Review Article

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A juggernaut of innate & adaptive immune cells in chronic hepatitis C

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Hepatitis C virus (HCV) is a small positive-sense, single-stranded RNA virus, the causal organism for chronic hepatitis. Chronic hepatitis leads to inflammation of liver, causing cirrhosis, fibrosis and steatosis, which may ultimately lead to liver cancer in a few cases. Innate and adaptive immune responses play an important role in the pathogenesis of HCV infection, thus acting as an important component in deciding the fate of the disease. Numerous studies have indicated that the derangement of these immune responses results in the persistence of infection leading to chronic state of the disease. Interactions between virus and host immune system generally result in the elimination of virus, but as the virus evolves with different evading mechanisms, it makes environment favourable for its survival and replication. It has been reported that HCV impairs the immune system by functional modulation of the cells of innate as well as adaptive immune responses, resulting in chronic state of the disease, influencing the response to antiviral therapy in these patients. These defects in the immune system lead to suboptimal immune responses and therefore, impaired effector functions. This review highlights the involvement or association of different immune cells such as natural killer cells, B cells, dendritic cells and T cells in HCV infection and how the virus plays a role in manipulating certain regulatory mechanisms to make these cells dysfunctional for its own persistence and survival.

Key words B cell - chronic hepatitis C - dendritic cell - immune response - natural killer cell - survival - T cell

Introduction

Hepatitis C virus (HCV), discovered in 1989, is a positive-sense, single-stranded RNA virus of 9.6 kb length and belongs to genus *Hepacivirus* of *Flaviviridae* family[1,2]. Transmitted by infected blood and body fluids, Hepatitis C is a globally prevalent disease affecting more than 180 million people around the world[3]. Owing to the high degree of genetic variability in the HCV genome, there are six major genotypes, and more than 70 subtypes of this virus are recognized[4,5]. In India, genotype 3 predominates in north, east and west India, whereas genotype 1 is common in south India[4]. HCV causes acute infection in about 20 per cent of infected individuals who do not need any treatment, whereas the remaining 80 per cent develop a chronic course of disease defined as the presence of detectable viral replication for at least six months, which needs to be treated[6]. No vaccine has yet been developed for HCV, while chemotherapy has been available for decades. The previously popular combination therapy of pegylated interferon (IFN) and ribavirin was not successful in about 25-30 per cent of chronic hepatitis C (CHC) patients. Response to therapy depends on various host as well as viral

related factors such as HCV genotype, baseline viral load, age, race, liver histology, body weight, gender, baseline gamma-glutamyltransferase level, baseline alanine aminotransferase level and insulin resistance[7]. New direct-acting antivirals (DAA) have been introduced in the treatment regimen[5], which includes NS5B inhibitors (sofosbuvir and dasabuvir) and NS5A inhibitors (daclatasvir, ledipasvir, ombitasvir, velpitasvir and elbasvir). These drugs were introduced in 2016 according to the American Association for the Study of Liver Diseases (AASLD) guidelines for Testing, Managing, and Treating Hepatitis[8].

Efficient activation of an immune response helps in determining the fate of HCV infection. A broad spectrum of activated T-cell clones is found to be circulating in acute infection, which helps in self-resolution of the disease in about 8-12 wk, whereas a weak T-cell activation with a narrow range of T-cell clones is detectable in patients with CHC, who are unable to clear the virus. Persistence of virus causes continuous inflammation of the liver, further leading to cirrhosis, fibrosis and hepatocellular carcinoma in a few. Both viral and host factors seem to play key roles in determining the fate of HCV infection. Among viral factors, viral sequence variations (genotype), quasi-species, high replication rate, viral insusceptibility to cytokines and emergence of escape mutants are prominent. Amongst the host factors, the polymorphism in human leucocyte antigen (*HLA*) and interleukin (*IL*) genes, factors affecting T-cell response, genetic variations in cytokine genes and their cellular receptors are included.

