (sBLyS) [25-27]. sBLyS is not capable of co-stimulating T-cell activation, however, it is still capable of regulating B-cell maturation, function, and survival [28,29].

mBLyS expression on mDCs is unchanged during chronic HCV infection, which indicates that mBLyS on mDCs may not be one of the molecules leading to impaired T-cell response during chronic HCV infection [34]. However, mBLyS expression on other immune cells is to be determined in future studies.

sBLyS levels in serum of HCV-infected patients are significantly higher than in healthy controls [35], and serum sBLyS level predicts the outcome of acute HCV infection [36]. sBLyS level is significantly increased in acute HCV-infected patients evolving to chronicity compared to those with a self-limited disease course, and thus a higher serum level of sBLyS is associated with persistence of HCV infection [36]. Patients with chronic HCV infection demonstrate extrahepatic manifestations, including mixed cryoglobulinemia, Sjogren's syndrome, B cell non-Hodgkin's lymphoma, and presence of auto-antibodies in sera [37]. The elevated serum level of sBLyS in these patients might contribute to these extrahepatic manifestations [35].

Inhibiting Molecules with Altered Expression during HCV Infection

The impaired CD4 T-cell responses to HCV are a defining feature of chronic hepatitis C [8,9,14,16]. Antigen-driven proliferation of CD4 T-cells is observed in patients who cleared HCV infection, but is inconsistently detected in patients who develop chronic hepatitis C. Chronic hepatitis C patients fall into two groups depending on HCV replication patterns during the first few months of infection. Patients in the first group are unable to mount an HCV-specific CD4 T-cell response and remain chronically infected. The second group of patients has strong HCV-specific CD4 T-cell activity and clear HCV RNA transiently from their serum. However, the CD4 T-cell activity weakens just before a rebound in viremia, which results in chronic infection [9,38]. The presence of killer DCs during chronic HCV infection possibly leads to the strong and then weakened CD4 T-cell in the second group of chronic hepatitis C patients [34].

DCs are the most potent professional APCs regulating T-cell responses [1,5]. Besides their well-known immunogenic function of stimulating T-cell proliferation, DCs also have an indirect tolerogenic activity by killing T-cells. Killer DCs express Fas ligand (FasL: CD178) [34,39-45], tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL: CD253) [46-49] and PD programmed death-1 (PD-1) ligand 2 (PD-L2: CD273) [34]. Killer DCs which have upregulated the expression of TRAIL can be found during measles virus (MV) [46,47] and HIV [48,49] infections. During HCV infection, DCs demonstrate upregulated expression of FasL and PD-L2, and are capable of regulating the apoptosis of T-cells [34].

Male patients have more aggressive HCV infection in general than female patients. Women tend to eliminate HCV more rapidly, have a lower rate of disease progression, and a lower mortality rate from HCV-related liver disease [50]. Although the mechanisms are not fully understood, killer DCs might explain this phenomenon. Male chronically infected HCV patients display more killing activity of mDCs than females. Indeed, mDCs from female chronically infected HCV patients do not demonstrate altered expression of FasL or PD-L2, and have a poor killing activity on T-cells [34].

Up-regulated expression of FasL on mDCs of chronic hepatitis C patients

DCs expressing Fas ligand (FasL: CD178) are referred to as killer DCs since they are capable of inducing T-cell apoptosis. CD4 and CD8 T-cells express Fas. The interactions of FasL/Fas lead to T-cell apoptosis [34,39-45]. Chronically infected HCV patients' mDCs demonstrate increased expression of FasL compared to healthy donor mDCs. Patients' mDCs are capable of killing both autologous and allogeneic CD4 and CD8 T-cells [34]. Of note, the FasL-expressing killer DCs in hepatitis C patients are not capable to kill T-cell lines which have poor TCR expression [34]. TCR interacts with HLA molecules and are crucial for the formation of the immunological synapse [51]. Formation of immunological synapse, which facilitates the cell-cell contact, is probably important for the FasL-Fas interaction between APCs and target T-cells and drives the specific killing of mDCs. At variance from FasL, however, the expression of membrane-bound TRAIL on mDCs is comparable in chronically infected HCV patients and healthy donors. The concentrations of soluble TRAIL (sTRAIL) in patient serum and in culture supernatants of patients' mDCs are also comparable to those of healthy donors [34]. This is different from what is seen in MV and HIV infections. MV induces TRAIL expression on DCs and these DCs induce the apoptosis of activated T-cells [46] and can kill tumor cells [47]. DCs represent a major target of MV. MV suppresses cell-mediated immunity by interfering with the survival and functions of DCs and T-cells [46]. TRAIL is not detected on the surface of DCs from MV infected patients. Therefore, cytotoxic activity of MV-infected DCs is mostly mediated by sTRAIL [47]. During HIV infection, killer DCs also produce TRAIL and induce the apoptosis of CD4 T-cell lines [48,49]. Stary et al. [49] have reported an upregulated expression of mTRAIL on pDCs during HIV infection. HIV infection is also associated with the upregulation of the apoptosis-transmitting receptor TRAIL R1 on activated CD4 T-cells and therefore these T-cells become more susceptible to TRAIL-dependent pDC-mediated killing. Moreover, pDCs have been reported to be killers of CD4 T-cells in HIV infection [49].

