Immunological mechanisms controlling hepatitis C virus infection

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1. Introduction

When hepatitis C virus (HCV) infection occurs, a subset of acutely infected individuals (nearly 15%–30%) can spontaneously eradicate the virus[1]. In addition, significant levels of natural immunity to HCV have been reported in studies of the chimpanzee model and in studies of reinfections in intravenous drug users[2,3]. Aided by a better understanding of the immunological correlates and mechanisms underlying the successful control of viral infection, the fundamental role of innate immune response in facing HCV infection has been emphasized[4]. Innate responses are observed early after HCV infection. The armaments which enable the body to fight HCV including type I interferons (IFNs), HCV specific CD4+ cells and CD8+ T cells, cytokine production, natural killer cells, dendritic cells, and the production of anti-HCV neutralizing antibodies. Toll-like receptors form an important element of the innate immune response, and there is considerable evidence for their crucial role in HCV infection. In order to limit the availability of the cellular components for viral amplification, apoptosis occurs. It involves caspases, the key effectors of apoptotic cell death. This article reviews what the immune system does, when HCV attacks the body.

1.1. Type I IFNs

Hepatitis C virus (HCV) infection is a significant global health problem, affecting over 150 million people worldwide. There is increasing evidence that a small percentage of individuals exposed to the HCV have the capacity to generate a strong cellular as well as humeral immune response against the virus and avoid persistent infection, and perhaps do so repeatedly after re-exposure. While the critical role of the adaptive immune system in HCV infection is well-established, the importance of the innate immune system has been recognized in more recent years. The immune system has many weapons to combat the HCV infection. These include type I interferons, HCV specific CD4+ cells and CD8+ T cells, cytokine production, natural killer cells, dendritic cells, and the production of anti-HCV neutralizing antibodies. Toll-like receptors form an important element of the innate immune response, and there is considerable evidence for their crucial role in HCV infection. In order to limit the availability of the cellular components for viral amplification, apoptosis occurs. It involves caspases, the key effectors of apoptotic cell death. This article reviews what the immune system does, when HCV attacks the body.

KEYWORDS
HCV, Cellular immunity, Interferon, T cells, Cytokine, Natural killer cells, Dentritic cells, Humeral immunity, Toll-like receptors, Caspases
IFN regulatory factor-3 (IRF-3), which is in association with many other factors regulating the expression of IFN-α[7]. After reaction cascades involving other proteins (e.g., Fas-associated death domain protein, caspase recruitment domain), retinoic acid-inducible gene-I–like ribonucleic acid (RNA) helicase signaling pathway, IRF-3 and IRF-7 are activated leading to the formation of IFN-α and IRF[8].

Moreover, endogenous IFN-αβ binds to a common receptor expressed at the surface of target cells, leading to the activation of signal transducer and activator of transcription (STAT) 1 and STAT2, which, together with IFN-stimulated gene factor 3, γ subunit/IRF-9, bind to IFN-stimulated response elements, thereby activating the transcription of IFN-α/β-inducible genes[9-11]. The inducible genes include genes encoding ribonuclease L and protein kinase R which degrade viral RNAs and block their translation. In addition to these genes, type I IFN activates the expression of more than 300 genes that also have antiviral functions, e.g., the 2′,5′-oligoadenylate synthetase, adenosine deaminase (adenosine deaminase, RNA-specific), and the MxA protein GTPases[12].

Cytotoxic T lymphocyte antigen-4 is a key negative regulator of T cell activation. Its inhibitory effect is due to reducing the production of interleukin (IL)-2 and arresting cell cycle progression[18].

The inhibitory receptor PD-1, a CD28 family costimulatory/coregulatory molecule, is highly expressed on virus-specific exhausted CTLs cells in comparison to functional memory CD8+ T cells[24]. Interactions between PD-1 and its ligands PD-L1/PD-L2 can inhibit antigen-specific T cell proliferation and effector function.

