Pathogenesis of Hepatitis C – HCV Consensus 2007

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The chronic hepatitis C virus (HCV) infects approximately 130 million people worldwide [1]. It is estimated that approximately 15% of HCV-infected individuals eliminate the virus spontaneously, that 25% develop a mild form of the disease, and that 60% develop the chronic progressive form [2]. The elimination or persistence of HCV infection depends on the balance between the effectiveness, specificity and rapidity of the innate and adaptive immune responses, as well as on the HCV replication rate [3]. Persistence of HCV can also be caused by infection at privileged (extrahepatic) sites, viral inhibition of antigen presentation, selective immune suppression, negative regulation of HCV gene expression, viral mutations, immune exhaustion of T cells and the incomplete differentiation of memory T cells [4,5].

Fibrosis is the principal complication of chronic hepatitis C, and it is estimated that 20% of patients develop cirrhosis over a period of 10, 20 or 30 years [2,6]. The progression of fibrosis increases morbidity and mortality in chronic hepatitis C [7], since it can lead to death due to complications caused by cirrhosis or hepatocarcinoma [2].

Various studies have associated the progression of fibrosis in hepatitis C with diverse factors such as: the kinetics and pathogenicity of HCV; host-HCV interaction; intrinsic host factors such as demographic profile, body mass index and diabetes mellitus; host exposure to external factors; and the form of HCV acquisition.

Life Cycle and Pathogenicity of HCV

Belonging to the Flaviviridae family, HCV is a small enveloped virus [8]. Its genome consists of one RNA molecule that is composed of two terminal regions, 5'- and 3'-untranslated regions, and between these there is a single open reading frame that encodes a polyprotein with approximately 3000 amino acids. This polyprotein cleaves at the N-terminal side of three structural proteins, the nucleocapsid (core), envelope 1 (E1) and envelope 2 (E2), all of which are involved in the architectural organization of HCV. At the carboxyl-terminal side, the polyprotein cleaves to six nonstructural proteins, NS2, NS3, NS4 (NS4A and NS4B), NS5 (NS5A and NS5B) and NS6, which are responsible for the life cycle of the virus [9].

After entering a susceptible host, HCV invades, infects and replicates within the blood stream, repeating the process in various tissues, as well as in peripheral B and T lymphocytes, as it proceeds to the liver by tropism, passing through various tissues such as those of the pancreas, thyroid, adrenal glands, spleen and bone marrow [10-12]. Since HCV can also directly infect the lymphatic tissue, its stimulation can lead to the development of B-cell lymphomas [13]. It is known that the liver is the principal site of HCV replication, and various studies have shown that this virus infects approximately 10% of hepatic cells [5]. Infection with HCV at extrahepatic sites can promote the appearance of HCV variants [14,15], thereby decreasing the chance that the immune system will recognize the virus.

To enter the host cell, HCV E2 and E1 proteins recognize and bond with the CD81 receptors present on the surface of hepatocytes and lymphocytes [16,17]. Circulating HCV particles are accompanied by low-density and very low-density lipoproteins, which prompts discussion in the literature regarding the possibility that low-density lipoprotein is also a viral receptor [15]. After the interaction of the virus envelope with the host cell membrane, HCV enters the cell through endocytosis. In the cytoplasm, the messenger RNA then undergoes translation, and polyproteins are processed; the HCV RNA then replicates, after which the new viral 'RNA's are packaged and transported to the surface of the host cell so that they can disseminate and complete a new cycle [18].

The HCV replication rate is high, approximately 1×10^{12} virions per day; this, together with its high mutation rate, estimated at 10^{-3} nucleotide substitutions per year, leads to great heterogeneity in its presentations, which are known as quasispecies [8]. The selection of and host adaptation to HCV quasispecies have given rise to distinct genotypes [19] whose classification is based on the similarity of the sequence of nucleotides: similarity below 69% characterizes a new viral type; and similarity between 75 and 80% characterizes a subtype [20].

The progression of fibrosis in chronic hepatitis C has been associated with the diversity of HCV quasispecies [21]. The production of new viruses is counterbalanced by the destruction of infected cells through tissue apoptosis or degradation in peripheral blood, since the half-life of the virus in peripheral blood is approximately 2.7 hours [7]. Experimental studies have shown that NS3 and NS5 proteins induce apoptosis in infected hepatocytes [22].

In individuals infected with HCV, the persistence of the virus can be attributed the large inoculum and the high rate of viral replication, which allow the virus to evade the host immune response [4,23]. There is controversy over whether the sequence of nucleotides is directly associated with more intense hepatic lesions [6,24,25].

