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Review

Immunological aspects controlling hepatitis C virus infection

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ABSTRACT

Hepatitis C virus (HCV) makes successful strategies to antagonize the host immune responses. A persistent HCV infection leads to chronic hepatitis and eventually causes cirrhosis and hepatocellular carcinoma. Spontaneous clearance of HCV infection is associated with a prompt induction of innate immunity. Host cytokines and innate immune responses play an important role in controlling HCV infection. Innate immune responses modulate adaptive immune responses. The inability of the immune system to eliminate pathogens often results in the development of a persistent viral infection. A persistent HCV infection results from inefficient innate and adaptive immunities with exhausted virus-specific T-cell responses. These responses have recently been shown to play a role in the context of antiviral therapy for chronic HCV infection. Recent introduction of direct acting antiviral (DAA) achieved sustained virological response in a majority of HCV-infected patients. Combination therapy with DAAs exhibiting a high barrier to resistance that target different segments of the HCV life cycle will be associated with a low risk of the emergence of resistance and improved efficacy related to curing HCV infection. Detection of HCV carriers, lack of immunity against reinfection, insufficient access to DAA therapy, uncertainty about the magnitude of viral resistance development, and continued risk for several liver damage are the major hurdle to overcome. Developing a protective vaccine for HCV is an unmet medical necessity. However, efforts to develop an HCV vaccine are hampered by viral factors such as HCV genomic diversity, the cell to cell spread of HCV, a high mutation rate, and the development of infectious lipoviral particles. Because the immune response to an HCV infection is protective, ongoing research to develop a safe and affordable vaccine will provide hope for millions of individuals at risk of HCV infection. Global eradication of HCV will not likely be possible without a robust vaccine.

KEYWORDS: hepatitis C virus, chronic hepatitis, innate immune responses, adaptive immune responses.

1. Introduction

Hepatitis C virus (HCV) is a major cause of liver disease. Approximately 177.5 million people in the world are infected with HCV, and annually 1.3-3.7 million new cases of HCV infection are estimated [1].

HCV leads to chronic hepatitis in more than 80% of acute infected subjects. An acute infection means that the body is able to clear the virus within 6 months of incidence, whereas in a chronic infection, the immune system is unable to nullify the threat and the virus is persistent. The search for protective immune responses has focused on the ~20% of patients who spontaneously clear HCV after acute symptomatic infection with high-level viremia and increased liver enzymes. The inability of the immune system to eliminate pathogens often results in the development of a persistent viral infection. A persistent HCV

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infection leads to chronic hepatitis and eventually causes cirrhosis and hepatocellular carcinoma [2]. The human immune system has developed two arms -innate and adaptive immunity- to act co-operatively, protecting against infection and limiting the damage caused by invading pathogens. Innate immunity acts immediately following infection, directing production of pro-inflammatory cytokines and orchestrating adaptive immunity [3]. HCV persistence in the host can be attributed to the ability of the virus to evade immune surveillance by means of viral mutation and an inhibition of innate immune cells such as dendritic cell (DC) and natural killer (NK) cells by HCV viral proteins, as well as by an alteration of the innate and adaptive arms of immune response [2]. A persistent HCV infection results from inefficient innate and adaptive immunities with exhausted virus-specific T-cell responses. Innate immune responses modulate adaptive immune responses through direct interactions and by using the exchange of signals between immune cells [4, 5, 6, 7, 8, 9]. These responses have recently been shown to play a role in the context of antiviral therapy for chronic d HCV infection [4]. Several viral proteins also appear to play a role in the evasion of host immune responses. In addition to infecting hepatocytes, HCV has been reported to infect DCs, B cells, and peripheral mononuclear cells [10, 11]. The genome of HCV encodes a single poly-protein, which is translated and processed into structural and non-structural proteins. These HCV proteins are the target of innate and adaptive immune system of the host. Retinoic acid-inducible gene-I (RIG-I)-like receptors and Toll-like receptors (TLRs) are the main pattern recognition receptors (PRR) that recognize HCV pathogen-associated molecular patterns (PAMPs). This interaction results in a downstream cascade that generates antiviral cytokines including interferons (IFNs). The cytolysis of HCV-infected hepatocytes is mediated by perforin and granzyme B secreted by cytotoxic T lymphocyte (CTL) and NK cells, whereas non-cytolytic HCV clearance is mediated by IFN-gamma secreted by CTL and NK cells. A host-HCV interaction determines whether the acute phase of an HCV infection will undergo complete resolution or progress to the development of viral persistence with a consequential progression to chronic HCV infection [2]. Host cytokines and innate immune responses play an important role in controlling HCV infection. HCV disturbs the activation of innate immune responses. Innate immune responses control adaptive immune responses. In contrast to hepatitis B (HBV), HCV may be completely cleared by immune responses. Key mediators of spontaneous HCV clearance are virus-specific T cells, which remain readily detectable in the circulation for decades after clearance. These findings have indicated that vigorous CD4 and CD8 T-cell responses are associated with HCV clearance and may protect against reinfection.

2. Immune response in HCV infection

2.1. Innate immune response in HCV infection

Innate immune responses generate IFNs, pro-inflammatory cytokines, complement activation, and NK cell response. Ultimately, these lead to the induction of a robust virus-specific adaptive immunity. Although the host innate immune system senses and responds to eliminate virus infection, HCV evades immune attack and establishes persistent infection within the liver. Spontaneous clearance of HCV infection is associated with a prompt induction of innate immunity generated in an infected host [12].

The innate immune response includes type I IFN in infected cells [13], which induces double-stranded RNA-dependent protein kinase (PKR) and other genes to induce apoptosis of infected hepatocytes, as well as to inhibit viral replication [14]. Compared to HBV, HCV initiates a better innate response due to the exposure of its genetic material in the cytoplasm.