Up-regulated expression of PD-L2 on mDCs of chronic hepatitis C patients

There are two ligands for PD-1, PD-L1 and PD-L2. The two ligands differ in their expression, and PD-L2 may have stronger biological activity than PD-L1. Being expressed much more broadly than PD-L2, PD-L1 is present on multiple blood cells and a wide variety of non-hematopoietic cells. PD-L2 is only expressed on DCs, macrophages and bone marrow-derived cultured mast cells [52]. Blocking PD-L2 on DCs results in enhanced T-cell proliferation, while the proliferation induced by blocking PD-L1 is more modest [53]. Blocking PD-L1 has been shown to restore T-cell functions in HCV-infected patients [54]. PD-L2 expression on mDCs from chronic hepatitis C patients is however significantly higher than that seen on mDCs from healthy donors while the level of PD-L1 expression is the same [34]. PD-L2 could therefore be more important than PD-L1 to regulate CD4 and CD8 T-cell apoptosis during HCV infection.

mDCs from chronic hepatitis C patients are capable of inducing T-cell apoptosis. Of note, both healthy CD4 T-cells and patient mDCs demonstrate more apoptosis after co-culture [34]. This phenomenon indicates that there might be a negative feedback of immune response against HCV infection. DCs kill T-cells, which lead to apoptosis of DCs, and then there are fewer DCs to activate T-cell response.

Specific antibodies against TCR, FasL, Fas, PD-L2 and PD-1 all partially abolish the killing effect of mDCs during chronic HCV infection. Furthermore, transwell membranes almost completely block the killing of patient mDCs [34]. This indicates that the cytotoxic activity of mDCs from chronically infected HCV patients requires cell-cell contact, and is primarily mediated by interactions between FasL/Fas and PD-L2/PD-1.

Up-regulated expression of programmed death receptor-1 (PD-1) on T-cells of chronic hepatitis C patients

The killing effect of mDCs is mediated by PD-1 expression on target T-cells [34]. Being an important molecule regulating T-cell response, PD-1 is a potential target of virus immune evasion and thus also a potiential target for immunotherapy against viral infections, e.g. HCV [55,56] and HIV [57] infections.

CD4 T-cells from chronic hepatitis C patients have a higher expression of PD-1 and a higher level of spontaneous apoptosis compared to healthy donor CD4 T-cells. The increased expression of PD-1 on T-cells from chronic hepatitis C patients indicates that they are more sensitive to apoptosis compared to healthy donor T-cells [34]. Correspondingly, HCV-specific CD8 T-cells undergo significant apoptosis in the peripheral blood during acute HCV infection and in the liver during chronic HCV infection [58]. This indicates that PD-1 is an important molecule associated with significant functional T-cell deficits in chronic HCV-infected patients [34,58].

CD8 T-cells are important effector T-cells, which kill virus-infected cells and thus are critical for virus clearance. Two major mechanisms lead to the poor CD8 T-cell response in chronic hepatitis C patients: (1) mDCs in these patients can directly kill CD8 T-cells through Fas/FasL and PD-1/PD-L2 interactions [34]. (2) Patient mDCs impair CD4 T-cells as shown above [20,34], and thus can indirectly weaken CD8 T-cell responses since CD4 Th cells are critical for the initiation and maintenance of CD8 T-cell responses.

In summary, the ability of mDCs to stimulate T-cell proliferation is impaired in chronically infected HCV patients compared to mDCs from uninfected controls [20], and the ability of mDCs to induce T-cell apoptosis is upregulated in these patients [34] (Figure 1). The function of mDCs is switched from immunogenic to tolerogenic during chornic HCV infection (Figure 2). T-cells express a higher level of PD-1, and demonstrate increased susceptibility to the killing effect of mDCs [34]. These changes are mediated by immune molecules, and eventually lead to the immune evasion of HCV [59].

Taken together, mDCs from chronically infected HCV patients display phenotypical and functional changes. Immune molecules play a fundamental role in regulating T-cell responses, and thereby determine the outcome of HCV infection. Immunotherapy designed to recover a normal profile of immune molecules expression may rescue T-cell responses and help to clear HCV infection.