There is some evidence of direct cytopathic lesion caused by HCV, including HCV-induced histological lesions with scant inflammatory infiltrate [26-28], fulminant hepatitis C after chemotherapy in liver transplants [29] and HCV-related acute cholestatic syndrome after renal transplantation [30].

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Studies suggest that specific genotypes, such as genotype 1, can be more cytopathic [31] or can induce more rapid progression of the disease than do other genotypes [32]. Genotype 1 has been shown to be the genotype most strongly associated with chronic HCV infection [33]. The risk of cirrhosis and hepatocarcinoma has been shown to be greater in individuals presenting genotype 1b than in those presenting genotypes 2 and 3 [34]. However, other authors have stated that HCV genotype and viral load do not influence the progression of the disease [2,6].

It is known that steatosis is a cofactor that influences the progression of fibrosis in chronic hepatitis C [35]. Various studies have directly associated steatosis with HCV genotype 3 [36,37]. Therefore, genotype 3 is considered cytopathic [38,39]. Kumar et al. identified the reduction of steatosis as the only variable predictive of the virological response to the treatment of chronic hepatitis C in individuals infected with HCV genotype 3 [38].

Innate Immune Response to HCV

The innate immune response to HCV is responsible for the activation of cytokines such as interferon (IFN) which activate antiviral proteins that inhibit the replication of the virus while the adaptive immune response to HCV neutralizes viral particles and destroys infected cells [40]. Studies of HCV-infected chimpanzees that eliminate the virus without the specific T-cell immune response suggest that, in some cases, the innate immune response might be sufficient to destroy the infection [41].

The RNA of HCV is recognized by the innate immune response through the Toll-like receptor which responds with the production of IFN-1α and IFN-1β [5,42]. IFN-1 stimulates the nitric oxide synthase enzyme that is expressed in hepatocytes and macrophages as the isoform inducible nitric oxide synthase [43]. Patients with HCV who are treated with IFN present higher levels of inducible nitric oxide synthase, which have been correlated with lower serum levels of alanine aminotransferase [44].

In addition, IFN-1 induces the production of various proteins such as protein kinase (PKR), 2',5'-oligoadenylate synthetase (OAS) and the Mx protein [44]. These proteins are responsible for the expression of the genes that inhibit the replication of this virus within hepatocytes in an attempt to destroy the infection [7,40]. When IFN bonds with the IFN receptor on the surface of the infected cell, it activates the Janus kinase, which induces phosphorylation of cytoplasmic proteins known as signal transducers and activators of transcription (STATs), specifically STAT 1 and STAT 2. The STATs form a dimer that directs itself to the cell nucleus where it forms a complex with the p48 protein, which is a stimulation factor for IFN-stimulated gene factor 3. That complex bonds with the IFN-stimulated response element ISRE, which is an RNA-polymerase promoter complex, and there is a stimulus of the genes responsible for the production of antiviral response proteins and of major histocompatibility complex (MHC) proteins [44].

Some individuals present genetic alterations in the STATs or in the Janus kinase that would impede the formation of antiviral proteins [45].

Various viral proteins have shown a capacity to escape the effect of IFN, as evidenced by the high rate of resistance to treatment with IFN-α seen among individuals with hepatitis C [44,45].

There are various characteristics of HCV that allow it to evade the innate immune response:

- The viral replication complex appears to be composed of a membrane that is highly resistant to *in vitro* proteases and nucleases, which protects HCV from detection by the innate immune response [40].
- The HCV core protein interacts with diverse cell factors, including the tumor necrosis factor (TNF) receptor, which decreases the cytolytic activity of T cells [46]
- Core proteins impede the antiviral activity of IFN, as do NS3/4A and NS5A proteins [40].
- The NS3/4A proteins can impede the recognition of the Toll-like receptor [5].
- The NS5A and E2 proteins can bind to PKR, thereby blocking its activity [5].
- Multiple mutations in the IFN-sensitivity-determining region (ISDR) modify the NS5A region, which inhibits the phosphorylation of PKR, thereby impeding its antiviral activity [45].
- The E2 region of HCV contains a sequence of eight amino acids identical to those of PKR, and this sequence is more common in genotype 1 than in genotypes 2 and 3, which probably accounts for the fact that individuals infected with genotype 1 present greater resistance to treatment with IFN [44].
- Mutations in the ISDR sequence of NS5A suppress the antiviral action of OAS.
- Levels of this protein are lower in nonresponders to treatment with IFN [44].