IFNs are the first line of defense against viruses and act directly on viral replication and indirectly *via* the activation of immune responses. The role of the hepatic IFN system and the adjuvant effects of fine-tuned innate immunity that serve as a key to successful vaccine development are important. The major players in HCV-induced immune responses are IFNs I and III, IFN stimulated genes (ISGs), NK cells, T cells, and antibody-type responses. Following an un-coating of HCV, TLR3 and RIG-I-like receptor (RIR) on HCV-infected hepatocytes sense HCV and respond by

generating type I and III IFN that inhibit the replication of HCV as well as activate NK cells. An interaction between the HCV dsRNA replication intermediate and ssRNA with RIG-I and melanoma differentiation-associated gene 5 (MDA-5) activate the Toll/IL-1R-(TIR) containing adapter inducing IFN-beta (TRIF) and mitochondria antiviral signaling protein (MAVS), which phosphorylate IFN regulatory factor 3 (IRF3) and IRF7 to induce type I and III IFN production [15, 16]. Additionally, a TLR3-mediated innate immunity is induced when TLR3 interacts with the dsRNA replication intermediate to activate TIR that phosphorylates IRF3 [17]. Type I (IFN-alpha and IFN-beta) and type III (IFN-lambda) IFN *via* their respective receptors phosphorylate STAT-1 and STAT-2 to generate ISGF3, a transcription factor that translocate into the nucleus, where they play a role in producing IFN-stimulated antiviral genes [17, 18]. A patient's responsiveness to IFN-alpha-based therapy may be assessed using the activation of innate immune cells. Innate immune response cells, such as NKT cells and NK cells, constitute a major cell population in the liver, and have the capacity to respond rapidly to chemokines and/or to altered cell surface marker expression on infected cells. Recent introduction of direct acting antiviral (DAA) achieved sustained virologic response (SVR) in a majority of HCV-infected patients [19]. NK cells are a large proportion of the granular lymphocyte population in the human liver, remain in a functionally hypo-responsive state, but rapidly induce an innate immune response to viral infection. NK cells either directly target infected hepatocytes, or act indirectly by influencing other immune cells such as DCs or T cells for virus clearance [20]. Innate immune cells may influence direct antiviral effector functions and assist with priming and modulating adaptive immune responses. NK cell activation may be mediated by inflammatory cytokines, such as type I IFNs and interleukin (IL)-12, which are commonly released in response to viral infections. NK cells are activated during acute hepatitis 8-14 weeks after infection when liver enzymes and viremia reach high levels [1]. NK cells are a principal part of the IFN-responsive innate population because they are more enriched among lymphocytes in the liver (30%) than in blood (5%~20%), and their percentage increases further in viral hepatitis. The NK cells of patients with chronic HCV infection show higher levels of signal transducer and activator of transcription 1 (STAT 1) and phosphorylated STAT 1 (pSTAT 1) than the NK cells of healthy controls, suggesting that they are activated by type 1 IFN. STAT 1 itself is a product of ISGs and its phosphorylation is an important part of signaling downstream of type I IFN receptors. The NK cells of chronically HCV-infected patients show the altered expression of activating and inhibitory receptors compared with those of healthy uninfected controls. The integration of all these signals results in the activation of blood and liver NK cells in HCV infection and an altered functional phenotype with increased cytotoxicity and decreased production of antiviral cytokines [5]. NK cells through the cytolysis of infected cells, cytokine production, and the activation of T cells results in an initial reduction in the systemic HCV viral load [21, 22]. This is followed by the activation of adaptive immunity, during which virus-specific $CD4^{+}$T, $CD8^{+}$T, and B cells are induced by antigen presenting cells (APCs), specifically DCs. DCs bind to the NKp30 receptor on NK cells and produce IL-12 and IL-15 that activate an NK cell, and activated NK cells secrete IFN-gamma and TNF-alpha that reciprocally enhance the maturation and antigen presentation of DC [23]. NK cells secrete TNF-alpha and IFN-gamma that inhibit HCV replication as well as cytolytic enzymes that destroy HCV-infected host cells. IFN-gamma is the major cytokine that NK cells secrete and is a critical factor for inhibition of viral replication. The cytolytic action of NK cell-released perforin/granzyme could cause collateral damage to host tissues. It is important to note the different subset of NK cells on the basis of the expression of CD16 and CD56. $CD16+CD56^{dim}$ NK cells are more cytolytic in nature, whereas $CD16\text{-}CD56^{bright}$ NK cells usually have a predominantly non-cytolytic phenotype [17]. HCV affects NK cell activity through direct cell-to-cell interaction *via* CD81 or NK cell receptors or in an indirect manner *via* cytokine or TRAIL release [21, 24, 25]. NK cells may influence the balance of Th1 versus Th2 response to an HCV infection. NK cell frequencies in peripheral blood

are reduced in chronic HCV infection when compared to healthy individuals. The functional impairment of NK cells is associated with the evolution of HCV chronicity.

Myeloid dendritic cells (mDC) produce IL-12 in response to HCV-mediated TLR-3 signaling and induce IFN-gamma secretion by NK cells [26]. Plasmacytoid DCs (pDCs) sense HCVRNA in exosomes generated from the infected hepatoma cells and secrete IFN-alpha which activates NK cells [27]. $CD11c^+$ mDC1, $CD141^+$ mDC2, and pDC are DC subsets involved in producing cytokines in response to an HCV infection. IL-12, IFN-lambda, and IFN-alpha are produced by mDC1, mDC2, and pDC, respectively in response to an interaction between HCV pattern-associated molecular patterns (PAMPs) and PRR on DC. These cytokines possess immuno-stimulatory properties [17].

HCV E2 protein binds to the NK CD81 receptor, decreasing the release of IFN-gamma and cytotoxic granules [28]. HCV NS5A protein stimulates monocytes through TLR-4 and induces secretion of IL-10, which subsequently stimulates the secretion of transforming growth factor (TGF)-beta and down-regulates NKG2D on the NK cell surface, resulting in functional impairment of NK cells [29]. HCV NS2 and NS5B proteins are also responsible for HCV-associated decrease in major immunogene complex (MIC) A/B, resulting in a loss of the C3/C4 complement components. This inactivation of the complement system leads to impairment of NK cell activation and attenuates adaptive immune response [30].

