Review

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Innate immunity and HCV

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Summary

Hepatitis C virus (HCV) infections become chronic in the majority of infected individuals, and chronic hepatitis C (CHC) can lead to cirrhosis and hepatocellular carcinoma. The innate immune system is central to host-virus interactions during the entire natural course of the disease. The HCV NS3/4A protease efficiently cleaves and inactivates two important signaling molecules in the sensory pathways that react to HCV pathogen-associated molecular patterns (PAMPs) to induce interferons (IFNs), i.e., mitochondrial antiviral signaling protein (MAVS) and Toll-IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF). Despite this viral escape mechanism, the innate immune system strongly reacts to HCV within the first days after infection. The sensory pathways, the type(s) of IFNs involved and the cellular source of IFNs are largely unknown. After 4–8 weeks, HCV specific T cells are recruited to the liver. IFN-γ-stimulated genes get strongly expressed in the liver. In about 30% of patients, the virus is eliminated during the acute phase of the infection by T cell-mediated antiviral mechanisms. In the remaining 70% of patients, HCV persists for decades. During this phase, T cell-derived IFN-γ cannot be detected any more in liver biopsies. Instead, in about half of the patients, hundreds of type I or III IFN-stimulated genes become again strongly expressed. However, this innate immune reaction is ineffective against HCV. Moreover, patients with constitutive IFN-stimulated gene (ISG) expression have a poor response to treatment with pegylated IFN-α (PegIFN-α) and ribavirin. The viral escape mechanisms that protect HCV from IFN-mediated innate immune reactions are not entirely understood, but might involve blockade of ISG protein translation at the ribosome, localization of viral replication to cells with refractory IFN signal transduction pathways or to cell compartments that are not accessible to antiviral IFN-stimulated effector systems. Recently, genetic variations near the *IL28B* (*IFN-λ3*) were found to be strongly associated with spontaneous clearance of HCV and response to treatment with PegIFN-α and ribavirin. The finding supports a central role of the innate immune response in host-viral interactions. The signaling pathways that link genetic variants of *IL28B* with immune answers to HCV remain to be elucidated. The present review article attempts to summarize current knowledge of some central aspects of the interactions of HCV with the innate immune system.

Keywords: Interferon; Hepatitis C virus; Innate immunity; Jak-STAT.
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Abbreviations: AHC, acute hepatitis C; CHC, chronic hepatitis C; DC, dendritic cell; HCV, hepatitis C virus; IFN, interferon; IFNAR, IFN-α receptor; IFNGR, IFN-γ receptor; IRF, interferon regulatory factor; ISG, interferon-stimulated gene; MAVS, mitochondrial antiviral signaling protein; MDA5, melanoma differentiation antigen 5; NK cells, natural killer cells; NKT cells, natural killer T cells; pDC, plasmacytoid dendritic cell; PAMP, pathogen-associated molecular pattern; PBMC, peripheral blood mononuclear cell; PHH, primary human hepatocytes; PIAS, protein inhibitor of activated STAT; RIG-I, retinoic acid inducible gene-I; TLR, toll like receptor; TRIF, Toll-IL-1 receptor domain-containing adaptor inducing IFN-β; USP18, ubiquitin specific peptidase 18; UBP43, ubiquitin-specific protease 43 kDa.

Introduction

Hepatitis C virus

Hepatitis C virus (HCV) infects 130–170 million persons worldwide [1]. HCV is parenterally transmitted, mainly due to injection drug use and unsafe transfusions and therapeutic injections [2]. Acute HCV infections (AHC) are often oligo- or asymptomatic [3]. In 70–80% of those infected, the virus persists and the infection becomes chronic. Spontaneous clearance of HCV is rare in the chronic phase of the infection. In most patients, chronic hepatitis C (CHC) leads to some degree of liver fibrosis, and in 15–25% of patients, cirrhosis develops after 10–40 years [4]. Patients with CHC and cirrhosis are at increased risk of liver failure and hepatocellular carcinoma development [5].