The liver cell populations that participate in the innate immune response are the natural killer (NK) cells, NK T cells, Kupffer cells and dendritic cells [5]. The NK cells respond minutes or hours after HCV infection by polarizing of the granules in the direction of the infected cells as well as by releasing perforins that fragment the nuclei of infected cells and induce apoptosis [47]. They inhibit viral replication with the production of IFN gamma (IFN-γ), which recruits intrahepatic inflammatory cells and stimulates the T-helper 1 (Th1) response [48], thereby inducing the necrosis or apoptosis of the HCV-infected cell [49]. Studies suggest that HCV inhibits receptor genes in the activation of NK cells, decreasing the activity of these cells by reducing their number and function in chronically infected individuals [40]. The NK cells also have the capacity to increase the functions of dendritic cells in the presence of hepatic cells, although that capacity is impaired in NK cells derived from patients with chronic hepatitis C, in which the production of interleukin (IL)-10 and transforming growth factor beta (TGF-β) can inhibit the activity of dendritic cells [50].

After HCV enters the host cell, the binding of the E2 glycoprotein with the CD81 receptor of NK cells inhibits the function of the NK cells [7,51], which alters the immune response to HCV infection. The E2 glycoprotein also inhibits cytotoxicity and the production of IFNγ by NK cells [40].

Various studies have suggested that the failure of dendritic cells to recognize HCV contributes to the persistence of hepatitis C [50,52-54].

Humoral Immune Response to HCV

After HCV infection, there is expression of the hypervariable NS1/E2 region on the surface of the virus, which stimulates B cells to produce high antibody titers of antibodies with the objective of destroying the permanence of the virus [44]. The appearance of anti-HCV antibodies is significantly delayed, and these antibodies can first be detected from 7 to 31 weeks after infection [7]. The host applies selective pressure on HCV, and this stimulates high nucleotide variation, as well as the appearance of mutations in the envelope proteins, from which the virus selects genomic variants in an attempt to eliminate the site of immune response recognition [55]. The great quantity of HCV quasispecies formed allows the virus to evade the humoral immune response, and the effect of HCV-neutralizing antibodies appears to be insufficient to control the infection [7], which therefore persists [15].

Similar to what occurs in auto-immune type 2 hepatitis, HCV can mimic the immune system, leading to viral escape or postinfection immunity [4,56]. Anti-HCV antibodies have been implicated in tissue damage due to the formation of immunocomplexes such as antinuclear antibodies [57], auto-antibodies that act against cytochrome P450 and antibodies that act against the liver and kidney [4]. The deposition of immunocomplexes has been related to the appearance of extrahepatic manifestations, such as arthritis, cryoglobulinemia [58], vasculitis, glomerulonephritis, Sicca syndrome and itchiness, all of which cause considerable morbidity [57].

There is evidence that HCV infection can be resolved by the cell response with specific CD4+ and CD8+ T cells when there is no formation of antibodies against this virus [59,60], showing that the humoral immune response is not always involved in the response to HCV infection.

Cell Response to HCV

Since there is a weak humoral immune response to HCV, it is believed that the reactivity of cytotoxic T-lymphocytes (CTLs) or CD8+ T cells is fundamental to viral elimination [61,62], and that impairment of this reactivity is one of the factors responsible for the chronicity of the infection [7,63,64]. The CD8+ T cells can eliminate HCV from the liver through two mechanisms: inducement of apoptosis in infected hepatocytes; and suppression of replication by the production of IFN-γ [22,65]. The CTL response is less vigorous in chronically infected patients than in those presenting acute infection [4]. This can be the result of immunologic tolerance or exhaustion of the CD8+ T cell response to the high viral load that persists in individuals chronically infected with HCV [4].

In addition to CD8+ T cells, CD4+ T cells seem to be involved in the viral damage mediated by the increased expression of MHC class II molecules. Some studies have attributed the vigorous and long-lasting response of CD4+ T cells to the elimination of HCV in the acute form the infection [4,66]. However, the loss of the specific CD4+ T cell reactivity to HCV has been associated with the persistence of the virus and the progression of liver damage [67,68].

In acute HCV infection, the peak in serum levels of transaminases corresponds with the cell response, which suggests that the hepatic lesion is immune-mediated [5,64]. It is known that, after activation, T cells initiate clonal proliferation by secreting cytokines and other substances that can affect hepatic function in a variety of ways [69].

Various cytokines act as mediators in the inflammation caused by chronic hepatitis C and have been related to hepatocyte death, i.e. cholestasis and fibrosis, and paradoxically play a role in regeneration following hepatic injury [69,70]. It is argued that the imbalance between the production of Th1 and Th2 cytokines is related to the progression of chronic hepatitis C. The expression of Th1 cytokines such as IL-2 and TNF-α has been shown to be related to the more aggressive presentation of hepatic disease, whereas the expression of Th2 cytokines such as IL-10 has been shown to be related to the milder presentation [71].