B7–H4 is a co-inhibitory molecule expressed by activated hepatic stellate cells (HSC). Unlike quiescent HSC, activated HSC did not induce proliferation of antigen-specific T cells[20].

Tim-3 is a membrane protein, which has shown to be a T cell exhaustion marker in humans infected with HCV. Recent studies have shown that higher expression levels of dual Tim-3 and PD-1 have been reported to correlate with impaired Th1/Tc1 cytokine secretion and diminished cytotoxic potential[21].

LAG-3 is a CD4 homologue. It is a transmembrane protein that binds major histocompatibility complex (MHC) class II, enhances regulatory T cell activity, and negatively regulates cellular proliferation, activation, and homeostasis of T cells. Many cells of the hematopoietic lineage, such as B, NK, γδ T cells, and activated and regulatory CD4+ and CD8+ T cells, as well as tumor infiltrating lymphocytes, express LAG-3. Studies have shown that LAG-3 plays an important functional role on CD8+ T cells by maintaining the tolerogenic state[22].

1.3. Cytokines

Cytokines and chemokines which are secreted by immune cells contribute to viral control, or liver damage. Examples are IFN-α/β, IFN-γ, tumor necrosis factor α (TNF-α), granulocyte macrophage–colony stimulating factor, IL-5, IL-13, IL-10, and transforming growth factor. They are able to render uninfected cells resistant to infection and cure the infected ones from the virus by stopping viral replication.

In vitro studies, IFN-γ inhibits amplification of HCV replicons in Huh-7 liver cells. In humans, the induction of IFN-γ–producing, antiviral CTL corresponds with the successful clearance of the HCV infection. Furthermore, the degree of viremia correlates inversely with the expression of IFN-γ in the livers of HCV–infected persons[25].

Granulocyte macrophage-colony stimulating factor is a cytokine that functions as a white blood cell growth factor[26]. Through binding to the IL-5 receptor, IL-5 stimulates B cell growth and increases immunoglobulin secretion, while IL-13 is a key regulator in humoral and adaptive immunity. IL-10 downregulates the expression of Th1 cytokines, MHC class II antigens, and costimulatory molecules on CD8+ T cells by maintaining the tolerogenic state[22].

Figure 1. Immune response to HCV infection.

1.2. CD4+ and CD8+ T cells

The HCV-specific T–cell response has been shown to play a crucial role in determining the outcome of primary HCV infection. HCV specific CD4+ cells, together with cytolytic T lymphocyte (CTL) cells enter the liver in parallel with the onset of acute hepatitis. HCV–specific CD4+ T cells can potentially act in multiple ways and are central to the initiation and maintenance of adaptive immunity. Two likely major roles are in providing help for CD8+ T cells by cytokine production, and activation of antigen–presenting cells. There are multiple other roles including direct antiviral effects, and a role in B cell maturation, and regulatory functions[13]. CTL cells could respond to HCV viral infection through a couple of mechanisms: the killing of infected hepatocytes by apoptosis or the secretion of perforin and granzymes[14].

Comparative studies in man have demonstrated that the broad and sustained CD8+ and CD4+ T cell response is associated with spontaneous viral clearance. Conversely, a weak and narrowly targeted T cell response is a hallmark of persistent infection[15-17]. When the latter occurs, T cells are exhausted and overexpress inhibitory molecules including cytotoxic T lymphocyte antigen 4, programmed death 1 (PD-1) inhibitory receptor, B7 family member B7–H4, T cell immunoglobulin and mucin domain–containing protein 3 (Tim-3), and lymphocyte activation gene–3 (LAG–3)[18-22]. Interactions of these molecules with their cognate ligands on various cells types result in reduced T cell propagation and function, together with tolerance to the antigens’ exposure[23].
macrophages. It also enhances B cell survival, proliferation, and antibody production. IL-10 can block nuclear factor-κB activity, and is involved in the regulation of the Janus kinase–STAT signaling pathway\[27\].