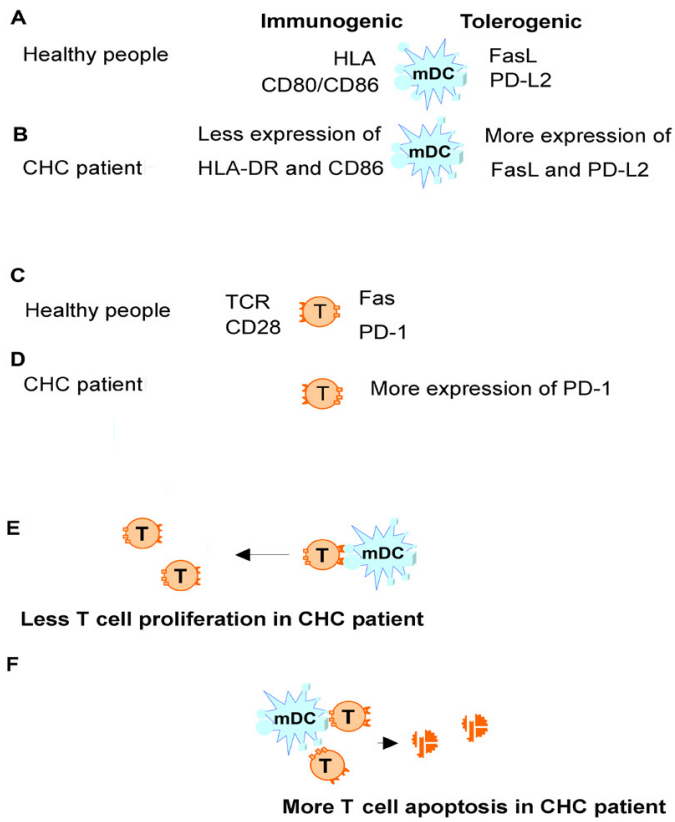

Figure 1: Model of mDC functions during chronic HCV infection [59].

mDCs in chronic hepatitis C patients express less activating molecules, HLA and CD86 [20]. mDCs in these patients express more inhibitory molecules, FasL and PD-L2 compared to mDCs in healthy donors [34] (A and B). CD4 T-cells in these patients have upregulated expression of the receptor PD-1 [34] (C and D).

mDCs in chronically infected HCV patients patients are less immunogenic in stimulating T-cell proliferation, and are more tolerogenic in inducing T-cell anergy and apoptosis [59].

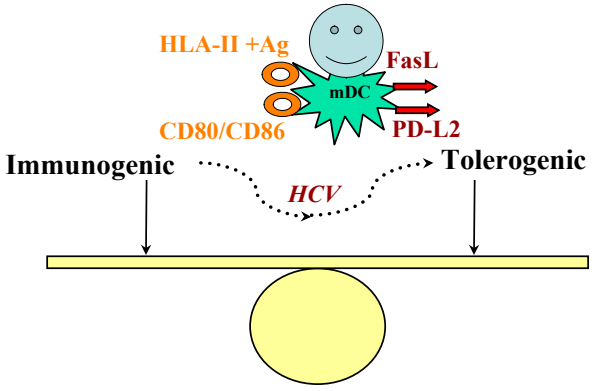

Figure 2: The altered expression of immne molecules in chronic HCV-infected patients facilitates the immune evasion of HCV.

The expression profile of immune molecules on immune cells regulates T-cell responses. On one hand, mDCs can be immunogenic and stimulate T-cell activation by expressing activating molecules (HLA-DR, CD86, etc.) [20]. On the other hand, mDCs can also induce T-cell anergy and apoptosis. Without enough 2nd signal (for example, CD86) for T-cell activation, mDCs provide the 1st signal only which leads to T-cell anergy [20]. Furthermore, mDCs can be tolerogenic by expressing inhibitory molecules such as FasL and PD-L2 [34]. During chronic HCV infection, the function of mDCs is switched from immunogenic to tolerogenic, which contributes to impaired T-cell responses and facilitates HCV persistence. The switch in DC function is a mechanism of HCV-induced immune dysfunction [59].

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Prediction of Treatment Response in Patients Infected with Hepatitis C Virus

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Keywords: HCV prediction; Direct-Acting Antivirals (DAAs) treatment outcome; Interferon (IFN)

Abstract

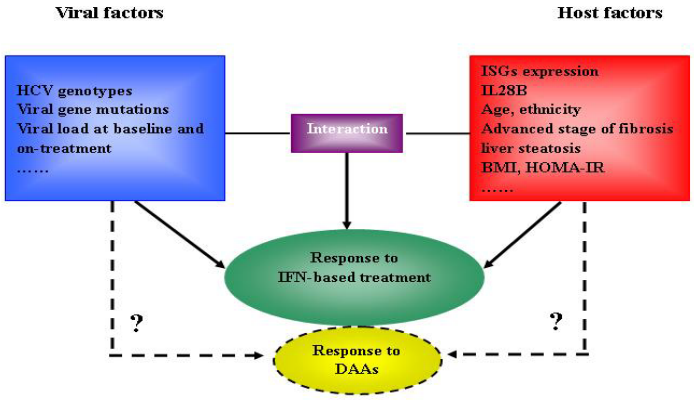
As a major world health problem, Hepatitis C Virus (HCV) infection causes various liver diseases. In the past decades, great efforts have been made in the development of novel therapy for HCV infection. Although newly developed Direct-acting Antiviral Agents (DAAs), such as HCV NS3/4A protease inhibitors Telaprevir and Boceprevir increased the response rate up to 80% for the most prevalent HCV genotype 1 infected patient, a significant proportion of patients still do not respond. Combination therapy with pegylated IFNα and Ribavirin (PEG-IFN/RBV) remains the first choice to treat chronic HCV infections in many developing countries. In consideration of the fact that resistance is now the unmet challenge in anti-HCV therapy, prediction of treatment outcomes becomes an important goal in HCV research. Patients would spare high cost and extensive side effects if prediction of the likelihood of treatment response prior to initiating therapy, or at least soon after starting therapy, could be made. In this chapter, we reviewed several host and viral factors affecting the likelihood of achieving sustained virological response (SVR) in patients receiving stranded PEG-IFN/RBV treatment or new DAAs- PEG-IFN/RBV triple therapy.