2.2. Innate immunity helping adaptive immune response

NK cells constitute a bridge between innate and adaptive immune responses. NK cell-mediated DC activation interplays in priming the adaptive immune response. During acute HCV infection, DCs of the host should interact with the viral proteins to contribute the $CD4^+$T and $CD8^+$T cell responses for clearance or persistence of infection. DCs expressing HCV core or NS3 protein show an impaired antigen presentation and maturation, which renders DCs unable to trigger T-cell activation [31]. An increased plasma level of HCV core protein in chronic HCV infection causes less IFN-alpha production due to a reduced frequency of circulating pDC [32]. In chronic HCV infection, the number of mucosa-associated invariant (MAIT) cells significantly decrease, and residual MAIT cells seem to suffer from immune exhaustion and senescence, which would contribute to the diminished innate defense and facilitate HCV persistence for liver disease progression [33].

Liver-infiltrating lymphocytes obtained from chronic HCV-infected patients suggest high levels of programmed cell death-1 (PD-1) and a low level of CD127 expression, resulting in a suppressed function of HCV-specific $CD8^+$T cells [34, 35]. These results suggest that regulation of the PD-1 pathway is essential for impairment of HCV core-mediated T-cell response. HCV-specific $CD8^+$T cell responses are critical for spontaneous viral resolution in acute infection, and help in maintaining HCV-specific $CD8^+$T cell responses by $CD4^+$T cells. HCV can induce myeloid-derived suppressor cells, cytokines like IL-10 and TGF-beta, resulting in a promotion of regulatory T cell (Treg) development and suppression of $CD4^+$T–cell function [36, 37]. HCV affects the antigen-presenting function of B cells, but not immunoglobulin (Ig) production, and dysfunction of adaptive immune response allows establishment of the persistent HCV infection in a host.

2.3. Adaptive immune response in HCV infection

Neutralizing antibodies (nAb) to HCV appear within 8-12 weeks and interfere with the interaction of CD81, low density lipoprotein receptor (LDLR), scavenger receptor class B type 1 (SRB1), and claudin-1 with HCV envelope glycoprotein E1 and E2 in early acute HCV infection [17, 38]. nAbs inhibit the binding of viral envelopes to host cellular receptors. nAbs to HCV inhibits the viral and cellular factors that promote HCV entry into host cells [39]. HCV E1 and E2 are the targets of nAbs. However, antibodies are short-lived and are not persistent during the chronic stages of the infection [40]. A mutation affecting the binding site of E2 on CD81 could result in the development of resistance to broad nAbs in an HCV infection [38, 41]. Because of the hyper-variable regions in E1 and E2 glycoproteins and high mutation rates, T cell

and B cell responses are short and quite inefficient. Due to a direct cell to cell transmission of HCV, it often escapes the antibodies and is difficult to neutralize. nAbs are thought to have a lesser role in controlling an HCV infection as they were detected more in chronic stages rather than acute infections [42]. HCV-infected individuals who cleared the infection in the acute phase demonstrated the presence of significant levels of HCV-specific $CD4^+$ and $CD8^+$T cells. $CD4^+$T cells provide help for priming $CD8^+$T cell response and activating DC *via* the action of IL-2 and IFN-gamma. The presence of HCV-specific $CD4^+$T cell responses during the acute phase of an HCV infection is associated with the control of viral replication. In chronic HCV infection, $CD4^+$T cells have a limited functionality due to an impaired proliferative capacity as a consequence of the HCV core-mediated suppression of IL-2 secretion [43]. In an acute HCV infection, HCV-specific $CD8^+$T cells perform cytolytic and non-cytolytic functions to mediate viral clearance. The $CD8^+$T response is enhanced *via* the assistance of $CD4^+$T cells during the acute stages of infection [13]. HCV-specific $CD8^+$T cells exposed to high viral loads in a chronic HCV infection exhibit a reduced ability to both proliferate and produce IFN-gamma [44]. The dysfunction of T cells occurs during a chronic HCV infection. There is evidence that suggests that T cells, especially $CD8^+$T cells need to be fully functional in order to successfully control chronic viral infections. Due to the inability of the immune system to control the viral load during chronic infections, significant levels of viral loads correlate with a persistent exposure of T cells to HCV, which render T cells exhausted [45]. Impaired HCV-specific $CD8^+$T cells were also observed to undergo massive apoptosis in the liver during the chronic phase [46]. The impaired function of both $CD4^+$T cells and $CD8^+$T cells correlate with the persistence of HCV infection [47]. IL-10 produced by macrophages, DC, regulatory T cells, and Th2 cells can suppress T cell function. An increased secretion of IL-10 has been observed for various chronic viral infections, including HCV [48, 49]. IFN-alpha-stimulated DCs are induced to express major histocompatibility complex (MHC)-class-I-related chain A/B that ligates with NKG2D-activating receptors on NK cells to induce NK cell activation. Additionally, DCs secrete IL-12, IL-15, and IL-18 that activate NK cells [50]. A reduction in NK cell frequency and cytotoxic function has been reported in the peripheral blood and livers of HCV-infected individuals. An increased expression of inhibitory receptors (CD94/NKG2A) on NK cell in chronic hepatitis C (CHC) patients coupled with a reduction in the proportion of natural cytotoxicity receptors (NKp30 and NKp46) on NK cells has been noted in HCV patients [51, 52]. NK cell cytotoxicity capabilities are impaired, and they secrete a significant amount of IL-10 and TGF-beta that skew the adaptive immune response by down-regulating the function of DC [51, 53]. It has been demonstrated that IFN-alpha therapy in patients with chronic HCV infection can restore NK cell functionality, thus indicating that HCV may suppress NK cell functions during the chronic infection phase [54]. DCs play an important role in initiating adaptive immune response to virus. However, a reduced frequency of mDC and pDC is observed during an HCV infection, and this can be correlated with an impaired capacity of these cells to activate T cells [55, 56]. mDC in the setting of an HCV infection have reduced the expression of co-stimulatory molecules (CD83 and CD86) and impaired the ability to secrete IL-12, which results in an impaired ability of mDC to present antigen to T cells [57, 58]. Dysfunctional DC in HCV infection is unable to induce cytokine-dependent NK cell maturation and T cell priming [59, 60]. Additionally, HCV-infected DC induces immune tolerance, since they secrete significant amounts of IL-10 with a consequential suppression of T cell responses [55, 61]. This is likely to result in a failure to maintain a sustained HCV-specific T cell immune response during chronic infection.