The normal anti-inflammatory cytokine milieu of the liver is disturbed by the antiviral immune response, which may activate stellate cells to produce matrix proteins and fibrosis-promoting cytokine. Transforming growth factor, another mechanism, which may contribute to liver injury in HCV infection is enhancing TNF-α-induced cell death by suppressing nuclear factor-κB activation through the action of core, NS4B, and NS5B\[28\].

The intrahepatic T cells from the individuals with chronic HCV infection produce almost 50 times more TNF-α than the ones who control this infection. Furthermore, TNF-related apoptosis-inducing ligand kills hepatocytes from virus-infected, inflamed livers via death receptors-4 and death receptors-5 but not from healthy ones\[29\].

### 1.4. DCs

DCs, the professional antigen presenting cells, represent the cornerstone cell part of innate immunity. They orchestrate the quality and effectiveness of downstream adaptive immune response (Figure 2).

![Figure 2. DCs and immune responses in HCV infection.](image)

Plasmacytoid DCs recognize HCV infection and produce IFN-α, which activates NK cells, Th cells, macrophages, and CTLs. Activated NK cells destroy the HCV-infected hepatocytes in a nonspecific manner, whereas CTLs destroy the infected hepatocytes in an antigen–specific manner. Myeloid DCs, which recognize dead hepatocytes, secrete IL-12, promoting the activation of NK cells, Th1 cells, and CTLs. Activated Th1 cells, in turn, promote DC maturation by interacting with the CD40L/CD40 ligand. Macrophages stimulated by type 1 Th1 cells produce TNF-α, which accelerates local inflammation. In humoral immune responses, Th2 cells activate B cells. Plasma cells differentiated from B cells secrete immunoglobulins to neutralize the circulating HCV. pDC: Plasmacytoid DC; mDC: Myeloid DC (adapted from Hiroishi et al\[30\]).

Found within the peripheral tissues and lymphoid organs, DCs are perfectly suited to detect and capture pathogens. Their antigen presenting capability is crucial for generation of CD4+ T cells, and priming B cells for antibody production. By production of CD40 and IL-2, DCs provide help to CD8+ cells. Since DCs express distinct sets of TLRs, it is likely that some viral components stimulate DCs through ligation of TLRs, presumably TLR 3, 7–9\[31\].

The two major DC subsets, the myeloid dendritic cells (MDC) and plasmacytoid dendritic cells (PDC), contribute to the immune mechanisms targeting HCV. MDC produce large amounts of IL-12 and IL-10 and make small amounts of IFNs, while PDC are specialized type I IFN–producing machines and express much lower levels of other cytokines. These released cytokines, efficiently prime both CD4+ Th cells and CD8+ CTLs\[32\].

Several studies indicate that DCs response to HCV in the early stage of infection is fundamental in determining the outcome of the disease. Chronic HCV–infected individuals show impairment of DC functions, which may include a reduced frequency of MDC and PDC, reduced IL-12 and IFN-α, and increased IL-10 production, accompanied by an impaired capacity to prime naïve T cells\[33\].

### 1.5. Anti–HCV nAb

Although the role of anti–HCV nAb was controversial in the pathogenesis of HCV infection, recent studies have indicated that nAbs are fundamental in determining HCV disease outcome. Viral clearance is associated with a rapid induction of nAb in the early phase of infection with some evidence that these nAb are broadly reactive, while chronic HCV infection is characterized by absent or low–titre nAb. Infection persists, despite the induction of cross–nAb in the later phase of infection\[34\].

Current understanding of the nAb response raised against HCV suggests that E2 is the major target, and that multiple epitopes within E2 may be targeted by both linear– and conformation–dependent antibodies. Predominantly, these neutralization epitopes overlap with CD81–binding sites and clearly demonstrate a role in inhibition of entry\[35\].