HCV interferes with the immune system via a number of mechanisms. It involves both innate and adaptive immune components of the immune system such as dendritic cells (DCs), natural killer cells (NK cells), B cells and T cells. The NK cells, which are known to play significant role in pathogen clearance because of their cytotoxic mechanisms using perforin and granzyme along with the production of cytokines such as IFN-γ and tumour necrosis factor (TNF)-α, are found to be dysfunctional in HCV-infected patients. NK cells pass maturation signals to DCs through crosstalk between the two components of innate immunity during any infection. The DCs are the most potent antigen presenting cells (APCs) in the body having capacity to activate the naïve T cells upon migration from periphery to regional lymph nodes. The NK cells have altered capacity to influence the functioning of DCs in CHC infection[9,10]. The dysfunctional DCs in such situations are unable to pass optimal activation signals to naïve T cells in CHC disease, leading to suboptimal adaptive immune response as observed in these patients. This results in reduced T-cell priming and proliferation, further leading to impaired differentiation and maturation. These defects lead to delayed trafficking of T cells to liver and therefore, impaired effector functions. This review highlights the involvement and association of different immune cells such as NK cells, B cells, DCs and T cells in HCV infection and how the virus manipulates to make these cells dysfunctional for its own persistence and survival in the human host.

Mechanism of HCV infection

The primary targets of HCV in liver are hepatocytes. The HCV genome replicates in the liver cells, mainly hepatocytes, but to some extent in DCs, B lymphocytes and hematopoietic cells also[11]. The early steps in HCV infection involve cell surface attachment, internalization, fusion and viral replication. The first attachment sites are heparin sulphate glycosaminoglycans followed by the interaction of viral envelope 2 (E2) with other host factors such as CD81, scavenger receptor class B type 1, claudin 1 and occludin. The most common route of endocytosis for HCV that entails internalization is clathrin mediated, which transports viruses along with receptors into early and late endosomes as evident by co-localization of HCV with Rab5a (an early endosome marker)[12]. An important signal (acidic *p*H) is given in endosomes that provokes penetration and uncoating. Fusion between viral and endosomal membranes is aided by fusion peptides embedded in viral envelope glycoprotein, resulting in the penetration of virus into the host. Fusion is followed by the release of viral genome into the cytosol where translation and replication take place. HCV particles are then assembled and released from the host cell[1].

Natural killer cells

NK cells, the cytotoxic lymphocytes are a major cellular component of innate immune response. These are known to provide an expeditious response to viral infected cells, stressed cells and tumour cells. Phenotypically, the NK cells usually express CD16 and CD56 markers on their surface[13]. In addition, the activation status of these cells is dictated by the balance between the signals from inhibitory [killer cell lectin-like subfamily A inhibitory form,

killer cell immunoglobulin (Ig)-like receptors (KIR), CD94/NKG2 receptor, leucocyte inhibitory receptors and Siglec 7] and activating receptors [killer cell lectin-like subfamily A activating form, natural cytotoxicity receptors (NCRs) which include NKp30, NKp44 and NKp46 and DNAM1]. Based on the expression of CD56, these cells are divided into two subpopulations: $CD56^{dim}$ and $CD56^{bright}$. Majority (90%) of the NK cell population constitute $CD56^{dim}$, are more cytotoxic, have high levels of perforin and distinctive repertoire of NK receptors and strongly express CD16 marker on their surface, whereas the other subpopulations (10%) constitute $CD56^{bright}$, are CD16, have weak cytotoxic function and are an important source of immunoregulatory cytokines such as IFN-γ and TNF-α[14].