Tregs have an important role to play in the viral persistence in a chronic HCV infection. In patients with CHC, the frequency of $CD4^+CD25^+$ T cells (TR cells) is reported to be high, and these cells can suppress virus-specific $CD8^+$ T cells *via* the action of immune-suppressive cytokines they secrete [62].

The cytolytic mechanism of viral clearance involves the activity of Fas ligand, perforin, granzyme, and TNF-related apoptosis inducing ligands (TRAIL)

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A Fas-FasL system in an HCV-infected liver is mediated by HCV-specific $CD8^+$T cells that express FasL HCV-infected hepatocytes that up-regulate the expression of FasL, which interact with Fas receptors to induce apoptosis of HCV-infected hepatocytes. The Fas-mediated apoptosis involves the activation of caspase-8 and caspase-9 and the subsequent activation of downstream caspase-3, -6, and -7 that cause cell death. Perforin and granzyme B released by activated CTL induce the apoptosis of HCV-infected hepatocytes *via* granzyme B cleaving pro-caspase [63, 64].

2.4. Activation of complement system

The complement system is a series of plasma proteins which work with the innate immune system for targeting and eliminating the invading pathogen. The complement plays a prominent role in the linkage of innate and adaptive immunity. HCV successfully escapes the complement response for persistent infection by regulating complement components. HCV suppresses C3, C4 and C9 complement synthesis and impairs membrane attack complex (MAC) for attenuation of MAC-mediated micro-bicidal activity [65]. C3 complement component is an important mediator of the humoral and T-cell immune response [66].

3. Virus strategy to survive

Infection with HCV is caused by a bilateral process of host-virus interactions. There are factors on both sides that contribute to clearance and chronicity. The virus strategy to survive is built on several basic features. HCV develops multiple strategies to escape or overcome the antiviral actions of IFN and makes chronic infection in the hos [67]. During chronic infections, an important feature is that immune responses towards targeted viruses are impaired or altered. Several mechanisms have been proposed for the failure in host immune responses to clear HCV infection. (1) The escape due to genetic variations, (2) the suppression of immune responses by HCV proteins, (3) the inhibition of innate immune responses during a chronic HCV infection, (4) the dysfunction of T lymphocytes, and (5) the involvement of Tregs in chronic HCV infection are factors that contribute to an impaired or altered immune response against HCV. An immunological escape due to genetic variations is a major immune evasion strategy used by HCV. The hyper-variable region 1 (HVR1), a small fragment spanning 27 amino acids of E2 on highly variable region of HCV genome, is a sequence mutation that plays a role in evading neutralization by HCV-specific antibodies [13, 68, 69]. HCV mutations located in NS3 and NS5 are targeted by $CD4^+$T cells, and these escape mutants to HCV-specific $CD4^+$T cell responses contribute to immune evasion [70]. Additionally, HCV genomic mutations in regions of the $CD8^+$T cell epitope have also been known to affect virus-specific $CD8^+$T cells by decreasing the T cell receptor (TCR) recognition of mutated peptides, impairing the binding affinity between epitope and major histocompatibility complex (MHC) molecule and weakening the ability of proteasomes to process HCV antigens [71, 72, 73].

HCV proteins play a significant role in chronic HCV infection. They exhibit an immune-suppressive activity on DC, NK cells, and T cells, which contributes to the establishment of a chronic HCV infection. HCV proteins may interfere with endogenous IFN and TLR responses. NS3/4A serine protease has been shown to interfere with RIG-I and TLR3 signaling, consequently interfering with endogenous IFN production [74, 75, 76]. HCV core protein degrades STAT1, and as such, inhibits the activation of STAT 1 [77, 78]. It also inhibits ISGF3 *via* the initiation of suppressors of cytokine signaling 3 (SOCS-3) expression, which impedes the binding of IFN-stimulated gene factor 3 (ISGF3) to the IFN-stimulated response elements (IRES) in the promoter regions of the ISG [79, 80]. The HCVNS5 protein impairs the ability of pDCs to produce IFN-alpha [55, 81, 82]. HCV core and E1 proteins inhibit DC maturation, which in turn, impairs the ability of DC to activate T cells. Furthermore, HCV core protein interacts with globular domain of C1q receptor (gC1qR), a complement receptor for C1q on DCs, to suppress production of IL-12, a key cytokine required for Th1 differentiation [83]. Likewise, the HCV core protein interacts with gC1qR on monocyte-derived DC to reduce IL-2 expression, consequentially inhibiting T cell proliferation [84]. Furthermore, the HCV core-mediated suppression of IL-2

production could contribute to an impaired differentiation of the central memory HCV-specific CD8 T cells into effector HCV-specific $CD8^+$T cells [85, 86]. The HCV core also down-regulates MHC and co-stimulatory molecule expression on DC, resulting in an impaired ability to prime HCV-specific $CD4^+$ and $CD8^+$ T cell response and facilitating the induction of IL-10 producing T cells [59]. The binding of HCV E2 proteins to CD81 on NK cells was shown to be associated with an impaired NK cell-mediated cytolytic function and an impaired IFN-gamma production [87].

4. Host factors in virologic failure

IFNs are the first line of defense against viruses and act directly on viral replication and indirectly *via* the activation of innate and adaptive immune responses. The role of the hepatic IFN system and the adjuvant effects of fine-tuned innate immunity that serve as a key to successful vaccine development are important. Type I IFN exerts its antiviral effects by inducing a wide array of ISGs [88]. The immunological programs established by CHC and rapid interference of the delicate balance by exogenous type I IFN may be associated with subsequent virological outcomes in CHC patients [89]. Liver diseases progress due to local immune responses aimed at hepatocytes infected with HCV, and the failure of triple-combination treatments with pegylated (PEG)-IFN-alpha, ribavirin (RBV), and a protease inhibitor (PI) has not yet been related to the exacerbation of alanine aminotransferase. PI-resistant variants that have been present for years as minor viral populations may not lead to a shift in immune dominance resulting in strong intrahepatic cellular immune responses and the production of associated inflammatory cytokines, which accelerate the progression of liver diseases. NS3-specific T-cell immune responses at the baseline may predict a sustained virologic response (SVR) by DAAs-based therapy, and resistance mutations before treatment do not play a significant role in anti-HCV combined therapy [90]. PIs of NS3/4A in HCV achieve antiviral potency by disturbing HCV polyprotein cleavage and may also neutralize HCV NS3 protease-mediated interventions in the innate immune system [91].