The antibody can exert their actions through a fragment crystallizable region–mediated recruitment of other components of the immune system, including antibody–dependent cell–mediated cytotoxicity, complement–dependent cytotoxicity and antibody–dependent cellular phagocytosis\[36,37\]. Given the potential antiviral effect of the antibodies, HCV has evolved multiple mechanisms for protection from antibody binding. One of these is glycosylation of receptor. Carbohydrates are poorly immunogenic and, therefore, do not stimulate the response of type B lymphocytes and simultaneously hide the underlying protein structures. HCV E2 protein contains up to 11 potential N–linked glycosylation sites. Specific glycans mask the CD81–binding site and, therefore, nAb epitopes. Lipid shielding may represent an additional strategy used by HCV to evade the antibody response. Current data suggest that key neutralizing epitopes are less accessible on lipoviral particles. More recently, HCV has been found capable of direct cell–to–cell transmission, which is largely resistant to antibody neutralization\[38\].


1.6. TLRs

TLRs, the family of pattern recognition receptors, function as primary sensors of the innate immune system to recognize microbial pathogens[39]. TLRs recognize the distinct structures in microbes, pathogen associated molecular patterns. Ligand binding to TLRs invokes a cascade of intra-cellular signaling pathways that induce innate immune response (Figure 3)[40].

Recent reports have uncovered the key molecules in the TLR-induced signaling pathways that lead to type I IFN induction, enhancing the antiviral activity of cytokines. This occurs via activation of IRF–3 and IRF–7 expression[42].

HCV infection constitutes an unwanted intrusion that needs to be eradicated by host cells. On one hand, one of the first protective barriers set up to prevent viral replication, spread or persistence involves the induction of apoptotic cell death that aims to limit the availability of the cellular components for viral amplification. The existence of numerous antiapoptotic products within the viral kingdom proves that apoptosis constitutes a major threat that should better be bypassed[43].

1.7. Apoptosis and caspases

Apoptosis depends completely on the host molecular machinery and to some extent on the virus itself[44]. In the host, it is carried out via two pathways: death receptor-mediated pathway, and mitochondrial apoptotic pathway. The two pathways involve a sequence of reactions; the common end point of both is the activation of the cascade of the intracellular proteolytic enzymes caspases (Figure 4).
and evolving equilibrium between HCV and host innate immunity involves in the protection of the body against HCV infection.

The overall data demonstrate complex, contradictory and evolving equilibrium between HCV and host innate immunity. Consequently, adaptive immunity are the determinants of final clinical outcomes ranging from resolution to chronic viral infection.

2. Conclusions

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Conflict of interest statement

I declare that I have no conflict of interest.

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Research frontiers

When HCV infection occurs, a subset of acutely infected individuals (nearly 15%–30%) can spontaneously eradicate the virus. In addition, significant levels of natural immunity to HCV have been reported in studies of the chimpanzee model, and in studies of reinfections in intravenous drug users. This review article makes an attempt to evaluate the role of innate immune response against HCV infection.

Related reports

HCV infection has been shown to influence both pathways. Among the different strategies developed by the virus to deal with apoptosis, one is based on attacking the cell directly. Another mechanism is to express proteins targeted by caspases, the key effectors of apoptotic cell death.

Innovations and breakthroughs

HCV infection constitutes an unwanted intrusion that needs to be eradicated by host cells. On one hand, one of the first protective barriers set up to prevent viral replication, spread or persistence involves the induction of apoptotic cell death that aims to limit the availability of the cellular components for viral amplification. The present review illustrates what the immune system does when HCV attacks the body.

Applications

This overall data demonstrate complex, contradictory and evolving equilibrium between HCV and host innate immunity. Consequently, adaptive immunity are the determinants of final clinical outcomes ranging from resolution to chronic viral infection.

Peer review

This is a good review article which showed and evaluated the role of innate immune response against HCV infection. The author tried to correlate the relation between different tools of innate immunity e.g. different types of cells, actions of some cytokines, anti–HCV and apoptosis in the process of immunity against HCV infection.
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