The NK cells have been shown to play a major role in both acute and chronic phases of HCV infection and also in post-transplant re-infection. It has been suggested that NK cells become dysfunctional in patients infected with CHC[15]. HCV interaction with NK cells *via* glycoprotein E2, which binds to CD81 receptor expressed on NK cells, results in the inhibition of NK cell activation, proliferation, cytokine production and cytotoxic granule release[16]. In addition, there are reports which suggest that HCV core protein alters the NK cell maturation and may influence the outcome of the disease[17]. Alterations in the phenotype and functions of these cells are observed in CHC infection[18]. In relation to the number of NK cells, it has been reported that NK cell frequency increases in diseased condition in liver[19], whereas other reports suggest that their number remains unchanged or low, with a change in subset population only, as the percentage of $CD56^{dim}$ cells becomes low, whereas $CD56^{bright}$ cells' frequency increases in the peripheral blood of patients infected with HCV[20,21]. The reduction in cell numbers and imbalance between different subpopulations could be due to NK cell homeostasis, impaired maturation of these subpopulations followed by change in expansion and survival of $CD56^{bright}$ and decreased survival of $CD56^{dim}$ population and a reduction in the serum IL-15 levels, which regulates NK survival, homeostasis and functions[22]. During HCV infection, alterations in receptor expression are also observed. Inhibitory receptor NKG2A and its ligand HLA-E were found to be upregulated on NK cells and hepatocytes, respectively, in chronic hepatitis infection[23], whereas, in a representative *in vitro* model of acute hepatitis C, the expression of these two markers was not found to be increased on NK cells or HCV-infected cells[24]. This may be due to differences in the experimental setting; while some studies report the expression on cells taken from circulation or liver biopsies, others have used *ex vivo* culture model[25]. Similar controversies have been reported regarding the expression of NCR (NKp30, NKp44 and NKp46)[20,26]. NK cells isolated from peripheral blood or liver derived from CHC-infected patients showed downregulated expression of NKp30 and NKp46 as compared to NK cells isolated from normal controls[27]. During acute HCV infection, the expression of activating receptor NKG2D was found to be increased with no change in the expression of NKp44[17,20]. The expression of another activating receptor NKG2C was found to be upregulated in NK cells of patients[28]. The reasons for discrepancy among the reports could be due to the differences in the ethnicity and gender of the study population and also due to the differences in the viral genotypes that could be prevalent among these populations[29]. Altered NK cell phenotype results in increased cytotoxicity and impaired production of IFN-γ[30]. Reduced levels of IFN-γ have been observed both in peripheral blood of CHC patients and Huh7.5 cells infected with HCV (short and long exposures)[18,24,27,30]. The possible reason is that the endogenous IFN-α given to patients infected with HCV promotes STAT1 expression and its phosphorylation as compared to STAT4. The reduced STAT4 phosphorylation further results in increased cytotoxicity and impaired IFN-γ response[31]. In contrast, these cells produce large amounts of immunosuppressive cytokines such as IL-10 and transforming growth factor-b[10]. Thus, the balance between activating and inhibitory receptors on NK cells, mainly the KIR and class 1 histocompatability-linked leucocyte antigen molecules (HLA-1), helps in determining the virus clearance[30,32]. Many KIRs such as 2DL2, 2DL3, 2DS1, 2DS2 and 3DL1 have been found to be associated with the outcome of HCV disease and efficacy of treatment[33,34]. The expression of NK cell receptor KIR2DL3 and its ligand HLA-C1 results in the spontaneous clearance of HCV[32,33]. KIR3DL1 expression is found to be decreased in HCV-infected individuals, suggesting that it might be involved with the regulation of HCV infection[30]. The expression of KIR3DS1 favours clearance of HCV genotype 1 and also gives protection against hepatocellular carcinoma[35]. Thus, KIR allelic variations and expression of KIR and HLA class 1 complexes

contribute greatly in determining the NK cell function, which further helps in controlling disease progression and in achieving sustained virological response in chronically infected patients.

B cells

HCV has been shown to cause dysregulation in B cells in terms of their activation, functions and phenotype[36]. The interaction of HCV E2 envelope glycoprotein with CD81 results in mimicking of B-cell co-receptor complex and thus reduces the threshold required for B-cell activation, leading to polyclonal B-cell activation[36]. This polyclonal activation leads to polyclonal proliferation, further leading to monoclonal proliferation of Ig-producing cells (IgMκ), which results in malignant transformation, lymphocyte proliferative disorders and development of lymphomas (B-cell non-Hodgkin lymphoma)[37]. Higher frequencies of B cells are observed in patients infected with HCV as compared to healthy volunteers, which correlated with the higher degree of inflammation and hepatic fibrosis[36]. The elevated B cells resembled naïve resting B cells, which expressed both IgM and IgD but no expression of memory B-cell marker (CD27). Highly elevated frequency of circulating CD5+ B cells has been reported from European and Israel study populations[36]. HCV also results in the overexpression of CD81 and expansion of CD5+ peripheral B lymphocytes causing over production of rheumatoid factor leading to rheumatoid arthritis, cryoglobulins leading to mixed cryoglobulinemia and other autoantibodies leading to other autoimmune disorders[38]. Thus, HCV-mediated expansion and activation of B-cell clones is one of the key factors in the immunopathogenesis of CHC infection.