The selection of a reference point for HCV sequences by immune responses is dependent on the HLA and T-cell repertoires. Mutations within HLA-restricted HCV epitopes enable HCV to evade host immune responses. HLA class II alleles are associated with spontaneous viral clearance and the persistence of HCV. HLA-DRB1*0101 [92, 93], HLA DRB1*0501 [94], and HLADQB1*0301 [93, 95, 96] are associated with the spontaneous clearance of HCV. However, HLADQB1*0201 [94] and HLADRB1*0301 [97] are associated with a persistence of HCV. Host cells during acute virus infection respond through pathogen recognition receptors (PRRs) and recognize viral pathogen-associated molecular patterns (PAMPS). The RIG-I-like receptors (RLRs) are cytoplasmic RNA helicases that function as PRRs for the recognition of HCV RNA following infection [98]. HCV antigens may regulate inflammatory regulators differently based on the cell types and the regulation of inflammatory regulators by HCV antigen may be a mechanism by which HCV target cells impair development of a strong adaptive immunity. Core expression may contribute to viral persistence by protecting infected hepatocytes from cell death through the suppression of apoptosis and inflammatory reactions to HCV viral infection [99]. The 5'-triphosphate of the poly-uridine core of the HCV RNA recognizes RIG-I and promotes conformational changes. These conformational changes activate type I and type III IFN production by triggering innate antiviral immunity to HCV infection. On the other hand, conformational changes in RIG-I cause interaction of mitochondrial-associated endoplasmic reticulum membrane (MAM) with MAVS. This interaction results in assembly of a signalosome complex that activates effector molecules, including the IFN regulatory transcription factor 3 (IRF3) and NFk-beta, to drive downstream innate immune signaling [98]. It is noteworthy that HCV NS3/4A protease interferes with RIG-I and TLR3 signaling by cleaving MAVS and TRIF, two human proteins known to play a critical role in innate immune response [75, 76]. HCV NS3/4A serine protease disturbs the phosphorylation and effector function of IRF-3, a principal antiviral signaling molecule. NS3/4A protease is a dual therapeutic target. An inhibitor of NS3/4A protease may disturb viral

duplication and restore IRF-3 activity to control HCV infection. The persistence of HCV infection is promoted *via* the viral ability to adopt adaptive mutations and duplicate as genetically distinct quasispecies, but may also be a result of the interruption of immune responses by HCV. IRFs are important transcription factors that initiate a cellular antiviral condition. Interruption of the IRF-3 pathway *via* one or more HCV proteins prevents the expression of IRF-3-activated genes. NS3/4A serine protease activity is required for the interruption of the IRF-3-pathway, which may be reversed *via* the antiviral inhibition of protease activity. IRF-3 induces the expression of many cellular genes that include type 1 IFNs. IFNs may enhance antiviral responses by including hundreds of ISGs. The inhibition of IRF-3 activation may not only advance persistent HCV infection after an initial infection, but also decrease the effectiveness of IFN treatments because many ISGs have IRF-3 target sites in their promoter/ enhancer regions. The antivirals that target HCV protease while disturbing viral replication by interrupting polyprotein processing may recover the responses of the IRF-3 pathway. In the early phase of HCV infection, the HCV NS3/4A protein cleaves MAVS and fails to transduce the RIG-I/MDA5 signal for IRF3-IFN-beta activation [100, 101]. The NS proteins of HCV, particularly NS3/4A, interrupt the induction pathways of type I IFN. Liang *et al*. revealed a novel mechanism for HCV to evade innate immune responses by disturbing TLR3-mediated IFN signaling through NS4B-induced TRIF degradation [102] Nevertheless, variance within cytotoxic T-lymphocyte (CTL) epitopes in NS3 protease is restricted *via* the fitness of HCV. Not all mutations at CTL-recognized epitopes are maintained during HCV infection [103]. NK cells are important components in innate immune responses to HCV infection. Killer cell immunoglobulin-like receptors (KIRs) are involved in regulating the balance between the activation or inhibitory functions of NK cells. KIRs and KIR-HLA contribute to the control of HCV infection by means of innate immune responses [104]. HCV E2 and NS5A protein interact with double-stranded (ds) RNA-activated protein kinase R (PKR) and disrupt PKR functions [105, 106]. The HCV core protein induces suppressor of cytokine signaling 3 (SOCS3) and SOCS1 expression, which blocks STAT1 function [107, 108]. HCV core and NS5A proteins suppress STAT1 phosphorylation in hepatocytes [108].

Polymorphisms of the IL28B (IFN-lambda) gene is protective against CH C and a predictor of response to IFN-based therapy. The genetic variation in the IFN-lambda 3 gene could be associated with a spontaneous clearance of an acute HCV infection. Genome-wide studies have shown that rs12979860 and rs8099917 are associated with a spontaneous resolution of an HCV infection. IFN-lambda polymorphism is linked to a persistence of HCV [109, 110, 111]. Genetic polymorphisms, such as SNPs near the IL28 B locus, modulate the production of cytokines, which may be related to the diversity in responses to HCV. The IL28B CC genotype (rs1297860 and rs5809917) may augment the early suppression of HCV by means of improving responses to endogenous IFNs that are produced as a result of the antiviral effects of DAAs therapy [112, 113, 104]. The IFN-lambda family of type III cytokines signal *via* Janus kinase (JAK)-signal transducers and activators of transcription pathways and promote an antiviral state by inducing the expression of several ISGs. IFN-lambda may be produced by dendritic cells, and apart from its antiviral effects on hepatocytes, may regulate the inflammatory responses of monocytes/macrophages, thereby functioning at the interface between innate and adaptive immunities [114].