The production of TNF-α is one of the earliest events in hepatic injury and is the 'trigger' for the production of other cytokines [72], as well as being implicated in the inducement of hepatocyte apoptosis in viral hepatitis [73].

The levels of cytokines such as IFN-γ, TNF-α, IL-6 and IL-8 are elevated in individuals with chronic hepatitis C [74-82], and some authors have shown that this increase is proportional to the extent of the damage, histologically [26,76,83,84].

There is evidence that IL-4 can modulate the immune response in HCV-infected individuals [75], principally through the activity of Th2 cells.

It has been shown that IL-10 can suppress proliferation in the Th1 and Th2 responses, as well as inducing anergy [85]. There is evidence that IL-10 levels increase in chronic hepatitis C [75]. Some studies report reduced inflammatory activity [86], and others report that administration of IL-10 to such patients causes fibrosis [87].

Various studies have shown that TGF-β is increased in chronic hepatitis C and is involved in the progression of fibrosis, which has been challenged by other authors [88,89]. It has been suggested that TGF-β and IL-10 act as immunosuppressive agents in the liver [90]. In addition, both have been shown to inhibit the immune response and regulate the activity of dendritic cells [91], which can establish a balance between the Th1 and Th2 responses in chronic diseases [92].

Host Factors Associated with the Persistence and Progression of Hepatitis C

In HCV infection, the genetic constitution and immune 'status' of the host are important factors in the persistence and progression of the virus [23,93], since they influence antigen recognition and presentation, as well as the type of Th response [94].

Some MHC class II alleles, such as DR5, have been associated with a lower incidence of cirrhosis in individuals chronically infected with HCV [94]. Rehermann et al. [95] identified CTLs restricted by histocompatibility leukocyte antigen A2 in 97% of chronic hepatitis C patients, compared with 2% of anti-HCV-negative controls. It is speculated that the MHC class II molecule presentation of antigens is deficient in HCV-infected cells, since some viral proteins inhibit the presentation of the antigen through IFN-induced negative immunoregulation [44].

Some pro-inflammatory cytokines appear to be associated with the viral infection response as well as with the expression of specific haplotypes [94], such as IL-10 haplotypes, which can be predictors of spontaneous elimination of HCV [96]. However, there is disagreement in the literature, since other authors did not find evidence for polymorphism in the studied genes being considered as a relevant factor in the elimination of HCV or in the response to treatment [97-99].

The influence of demographic data such as age, gender [6,34] and 'race' [100,101] in the progression of hepatitis C can be due to genetic variations existent among those. Some studies report that HCV positivity increases with age [34,101], thereby leading to a greater chance of progression of the disease [6,34]. The male gender is more prevalent in most studies on hepatitis C [6,101] and, in addition, it was associated with the progression of the disease to cirrhosis [6,34]. Some studies suggest that Afro-Americans, due to a greater propensity to chronicity, resistance to treatment (higher percentage of genotype 1) and development of hepatocarcinoma, present a worse evolution of hepatitis C than do Caucasian-Americans [100,102]. Analyzing 99 chronic HCV-infected individuals and 31 individuals who had spontaneously eliminated HCV, Sugimoto et al. [103] found evidence that the CD4+ T-cell response was less vigorous in Afro-Americans than in Caucasian-Americans, with a predominance of the Th2 response and maintenance of the infection. The evolution of hepatitis C in different ethnicities could be due to genetic factors, such as the presence of HLA class II alleles, which could define the spontaneous elimination of HCV [104].

There are various extrinsic host factors that are related to the progression of chronic hepatitis C: alcohol abuse; smoking [6,34,105-109]; the endovenous acquisition of HCV; and coinfection with other viruses such as HIV, HBV and human T-cell lymphotropic virus [6,110,111].

The prevalence of HCV infection is higher among individuals who consume alcohol [112,113]. Studies suggest that alcohol increases the ability of HCV to enter and persist within the organism [112]. Other studies argue that alcohol intake affects some components of the immune response [112] and can alter the inflammatory response of cytokines, thereby increasing viremia, which can be an important cofactor in the development of hepatocarcinoma [114]. In addition, alcohol intake in HCV-infected individuals increases hepatic steatosis and induces apoptosis [107,112,115].

Smoking, in addition to increasing inflammatory activity and hepatic fibroses [109], can induce direct injury to the liver, as well as causing indirect damage (toxic effect), and can have immunological effects (production of IL-1, IL-6 and TNF-a, which cause liver damage).

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