Dendritic cells (DCs)

DCs are professional APCs that are involved in the uptake of antigens and their processing and presentation to naïve T cells for the generation of adaptive immune response against the pathogens. Two major types of DC have been defined: the myeloid DC (mDC) and plasmacytoid DC (pDC). The mDC are phenotypically positive for BDCA1 and are the major stimulators of T cells having TLR4 expression and secrete IL-12 cytokine. Whereas pDC (BDCA2+) are major producers of type1 IFN-α and -β and express TLR9[39]. HCV uses dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin, the major receptor present on DCs, to enter the DCs and thus uses these cells as reservoirs[40]. There are many reports which suggest that DCs lose their functions in terms of their maturation capacity and antigen presentation, in patients infected with CHC[41,42]. The expression levels of co-stimulatory molecules (CD80 and CD86) and MHC class II molecules (HLA-DR and HLA-DQ) are lowered in patients infected with CHC as compared to normal volunteers[43]. There are many controversies regarding the functional, maturation and allostimulatory effects of blood DCs. Many studies including our laboratory have shown functional impairment in both sets of DCs (myeloid DCs as well as pDCs) in patients with CHC, whereas other studies have reported no such impairment[44,45]. Delineating the mechanism of DC impairment, the findings from our laboratory show that the HCV facilitates the downregulation of the expression of some regulatory genes responsible for the maturation and functioning of DCs such as genes for co-stimulatory molecules (*CD80*, *CD86* and *CD40*) and MHC class II molecules. At the same time, the virus modulates the upregulation of the expression of certain negative regulatory genes of JAK-STAT signalling such as *SOCS*, *IDO* and *PD-L1*[44]. Thus, the HCV makes the DCs as poor stimulators of T-cell proliferation, displaying deficient allostimulatory capacity. It has been reported that monocyte-derived DCs from patients infected with CHC fail to secrete IFN-α on poly I: C/IFN-β stimulation[46]. A longitudinal study conducted on a cohort of CHC patients who were offered antiviral treatment, further indicated that the response to treatment was directly associated with the functional status of myeloid DCs, as it was shown that the functional impairment of DCs seen at treatment-naïve stage seemed to be reversed when the same patient achieved the sustained virological response (SVR), and this was not true in patients who failed the treatment[47]. This proposed hypothesis was found to be valid irrespective of the treatment regimen used in the CHC patients. Besides, IL-10 which plays an important role in the pathogenesis of HCV showed an increased secretion in monocyte-derived dendritic cells from patients who did not achieve SVR[48]. Increased IL-10 level is the possible cause of defects in allogenic T-cell stimulation as well as mDC maturation in patients infected with HCV[46]. Individual HCV proteins such as Core, NS3, NS4, NS5 as well as fused polyprotein (Core-NS3-NS4) impair the functions of both immature DCs (iDCs) and mature DCs by downregulating the expression of co-stimulatory and antigen presenting molecules, reducing IL-12 secretion, and also inducing the expression of FasL-mediating apoptosis, interfering with allostimulatory capacity,

inhibiting toll-like receptor (TLR) signalling and inhibiting nuclear translocation of nuclear factor-κB in DCs[49]. Therefore, it suggests that DC impairment induced by different HCV proteins might result in suboptimal immune response allowing for persistent HCV infection. HCV proteins such as core and NS3 have an inhibitory effect on DC maturation as shown in peripheral monocyte-derived iDCs, however this may not represent the *in vivo* situation of DCs in HCV patients[47]. A study has demonstrated that HCV non-structural proteins, particularly NS4, can change the iDC phenotype and reduce antigen-specific T-cell stimulatory function with reduction in the production of Th1 cytokines[50]. Thus, the available literature points towards the HCV-mediated downmodulation of the DC functions possibly because of the imbalance created by the dysregulation of the expression levels of negative and positive regulators of DC maturation and antigen presentation.