IFN-alpha stimulated DCs are induced to express MHC-class-I-related chain A/B that ligates with NKG2D-activating receptors on NK cells to induce NK cell activation. Additionally, DCs secrete IL-12, IL-15, and IL-18 that activate NK cells [50]. A reduction in NK cell frequency and cytotoxic function has been reported in the peripheral blood and livers of HCV-infected individuals. An increased expression of inhibitory receptors (CD94/NKG2A) on NK cell in CHC patients coupled with a reduction in the proportion of natural cytotoxicity receptors (NKp30 and NKp46) on NK cells has been noted in HCV patients [51, 52]. NK cell cytotoxicity capabilities are impaired, and they secrete a significant amount of IL-10 and TGF-beta that skew the adaptive immune response by down-regulating the function of DC [51, 53]. It has been demonstrated that IFN-alpha

therapy in patients with chronic HCV infection can restore NK cell functionality, thus indicating that HCV may suppress NK cell functions during the chronic infection phase [54]. DCs play an important role in initiating adaptive immune response to virus. However, a reduced frequency of mDC and pDC is observed during an HCV infection, and this can be correlated with an impaired capacity of these cells to activate T cells [55, 56].

Cytokines in host and innate immune responses play principal roles in controlling HCV infection. IL-1beta and IL-18 have important roles in combating the invading pathogen as part of the innate immune response. The production of IL-1beta and IL-18 is a tightly regulated process which requires two distinct signals for activation and release [115]. The first signal leads to NFk-beta activation and synthesis of pro-IL-1beta and proIL-18 mRNA in a TLR signal-dependent manner. The second signal involves activation of caspase-1, which cleaves pro-IL-1beta and pro-IL-18 into biologically active forms.

During the acute-phase inflammatory response, TNF-alpha, along with other cytokines, is produced to activate endothelial cells and leukocytes. They influence the function of other cells involved in adaptive immune responses. In the liver, macrophages and Kupffer cells are the main source of TNF-alpha [116]. The liver comprises mostly hepatocytes which can produce TNF-alpha during chronic HCV infection.

TLRs are germline-encoded molecules and are the key components of the innate immune system which recognize endogenous danger-associated molecular patterns (DAMPs) and exogenous PAMPs. HCV core and NS3 proteins trigger the TLR2 signaling pathway and activate inflammation [117]. On the other hand, both TLR3 and TLR7 play roles in sensing of HCVRNA. TLR3 is expressed in liver cells (hepatocytes and Kupffer cells) from HCV infection. TLR3 signals are transduced through the TLR domain containing adaptor-inducing IFN-beta (TRIF) leading to activation of the transcription factors IRF3 and NFk-beta for induction of innate immunity [118, 119]. Both activation and suppression of TLRs may be necessary to strengthen the anti-HCV immune response for limiting virus replication. Thus, the status of TLR signaling defines the type and strength of the anti-HCV immune response, and the outcome of infection.

5. Strategy for failure in treatment

The earlier HCV is eliminated, the better the long-term effects and outcomes achieved [120]. Therapy providing the greatest viral suppression leading to an extended rapid virologic response may be preferable as an induction approach [121, 122, 123]. In order to achieve SVR, it is necessary to (1) prevent the HCV yield and ensure rapid initial reductions in HCV RNA; (2) sustain viral obstruction throughout the treatment; and (3) cause a significant and slower second phase reduction in HCV RNA, resulting in the gradual clearance of liver cells infected with HCV *via* cell death or HCV elimination. It has been hypothesized that reductions in HCV RNA in the second phase are caused by adaptive immune responses through sustained viral inhibition. SVR may only be completed if the reduction in HCV RNA in the second phase is gradual and therapy continues for a sufficient duration to ensure that all infected cells are eliminated or cured.

The added ribavirin (RBV) improves reductions in HCV RNA in the second phase induced by means of PEG-IFN-alpha, thereby accelerating the elimination or cure of cells infected with HCV *via* unknown molecular mechanisms. Another hypothesis is that the restoration of innate immune responses by the clearance of HCV plays a vital role in the elimination of residual HCV genomes from these cells. These patients may be treated with PEG-IFN-alpha plus RBV (PR) as a lead-in treatment to restore wild type frequencies of HCV before attempting further DAAs therapy [124].

Early virologic elimination by induction therapy with natural IFN-beta for 24 weeks prior to PI with PR restored innate immune responses associated with adaptive immune responses, as shown by significant decreases in CXCL-10, CXCL-8, and CCL-4 and significant increases in IL-12 and IL-15, leading to SVR. Induction therapies linked to reductions in HCV RNA levels prior to the initiation of triple therapy with PIs with PR may be used to treat difficult-to-cure CHC patients with genotype 1b and a high viral load. HCV elimination may result in the restoration

of innate and adaptive immune responses. However, many studies have focused on the induction of the persistent suppression of HCV linked to the restoration of innate immune responses resulting in SVR. Furthermore, IFNs have no resistance to HCV, which is different from DAAs [112, 113, 104, 121]. The presence of resistance-associated variants (RAVs) to NS5A inhibitors does not reduce the effectiveness of NS3 inhibitors or PR, and PI and PR indicate the selection of an optional therapy to combination therapy with DAAs against Y93H RAV [121, 122, 123]. The efficacy of treatments may be enhanced by the addition of RBV and/or extending the length of therapy. Until recently, available therapeutic options for HCV infection were limited to PEG-IFN and RBV (PR) for all genotypes with a sustained virologic response (SVR) achievable in a subset of treated HCV-infected individuals [125]. Although the treatment options for HCV infection is rapidly moving away from IFN-based regimens, a highly effective, short course IFN-containing treatment is still a viable option for a subset of patients for whom there are no alternative treatment options available. There are four classes of DAA that are being used in different combinations for all HCV genotypes and that form the mainstay of anti-HCV therapy [126]. In comparison to IFN, DAAs are safer and more efficacious with concomitant improvement in SVR and reduced treatment duration. IL-1beta induces the chronic activation of innate immune-mediated inflammation [127, 128]. DAA pharmacotherapy has been shown to reduce the innate immune activation through reduced production of IL-1beta as well as reduced phosphorylation of NFk-beta. This translates to a reduced inflammation with a consequential reduction in liver fibrosis and damage. The reduction in the expression of CXCL10 and CXCL11, chemokines that recruit innate immune cells, is observed with DAA pharmacotherapy. Furthermore, DAA therapy is associated with a normalization of NK cell function [129]. The reduced secretion of these chemokines along with the normalization of NK cell function correlates with a reversal of dysregulated innate immunity leading to reestablishing homeostasis of the innate immune system [130]. DAA-mediated removal of HCV antigens could have contributed to a restoration of the proliferative capability of exhausted HCV-specific $CD8^+$T cells in the majority of patients with a SVR 12 weeks after cessation of treatment (SVR12). A DAA-mediated cure of HCV is associated with the normalization of innate immunity with a partial restoration of exhausted HCV-specific $CD8^+$T cells that express low levels of PD-1 [131].