Introduction

Hepatitis C Virus (HCV) infection is a major world health problem with a prevalence of about 170 million worldwide, representing 3% of the world's population [1,2]. In most cases (60-85%), HCV infection progresses to chronic liver diseases, which are associated with cirrhosis and hepatocellular carcinoma (HCC) [3]. HCV remains the most common newly diagnosed cause of liver diseases and the most common reason for a liver transplant in North American and Europe [4].

HCV has been discovered in 1989 and great progress has been made in the understanding of its virology, viral life cycle and host immunity. Unfortunately, antiviral therapy is still suboptimal. The current standard of care (SOC) is the combination therapy with pegylated Interferon-α and ribavirin (PEG-IFN/RBV) [5]. Although a sustained virological response (SVR), defined as undetectable HCV RNA 6 months after treatment completion, can be achieved in most (>90%) acute HCV infections, the SVR rates drop dramatically in chronic HCV infection. This combination therapy can cure HCV in only 30%-70% of patients chronically infected with HCV depending on different genotypes [6-9]. In addition to unfavorable response rates, high cost, long duration and extensive side effects inherent to the IFN-based regimen urge us to explore new antiviral strategies. Most recently, with the increased understanding of the HCV life cycle and the solution of crystal structures of HCV proteins, Direct-acting Antiviral Agents (DAAs) against HCV viral protease or RNA polymerase were developed, and this opened a new era for HCV therapy. At present, DAAs mainly consist of HCV protease inhibitors, polymerase inhibitors and HCV non-structure protein 5A (NS5A) inhibitors [10]. Two NS3/4 protease inhibitors (PI), Telaprevir and Boceprevir, were already approved to treat HCV genotype 1 patients [11] in 2011. Although DAAs in combination with PEG-IFN/RBV (triple therapy) improve the SVR to some extent, the apparent limitations include low genetic barrier to resistance. Genetic barrier is used to define the number of amino acid substitutions required to confer full resistance to a drug. DAA with a low genetic barrier usually requires only one or two amino acid substitutions to result in drug-escape mutants of HCV during treatment. Moreover, new medications, such as host-targeting agents which are involved in various HCV life-cycle, target HCV receptor-mediated entry (e.g.: CD81 [12], SR-B1 [13-15]), RNA replication (e.g.: miR122 and virion assembly (e.g. Glucosidase [16]). Considering the cost issue in most developing countries, less expensive natural compounds including Chinese herbal formulations are being explored but they are still in early development.

In consideration of the apparent limitations of current HCV therapy, especially high failure rate, prediction of treatment outcomes prior to the initiation of treatment and understanding of the molecular mechanism of HCV resistance are two important goals in HCV research. Patients would benefit if prediction of the likelihood of a treatment response prior to initiating therapy, or at least soon after starting therapy, could be made. In fact, numerous host and viral factors have been used to predict treatment response to interferon-based therapy (Figure 1).



IFN: Interferon; PEG-IFN/RBV: Pegylated-Interferon-α and Ribavirin; DAAs: Direct-acting Antiviral Agents; ISGs: IFN Stimulated Genes; IL28B: Interleukin 28B; BMI: Body Mass Index; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

Figure 1: Two major groups of parameters, viral factors and host factors, as well as their complex interaction, are associated with treatment outcomes of standard PEG-IFN/RBV in patients with chronic HCV. However, the predictive effects of them on new developed DAAs- PEG-IFN/RBV triple treatment remain to be elucidated.

Viral Factors to Predict IFN-based Treatment Response

HCV genotype

According to a recent international nomenclature of classification [17], HCV contains six major genotypes (1~6) that differ from each other by 30-35% of nucleotide sequence. Each group consists of a series of subtypes differing in 20-25% nucleotides [17]. Different areas in the world have different genotypes or subtypes distribution (reviewed in [18]). Numerous studies [7,19-22] indicated that the SVR rate in patients infected with HCV genotype 1 and 4 is 40-50%, much lower than that in patients infected with genotype 2 and 3(about 75%). Therefore, HCV-1 and 4 are defined as difficult to treat genotypes. Why different HCV genotypes respond to IFN-based treatment differently remains elusive. One study [23] published in 1999 argued that the HCV E2 envelope protein could inhibit PKR (RNA-activated protein kinase R) activity *In vitro* through the PePHD (PKR-eIFα phosphorylation homology domain) whose sequence was similar to the phosphorylation domains of PKR. This similarity is greater in HCV genotype 1 compared with that in genotype 2 or 3. As such, E2 was speculated to contribute to the resistance of HCV genotype 1 to PEG-IFN/RBV [23]. However these findings could not be reproduced by other studies [24,25]. Besides HCV genotype 1, whose responses to the treatment are apparently improved by the recent development of protease inhibitors [26,27], HCV genotype 4 become the most "difficult to cure" genotype of HCV [28]. HCV genotype 4 is predominating in the Middle East and Africa, especially Egypt [29]. Although the specific mechanisms are still unclear, the slow viral dynamics of HCV genotype 4, which is similar to those of HCV genotype 1 and slower than those of HCV genotype 2 [30], indicated that the therapeutic strategies should be seriously considered in relation to the genotype.