T cells

T lymphocyte plays a central role in the cell-mediated immunity against infections. The frequency of CD4+ and CD8+ cells as well as their ratio indicate the state of cellular immune function in an individual. The status of CD4+ and CD8+ cells during HCV infection is strongly correlated with the clinical profile of disease[51]. It is seen that during CHC infection, the CD4/CD8 ratio is disturbed. The number of CD8+ cells is increased with a concomitant decrease in the frequency of CD4+ cells and CD4/CD8 ratio in patients infected with CHC[52]. The number of naïve CD4+ cells was also found to be reduced in HCV-infected individuals as compared to healthy controls[53]. HCV-specific CD8+ T cells are the major effectors in HCV infection as already reported[54]. During the acute stage of HCV infection, CD8+ T-cell responses are vigorous and multiple epitopes are targeted, whereas in chronic stage of infection, these responses are weak and only a few epitopes are targeted[51]. Defective priming of CD8+ T cells is considered to be the major cause in CHC, as the responses are weak and lack multispecific CD8+ T-cell responses[55,56]. This impaired priming of CD8 cells is hypothesized to be the direct consequence of functionally impaired APCs such as DCs and macrophages in CHC[42]. The functional exhaustion of CD8+ T cells is reported to be another mechanism of T-cell failure in CHC[57]. The exhaustion could be either due to high viral load or by HCV core protein which impairs T-cell activation *via* interaction with its complement receptor gC1qr (membrane bound)[58]. The role of regulatory T cells (CD4+CD25+Foxp3+) and virus-specific IL-10-producing cells has been defined in HCV infection. Higher frequency of these cells is reported in patients chronically infected with HCV as compared to normal healthy volunteers and in patients who have successfully resolved infection[59]. T regulatory cells suppress the proliferation as well as cytokine (IFN-γ) secretion of HCV-specific T cells *in vitro*[60]. In addition, IL-10-producing virus-specific T cells have also been found in the liver of HCV-infected individuals[61]. These cells produce a large amount of suppressive cytokines. Studies have reported that IL-10 polymorphism is associated with viral persistence or viral clearance and thus the outcome of the disease[62,63]. Furthermore, lack of homing to the liver is another mechanism of failure of T-cell response. Some virus-specific CD8+ T cells are limited to peripheral blood, whereas only a few are found in liver, so it might be possible that these virus-specific cells have defective homing or their deletion in liver can also be a contributing factor for T-cell failure causing viral persistence[64]. HCV has also been shown to result in the impairment of virus-specific effector memory T cells, as the expression of CD27 is lowered in response to TLR stimulus[55]. Impaired CD27 signalling is known to contribute to the lack of memory T-cell pool. CHC infection is found to be associated with virus-specific T-cell response of limited clonal diversity, as majority of these T cells are not specific for HCV antigens and express inhibitory molecules such as programmed cell death protein 1 (PD-1) and mucin domain-containing protein 3[65]. HCV-specific CD8+ T cells have been shown to express high levels of PD-1, the inhibitory receptor in liver as well as in peripheral blood[66]. The blockage of the interaction between PD-1 and its ligand PD-L1 by antibodies led to the restoration of proliferation, cytokine secretion and cytotoxicity by the exhausted CD8+ T cells in acute and chronic infection *in vitro* and also to substantial decrease in viral load[67]. Host HLA class 1 molecule, which helps in the recognition of antigens by CD8+ T cells, is also thought to be associated with viral persistence and clearance[68]. HLA class 1 allele B8 is associated with viral persistence, whereas alleles A3, B27 and Cw*01 are associated with early viral clearance[54]. HLA-B27 shows the strongest protective effect, as 80 per cent of women bearing this allele successfully cleared the infection and only a few developed chronic infection[54]. Another population study in Caucasians, African-Americans and West

Africans has also shown an association between HLA-B57 and viral clearance[69]. Thus, the available reports indicate that the lack of virus-specific CD8+ T-cell response in liver during CHC infection may be the result of multiple mechanisms. Functionally impaired APCs, functional exhaustion due to higher expression of PD-1 and certain HLA genotypes may be the major contributors. Further research on carefully selected cohorts of such patients could reveal some novel targets of intervention.

Conclusions

The nature of host immune response plays an important role in deciding the clinical profile of hepatitis C infection. While it has been established that HCV impairs the immune system by modulating its cells, resulting in the chronic phase of the disease, it also influences the response to antiviral therapy in these patients. These findings vouch for the search of novel molecules that may be used as adjunct to the existing antiviral drugs for better management of this disease.

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