Sofosbuvir (SOF) plus PR for 12 weeks needs to be considered as a treatment option for patients infected with HCV genotype 3. The currently approved regimen in the United States for the treatment of HCV genotype 3 infection is a 24-week regimen of SOF and weight-based doses of RBV [132]. There is currently no FDA-approved treatment for prior DAA failures. SOF-based therapy appears to be the basis of saving treatment, even in patients who do not respond to SOF-based treatments. DAAs control HCV replication by at least two distinct mechanisms: (1) the direct inhibition of viral replication by antagonizing the function of viral proteins, and (2) the restoration of the endogenous IFN system *via* the robust introduction of ISGs. Therapeutic IFNs may maintain their position upon the emergence of difficult-to-treat HCV that is resistant to DAAs. In patients infected with HCV genotype 1, SOF may be used in combination with PEG-IFN/RBV, RBV alone, ledipasvir (LDV), or LDV and RBV. The combination of elbasvir and grazoprevir, with or without RBV, was highly efficacious in inducing an SVR12 in patients with HCV genotype 1, 4, or 6 infection [133]. The retreatment of patients, who previously did not respond to DAA therapies, with SOF-velpatasvir or/and plus RBV for 24 weeks was tolerated well and effective, particularly among those infected with HCV genotype 1 or 2 [134]. The combination of Glecavir and Pibrentasvir was highly efficacious and well tolerated in patients with HCV genotype 1 and genotype 2 infection, and prior failure of DAA-containing therapy [135]. Treatment lengths varied between 8 and 24 weeks. The only treatment choice currently available for HCV genotype 2 or 3 failure is a longer length of treatment with SOF and RBV or the addition of PEG-IFN to the regimen. Treatments with drug combinations are sufficient to ultimately control the emergence of resistance-associated substitutions (RAS) in HCV. IFNs exert broad antiviral effects

and contribute to the clearance of resistant HCV. Therefore, IFNs may play a role in the treatment of patients with DAA resistance and enhance the success of retreatment with DAAs. Although the guidelines for the treatment for patients with emergent resistant-associated variants (RAVs) have not been established, DAA therapy should be discontinued in such patients [124].

HCV infection is critically dependent on host lipid metabolism, which contributes to all stages of the viral life cycle, including virus entry, replication, assembly, and release. 25-Hydroxycholesterol (25HC) plays an important role in regulating lipid metabolism, modulating immune responses, and controlling viral pathogens. The synthesizing enzyme of 25HC, cholesterol 25-hydroxylase (CH25H), efficiently inhibited HCV infection. CH25H inhibits HCV infection by suppressing the maturation of the transcription factor sterol regulatory element-binding proteins (SREBPs), which are critical transcription factors for host lipid biosynthesis. CH25H is not an IFN-stimulated gene in humans, but shows a primary and direct response to viral infection. CH25H constitutes a primary innate response against HCV infection by regulating host lipid metabolism. The manipulation of CH25H expression and functions represents a novel strategy for anti-HCV therapeutics. CH25H induction constitutes a part of the host innate immune response. The introduction of CH25H indicates an important host innate response against virus infection and highlights the role of lipid effectors in host antiviral strategies [136]. An increasing number of microRNAs (miRNAs) have been reported to control HCV replication and infection by interacting with the HCV genome directly or by controlling host innate immunity to build a non-specific antiviral state within cells. The potential application of miRNAs as a therapeutic choice for the treatment of HCV infection is being developed [137].

6. Hepatitis C vaccine development

HCV infections are rarely symptomatic before the onset of advanced liver disease, and HCV screening is rare in most parts of the world. Therefore, the majority of individuals with HCV infection are not identified. Although DAAs have fueled optimism for global control, the high cost of current DAAs, which are unaffordable in resource-limited nations with a high prevalence of HCV, is another compelling reason to intensity efforts to develop an affordable and effective vaccine. Vaccination strategies that either provide sterilizing immunity or protective immunity against the development of viral persistence upon reinfection would be immensely beneficial particularly in high risk groups who are most likely to be re-infected with HCV [138]. An effective preventive vaccine will have a significant effect on the incidence of HCV and result in major advances toward global HCV control. However, there are barriers to development, including limitations to HCV culture systems, virus diversity, limited models, and at-risk populations for testing vaccines as well as an incomplete understanding of protective immune responses. Another barrier to HCV vaccine development is the lack of *in vitro* systems and immune-competent small animal models that facilitate investigations on whether vaccinations induce protective immunity. Although an incomplete understanding of protective immunity against HCV is a barrier to vaccine development, previous studies have provided substantial evidence to show that protective immunity does exist. Adjuvant envelope or core protein and virus-vectored non-structural antigen vaccines have been tested in healthy volunteers who are not at risk for HCV infection. Viral vectors encoding non-structural proteins are the only vaccine strategy to be tested in at-risk individuals [139]. The hepatic IFN system and adjuvant effects of fine-tuned innate immunity that serve as a key to successful vaccine development are necessary. Therefore, more detailed information on innate immunity and its cross-talk with adaptive immunity may ultimately overcome this significant threat to public health [140]. A clearer understanding of the mechanisms by which antigen-specific immune cell populations mediate long-term protection is of importance. Highly variable HCV represents a major obstacle to vaccine development. Vaccine strategies to overcome the enormous diversity of HCV need to generate broad immune responses that are capable of responding to abundant variations. The potential application of HCV proteins to develop vaccines and specially the use

of precise epitopes of structural proteins or various linear or conformational epitopes of core, E1, E2, NS3 and NS4 and different T-cell stimulating epitopes of core, E1, E2, and NS3 regions as immunizing agents have been disclosed [141].