Viral load

In a study of 88 HCV genotype 1 patients (from the Italian Hepatitis C Cohort Study, ITAHECS) [31], one of the independent predictors of RVR (Rapid Virological Response) and SVR was pretreatment HCV RNA <400000 IU/ml. The predictive effect of baseline viral load was confirmed by several other studies, even in the studies of patients co-infected with HCV and HIV [32] or in population with recurrent HCV genotype 1 infection after living donor liver transplantation [33]. Interestingly, the current threshold of basal HCV RNA is mainly identified in patients with HCV genotype 1. In patients with HCV genotype 2 or 3, the basal HCV RAN is less useful in predicting response. It is not surprising because the viral load reflects the complex virus-host interaction which can be affected by HCV genotypes. In addition to the pre-treatment baseline viral load, on-treatment viral variables are also useful in predicting the probability to achieve SVR. Viral response at week 4 of therapy (RVR) has been identified as an important predictor of SVR in patients infected with HCV genotype 1 [34] or 2 [35]. Recent studies are looking for much earlier viral response after initiation of therapy to predict SVR. Laufer et al. [36] found that the reduction in HCV viral load from baseline to 24h of <1.4 had a negative predictive value for achieving SVR of 100% in HIV/HCV genotype 1 patients. Using the changes in HCV viral load in the first 14 days after PEG-IFN/RBV treatment, Jun Itakura et al. [37] even derived a prediction equation to predict when the patients will achieve HCV RNA negativity. Yuki Wada et al. [38] analyzed 64 HCV genotype 2 patients with high viral load and reported that the HCV core antigen level at 1 week after treatment initiation could predict SVR. These studies indicated that viral load at baseline or viral dynamics during treatment can be used to predict treatment response in HCV patients.

Viral gene mutations

HCV gene mutation contributes to treatment response. HCV nonstructural protein 5A (NS5A) is the most studied gene discriminating the non-responders (NRs) from responders (Rs) to PEG-IFN/RBV therapy. There are at least 3 functional domains of NS5A involved in IFN resistance: ISDR (Interferon Sensitivity-Determining Region), PKRBD (PKR Binding Domain) and V3 (Variable region 3) in the C terminus. In 1995, Nobuyuki Enomoto et al. [39] identified the COOH-terminal of the NS5A region as ISDR for the first time. Their findings argued that 4 or more mutations in the region (known as "mutant type") were associated with high SVR rate in Japanese patients chronically infected with HCV genotype 1b. However, this association has become a subject of long controversy. Some observations [40-43] supported the findings, while others [44-47], especially those performed in Europe and United States, were conflicting. Several factors may contribute to this contradictory observation: 1) epidemiological differences, such as HCV genotypes and strains in which the frequency of ISDR mutations is relatively low; 2) treatment protocol and different types of interferon used; 3) ethnicity and host genetic background; 4) viral gene mutations in regions other than ISDR. Furthermore, not only the total number of ISDR mutations, but also the

sites of those mutations may affect the SVR rate [48]. In fact, total number and position of amino acid substitutions in the ISDR affects HCV replication *In vitro* [49]. PKRBD, consisting of ISDR and additional 26 distal amino acids, is required for the binding of NS5A to PKR. This NS5A-PKR interaction inhibits PKR activity and resulting in suppression of PKR-mediated eIF-2α phosphorylation. Recent studies [44,50,51] reported that variations in PKRBD were independent predictors of treatment outcomes to PEG-IFN/RBV.

Although the core region of HCV is conserved, mutations of amino acid (aa) 70 and aa 91 are frequently observed. Several studies [41,42,52] have reported that sequence changes in the core region were associated with SVR rate. However, most of them were performed only in patients with HCV genotype 1b, and the predictive effect of mutations in the HCV core region in other genotypes and subtypes still remained to be determined.

Host Factors to Predict IFN-based Treatment Response

Pretreatment ISGs expression in the liver and blood

Gene expression profiling studies that looked at the effects of HCV infection on the host liver have often been aimed to associate changes in the expression of a subset of genes with clinical outcomes or treatment responses. To identify intra-hepatic protein expression that may be used as predictors, MacQuillan et al. [53] scored baseline hepatic human myxovirus a protein 1 (MxA) expression, and found that it was significantly higher in NRs compared to Rs. However, the baseline expression of another IFN- α induced protein, PKR, was not different in groups divided by treatment response. Another study [54] performed in 61 Italian patients with chronic HCV also revealed that lower hepatic PKR mRNA levels prior to therapy was associated with SVR. With the development of microarray gene-expression profiling, a high throughput method that allows simultaneously examine gene expression changes at the transcript (mRNA) level, it is much easier to look at the host response to HCV infection at the whole genomic scale. For instance, Chen et al. [55] used a 19,000 cDNA microarray to study pretreatment liver biopsy specimens taken from patients with chronic HCV who were subsequently treated with PEG-IFN/RBV. They identified 18 genes whose expression levels were consistently and statistically different between treatment Rs and NRs. This response signature, including eight high expression ISGs prior to treatment, ISG15, ISG16, OAS2, OAS3, IFIT1 (IFN-induced protein with tetratricopeptide repeats), MxA, USP18 (Ubiquitin-Specific Protease 18) and CEB1 (Cyclin E Binding Protein 1), accurately differentiate Rs and NRs in 96.8% (30/31) of patients with HCV. Similarly, Asselah et al. [56] also found 3 ISGs (IFI-6-16, IFI27 and ISG15) whose pretreatment high levels of expression were correlated with poor response in two independent cohorts by using real-time PCR analysis. Therefore, significant positive association between "high ISG" phenotype and unfavorable treatment outcome was confirmed. We can postulate that up-regulation of ISGs reflects a pretreatment activation of IFN-α signaling pathway, making the NRs react bluntly to further IFN-α treatment.

Although a certain correlation between elevated pretreatment ISGs expression in liver and failure of anti-HCV therapy was demonstrated, it is necessary to develop an easier predictive test with fewer invasions than liver biopsy based test. Besides the liver tissue, it is likely that strong HCV signal can also be found in blood.

As reported by Gerotto et al. [54], baseline PKR mRNA up-regulation in Peripheral Blood Mononuclear Cells (PBMCs) was more frequently observed in future NRs, independent of age, gender, HCV load and genotype, histological activity and stage of liver disease. Lempicki et al. [57] studied the role of gene expression pattern in PBMCs in 29 patients co-infected with HIV and HCV. They reported 79 genes that identified all 10 NRs, 8 of 10 ETRs (end-of-treatment response, but not SVR), and 7 of 9 patients with SVR. In 17 post-treatment samples one hundred and five genes correctly classified all 9 ETRs and 7 of 8 patients with SVR. Among them were 20 IFN-stimulated genes whose baseline (before treatment) expression levels were higher in treatment NRs than in ETRs or SVRs. Most recently, Tao Huang and his colleagues [58] utilized the HG-UI33A Gene Chip containing 22283 probes to analyze the global gene expression in PBMCs at different time points before and during the early stage of treatment. The prediction accuracy of their time-dependent diagnostic model is 100% and 85.7% in 36 Caucasian American patients and 33 African American patients, respectively. Although there is a certain limitation that the authors evaluated the response without concerning SVR, this study provided a new insight into the time series gene expression profiling of prediction. However, Sarasin-Filipowicz et al. [59] reported conflicting result of the study conducted in 16 Caucasians with HCV genotype-1 or non-genotype-1. Surprisingly, they found that the difference in the up-regulation of ISGs was not pronounced in the blood between Rs and NRs, unlike intra-hepatic ISGs expression pattern.

Although ISGs were elevated in NRs before treatment, identical ISGs expressions were observed in Rs and NRs/ ETRs after therapy [54,57,59,60], suggesting that maybe not ISG expression itself, but changes before and after treatment influenced the response. Importantly, given that patients infected with HCV genotype 2 or 3 were much easier to achieve SVR, the finding [59] that baseline ISG expression levels were significantly lower in subjects with HCV genotype 2 and 3 than with genotype 1 and 4 indicated that pretreatment activation of IFN-α system may be correlated with HCV genotypes. Whatever the reason, endogenously activated ISGs in chronic HCV may be a biomarker of immune dysfunction rather than antiviral effect of IFN-α. In addition, differential gene expression pattern in pre-treatment Rs and NRs may come from different cell types. Using immunohistochemical staining method, Chen et al [61] stained the pretreatment liver biopsies from HCV infected patients with ISG15 and MxA antibodies. They found that increased expressions of these ISG proteins in hepatocytes predicted treatment non-response while in macrophages predicts SVR. This finding may change our traditional way to look at chronic HCV infection, and will help us further understand the role of ISG expression in HCV replication/production and virus resistance to therapy. This cell-type specific protein expression pattern may provide a new way to predict response to IFN-based therapy. Exploring why differential expression of a subset of ISGs in different cell types (hepatocytes Vs macrophages) predicts treatment outcome is critical to understand the molecular mechanism of IFN resistance in HCV.

Host interleukin 28B (IL28B) genotype and SVR

Genome-wide association studies (GWAS), which allow an unbiased sampling of variations in genes across the entire genome without a hypothesis, have independently revealed that some SNPs around IL28B on chromosome 19 which encodes IL28B (IFN- λ3) contributed to both treatment-induced and spontaneous HCV clearance.

Ge et al [62] employed GWAS to study a population of patients involving 871 European, 191 African and 75 Hispanics with chronic HCV genotype 1 infection. They showed that Rs12979860 (located ~3 kb upstream of IL28B) was associated with a two fold increase in SVR rate, taking other clinic factors into account. They further compared the efficacy of PEG-IFN/RBV treatment in association with