Three major approaches have been adopted for vaccine design against HCV. The traditional approach uses recombinant envelope proteins to induce nAb. The second approach uses virus-like particles (VLPs) that express HCV structural proteins to induce both humoral and cellular immunity. The third and most promising approach is designing an HCV vaccine that would induce a potent T cell immune response. A combination of viral vector prime and DNA or recombinant protein boost is preferred to prevent neutralization of the subsequent boost by vector-specific antibodies that could have been generated during priming. The first vaccine uses heterologous prime/boost regimens with chimpanzee adenovirus AdCh 3 and a rare strain of human adenovirus (AD6) expressing the entire NS region of genotype 1b BK strain (NS-5B). This vaccine was tested in a phase I clinical trial in humans (Clinical Trials.gov NCT01436357). The second vaccine uses a regimen of priming with Ad6 encoding NS3-5B of genotype 1b BK strain and boosting with NS3-5B-encoding plasmid DNA [142]. A human prophylactic T-cell based HCV vaccine induced the production of both $CD4^+$ and $CD8^+$ T cells. This vector-based vaccine that encoded non-structural proteins uses a replicative defective Simian adenoviral vector as a prime and modifies vaccina Ankara (MVA) as a booster [143]. The selection of antigens that maximize the induction of T-cell and antibody responses that elicit successful responses remains an active area of research. (1) An HCV genomic variability with seven distinct genotypes with more than 65 subtypes which differ in nucleotide sequence, (2) a high error prone mutation rate of HCV with the capability to escape selection pressure by neutralizing antibodies and $CD8^+$ T cells [144], (3) a high mutation rate occurring in the hyper-variable region 1 of E2 along with the potential of HVR1 to interfere with the binding of antibodies to E2 [145], (4) the cell to cell transmission of HCV constituting a considerable hindrance to developing B-cell-based HCV vaccines that induced broad cross-neutralizing antibodies since HCV could avoid the extracellular component [146], and (5) HCV in circulation binding to plasma lipoprotein to form an infectious hybrid lipoviral particle (LVP) that promotes viral persistence and a high infection by limiting the access of nAbs to envelope glycoprotein are factors that poses a significant challenge to developing an effective HCV vaccine [147]. An HCV vaccine that can generate cross-nAbs and cell-mediated immune responses should be the goal. Two vaccines targeting the antibody or T-cell responses are currently in preclinical or clinical trials. Next-generation vaccines will likely involve a combination of these two strategies [148]. A prophylactic HCV vaccine is an important part of a successful strategy for global control of HCV infection. In the future, the successful control of HCV infection will most likely require a combination of large-scale screening to identify infected individuals, the treatment of infected individuals, and prevention and harm-reduction strategies for those who are uninfected and at risk [139].

7. Conclusion

The pathogenesis of HCV infection is strongly influenced by the nature of host antiviral immunity. Host immune responses play a key role in defining the clinical outcomes of HCV infection. HCV makes successful strategies to antagonize the host immune responses and often persists as chronic infection leading to life-threatening end-stage liver disease. The HCV antigen is sensed to the host by the innate immune system and would inhibit or prime the adaptive immunity. A persistent HCV infection results from inefficient innate and adaptive immune systems. HCV disturbs the activation of innate immune responses.

Innate immune responses modulate adaptive immune responses. Innate immune responses generate IFNs, pro-inflammatory cytokines, complement activation, and NK cell response. Ultimately, these lead to the induction of a robust virus-specific adaptive immunity. Although the host innate immune system senses and responds to eliminate virus infection, HCV evades immune attack and establishes persistent infection within

the liver. Spontaneous clearance of HCV infection is associated with a prompt induction of innate immunity generated in an infected host [12]. NK cells constitute a bridge between innate and adaptive immune responses. NK cell-mediated DC activation interplays in priming the adaptive immune response. DCs or macrophages are critical for antigen presentation, and regulation of these cells may impair adaptive immune response. Elucidating the mechanisms by which HCV fails to activate DC or modulate macrophage function and impair generation of a strong adaptive immunity will identify strategies for prevention of viral persistence. $CD4^+$T cells provide help for priming $CD8^+$T cell response and activating DC *via* the action of IL-2 and IFN-gamma. HCV-infected individuals who cleared the infection in the acute phase demonstrated the presence of significant levels of HCV-specific $CD4^+$ and $CD8^+$T cells. Cytokines in host and innate immune responses principally function to modulate infection with HCV. Combination treatment with DAAs showing a high barrier to resistance that target different stages in the life cycle of HCV may be associated with a low risk of the appearance of RAVs and increased efficiency related to curing infection with HCV. Saving treatment has until now been a field of research that is emerging, and individualized treatments for patients infected with HCV are needed.

However, recent reports suggest emergence of viral resistance against these therapeutic compounds [149]. This may allow continued HCV transmission in high-risk groups and resource-constrained setting due to limited surveillance. In addition, HCV infection often causes a silent disease and late diagnosis may lead to progression of advanced liver disease. Further, detection of HCV carriers, lack of immunity against reinfection, insufficient access to DAA therapy, uncertainty about the magnitude of viral resistance development, and continued risk for several liver damage are the major hurdle to overcome. Host-HCV interactions could pose a challenge to developing an HCV vaccine [2]. We also need to identify steps for augmenting immune responses, and developing a protective vaccine for HCV is an unmet medical necessity. However, efforts to develop an HCV vaccine are hampered by viral factors such as HCV genomic diversity, the cell to cell spread of HCV, a high mutation rate, and the development of infectious lipoviral particles.

Because the immune response to an HCV infection is protective, ongoing research to develop a safe and affordable vaccine will provide hope for millions of individuals at risk of HCV infection. Global eradication of HCV will not likely be possible without a robust vaccine.

CONFLICT OF INTEREST STATEMENT

The author has no conflict of interest to declare.

ABBREVIATIONS

HCV, hepatitis C virus; DC, dendritic cell; NK cell, natural killer cell; TLR, Toll-like receptor; IFN, interferon; CTL, cytotoxic T cell; IRF, interferon regulatory factor; IL, interleukin; APC, antigen presenting cell; TNF, tumor necrosis factor; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; TGF, transforming growth factor; PD-1, programmed cell death 1; Treg, reguratory T cell; nAb, neutralizing antibody.

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