IL28B genotype between African-Americans and European-Americans. They found that more favorable IL28B genotype was found in European than African populations, which explain better treatment outcomes in European-Americans to some extent. Tanaka et al. [63] investigated genetic predictors through a two-stage design, the first stage of GWAS in 154 Japanese with HCV genotype 1, and the second replication stage in an independent population of 172 patients. Strongest association was observed between SVR and Rs8099917/ Rs12980275 in two stages. 6 other SNPs also achieved the suggestive genome-wide threshold in both the GWAS cohort and replication cohort. Suppiah et al. [64] also used a two-stage approach consisting of an initial GWAS stage in 293 Australian and a followed stage in Western European. They identified Rs8099917 as the variant most strongly associated with SVR. A distinct 6-alleles haplotype block (tagged by Rs8099917) encompassing regulatory regions of both IL28A and IL28B [64] indicated a relationship, if any, between these polymorphisms and genes expression. A fourth GWAS was conducted in European patients chronically infected with genotype 1 or 4 [65]. In addition, the research was extended to both HCV mono-infected and HCV/HIV co-infected populations. In their study, the minor allele of Rs8099917 was identified in 58% of patients who failed to respond, and defined as a risk factor associated with progression to chronic HCV infection, regardless of co-infection with HIV or not. Ochi et al. [66] tested for SNPs that were associated with SVR in Asian ancestry with HCV genotype 1b or 2a. Two SNPs (Rs8099917 and Rs12979860) in IL28B have been documented to affect the outcome of PEG-RBV combination or IFN monotherapy therapy. They also found that haplotypes, including 4 novel SNPs, showed more accurate in predicting treatment outcomes than any single variant.

Taken together, all the studies confirmed that genetic variation in the IL28B region was associated with SVR, suggesting that patients with the "bad" allele must wait for new antiviral therapy. Based on the data acquired from GWAS, targeted studies were performed using a candidate gene approach, in which particular polymorphisms of IL28B are chosen to be tested for the association with treatment efficacy. As the most widely explored genetic variant in IL28B, Rs12979860 CC genotype was associated with successful treatment outcomes in most studies. In contrast, the predictive effect of Rs8099917 is still controversial (Table 1). However, these studies confirmed a significant association between IL28B genetic variation and response to treatment. Further studies should be done to explore the mechanism underlying this close association.

Single nucleotide polymorphisms	Subjects	Comment	Reference
Rs12979860	268 Caucasian (HCV genotype 2/3)	CC genotype is associated with SVR, especially in patients without RVR	[61]
Rs12979860 and Rs8099917	817 Japanese (HCV genotype 1b)	Rs12979860 CC is associated with SVR and NVR	[62]
Rs12979860, Rs8099917 and Rs12980275	645 Germans (HCV genotype 1/2/3)	Rs12979860 CC is associated with SVR in HCV genotype 2/3-infected patients	[63]
Rs12979860	284 Spanish (HCV genotype 1 and non-1), 69 Spanish (spontaneous clearance), 378 healthy control	Rs12979860 CC is associated with SVR and spontaneous resolution	[64]
Rs12979860	1171 Caucasians, 300 African Americans, and 116 Hispanics (HCV genotype 1)	Rs12979860 CC is associated with SVR and RVR, especially in patients without RVR	[65]
Rs12979860 and Rs8099917	682 white patients (HCV genotype 1/2/3/4)	Rs12979860 CC is associated with SVR in HCV genotype 1/4-infected patients	[66]
Rs12979860 and Rs8099917	109 Caucasian (HCV genotype 1/2/3/4)	Rs12979860 CC and Rs8099917 TT are respectively associated with SVR	[67]
Rs12979860	178 Caucasians (HCV genotype 1/2/3), 53 African American (HCV genotype 1)	Rs12979860 CC is associated with SVR only in Caucasians	[68]
Rs12979860 and Rs8099917	69 Caucasians (HCV genotype 3)	Rs12979860 and Rs8099917 are not associated with SVR	[69]
Rs8099917	160 Spanish (HCV/HIV-1 co-infected, HCV genotype 1/3/4)	Rs8099917 G allele is associated with treatment failure	[70]
Rs8099917	67 Japanese (HCV genotype 1/2)	Rs8099917 TT is associated with SVR	[71]
Rs8099917	719 Japanese (HCV genotype 2a/2b)	with PEG-IFN/RBV cure, Rs8099917 TT is associated with SVR in 2b genotype; with IFN cure only, Rs8099917 TT associated with SVR in 2a genotype	[72]

SVR: Sustained Virological Response; NVR: Non-Viral Response; RVR: Rapid Virologic Response; PEG-RBV: Peg-Interferon and Ribavirin.

Table 1: Genetic association studies of IL28B and treatment outcomes.

Correlation between ISGs expression and IL28B SNPs

Both the pretreatment baseline hepatic ISG expressions and SNPs of IL28B predict treatment outcomes to IFN-based therapy in patients chronically infected with HCV. Because IL28B encodes a type III IFN (IFN- λ3) that shares the same down-stream Jak/STAT signaling pathway with type I IFNs, increased pretreatment hepatic ISG expression may have a close link with IL28B SNP [67]. Some previous studies provided convincing data for this possibility. For instance, Honda et al. [68] reported that hepatic ISGs were up-regulated in Japanese chronic Hepatitis C patients with the unfavorable Rs8099917 genotype (TG or GG). In an American population, Urban et al. [69] also validated the correlation between high pretreatment ISGs and minor Rs12979860 genotype. Because both Rs8099917 and Rs12979860 lie in upstream section of IL28B gene, it is likely that they may influence IL28B transcription and synthesis. Tanaka et al. [63] and Suppiah et al [64] found positive correlation from two independent experiments studies in 20 HCV paitents and 49 healthy volunteers, respectively. They measured IL28B mRNA in PBMCs of individuals, and found lower IL28B mRNA level in individuals with miner G allele of Rs8099917. However, both Honda et al. [68] and Urban et al. [69] failed to find any association between Rs12979860 genotype and levels of liver IL28B mRNA expression. Therefore, the date on the association between IL28B genotype and gene expression are conlicting. One possibility is that IL28B expression may be tissure-specific, but most previous studies didn't test IL28B level in the hepatic tissuues and PBMCs simultaneously. Another factor contributing to this contradiction may be that detection of IL28B mRNA levels cannot reflect mature IL28B protein. However, precise mechanism of the phenomenon remains to be elucidated. On another hand,

Dill et al. [70] measured variation of IL28B (Rs8099917 and Rs12979860) and quantified the ISGs expression in liver biopsies from 109 Caucasian patients with Chronic Hepatitis C. They concluded that IL28B genotype and ISGs level are independent predictors of SVR, indicating other potential pathways to explain the complex host immune responsevniss to HCV.

In vitro, IFN-α induced early increases and rapid decrease in levels of known ISGs, whereas IFN-λ- induced ISGs peaked steadily [71]. Sirén et al. [72] found that IFN-α up-regulated TLR-dependent IL28 expression in macrophages by enhencing the expression of TLR3, TLR4, and TLR7. Ank et al. [73] generated type I IFN receptor-deficient mice, and observed a positive feedback on type III IFN expression mediated by type I IFN receptor system in the mice. Consequently, it is reasonable to hypothesize that type I IFN and type III IFN may interact with each other through a TLR-dependent pathway, which determined the complexity in ISGs expressions and genetic variations of IL28B. Although little is known about the mechanism of the association between IL28B polymorphism and ISGs, these observatons had great biological significance, opening up a new field to search for the "predicting puzzle".

Finally, we are left with the question of which one is the better predictor of treatment outcomes, the ISG expression pattern or the IL28B genotype? A study from Dr. McGilvray' s team in Toronto [74] tested the IL28B SNP rs12979860 and evaluated MxA in hepatocytes and macrophages of HCV patients with known IFN treatment outcomes. Data from this study suggested that absence of MxA staining in macrophages predicted treatment failure with higher accuracy than IL28B SNP Rs12979860. This observation was replicated by Micheal T. Dill et al. [75] from 109 patients choroniclly infected with HCV although L28B CC genotype appears to be associated with low ISGs, there is no causal link bewteen them, and they are independent predictors for treatment response. However, the detailed mechanism involved in the interaction between them remain to be elucidated.

Other host variables associated with SVR

In additon to all the host factors discussed above, age, race, pathological situation, metabolism and biochemical and immune status have been reported to be associated with SVR. Younger age [44,76] and Asian ethnicity [77-78] independently predict better response to PEG-IFN/RBV, while advanced stage of fibrosis [79], presence and severity of liver steatosis [80], high body mass index (BMI) [81] and high homeostasis model assessment of insulin resistance (HOMA-IR) [82,83] predict poor response. Furthermore, biochemical factors, such as low ratio of serum γ-glutamyl transferase/alanine transaminase (γ-GT/ALT) [84] or low γ-GT [52], high levels of anti-NS4a and anti-NS5a [85], eotaxin and macrophage inflammatory protein (MIP)-1b [86], apolipoprotein B-100 (apoB-100) [87], interleukin (IL)-12 and IL-18 [88] have been reported as positive predictors for favorable response.

Prediction of Treatment Response to DAAs

Both high replication rate of HCV and lack of proofreading activity of HCV-RNA-dependent RNA polymerase (RdRp, NS5B) contribute to the large population of quasispecies of HCV that include variants with suppressed susceptibility to DAAs. Therefore, a number of host or viral factors that were previously shown to be associated with outcomes of PEG-IFN/RBV treatment are further investigated to estimate their roles in predicting response to DAAs-PEG-IFN/RBV triple therapy. Unfortunately, most of them failed to predict treatment response to DAAs. For instance, protease inhibitors led to a rapidly undetectable HCV RNA in the majority of patients [26,27,89], making the on-treatment viral kinetics less useful to tailor the treatment. Although some specific parameters, such as HCV genotype 1(reviewed in [90]), LDLC [91], IL28B (reviewed in [92]) and pre-existing variants [93,94], are proposed as potential tools for pretreatment decision, it is impossible, in our opinion, that any of these parameters alone will be good enough to predict treatment response and for decision-making in the clinic. Therefore, optimized combination of several host, viral and pharmacologic factors need to be investigated and further validated in the clinic.

Conclusion

Numerous viral and host variables are reported to be associated with HCV treatment response, providing us with not only potential predictors of treatment outcome, but also evidence for possible mechanism of IFN resistance. Recent findings about ISGs and HCV treatment outcome may change our traditional way of understanding chronic HCV infection, and will help us further understand the effect of ISG expression on HCV replication/production and the paradoxical effects of type I IFN in viral persistence. Further studies need to be performed on an overall pattern taking both viral and host factors into account, which may be the more reliable method to predict HCV treatment response rather than one or two independent predictor(s).

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