HCV is a positive-strand enveloped RNA virus belonging to the family *Flaviviridae*. HCV isolates are classified into 6 major genotypes (numbered from 1 to 6) that differ in the sequence of the 9.6 kb genome by 30–35% [6]. Within genotypes, subtypes (designated with small letters, e.g., 1a, 1b) differ in their sequence by 20–25%. HCV infects humans and chimpanzees. Hepatocytes are the main target cells of HCV. Virus entry into hepatocytes requires multiple cellular factors including scavenger receptor type B1 (SR-B1), CD81, claudin-1, and occludin [7]. Reliable and widely reproducible methods to detect viral RNA or proteins in liver samples of infected patients are lacking. It is therefore still a matter of controversy what percentage of hepatocytes is simultaneously infected at any given time point during acute and chronic infection with

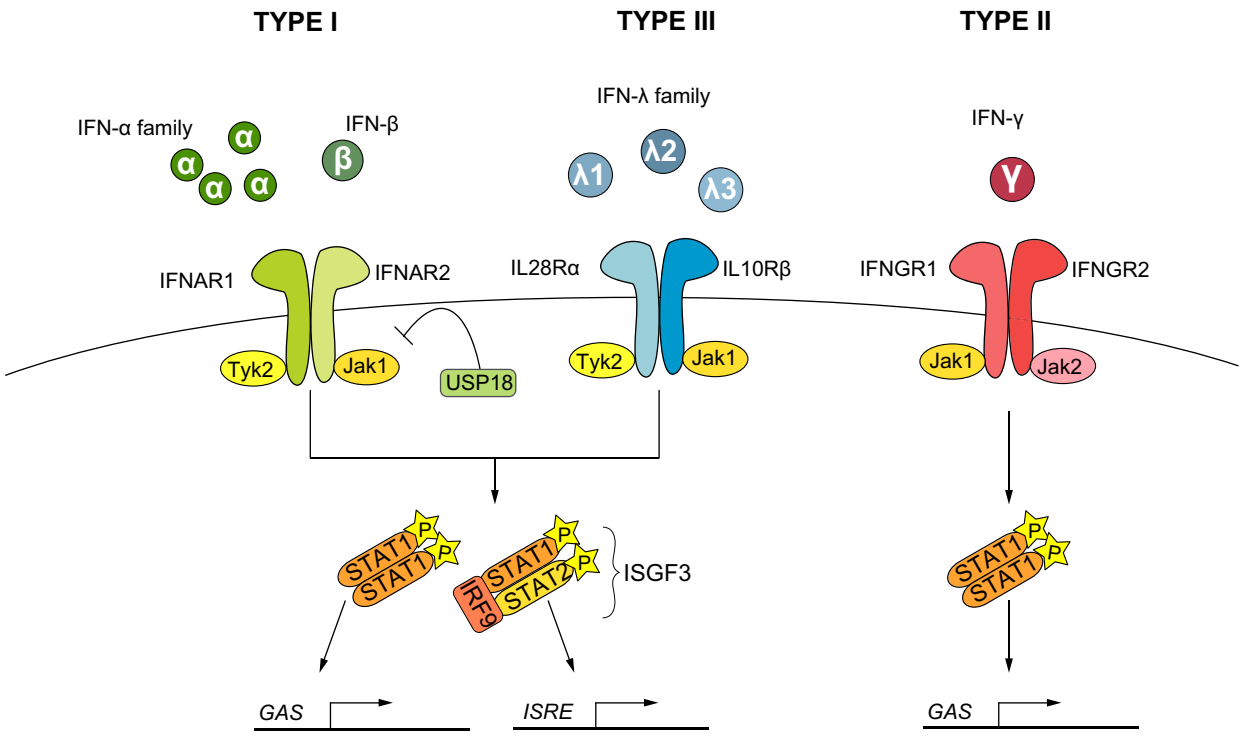

Fig. 1. IFN signaling through the Jak-STAT pathway. Type I (IFN-αs and IFN-β) and type III (IFN-λs) IFNs bind to distinct receptors, but activate the same downstream signaling events, and induce almost identical sets of genes mainly through the activation of IFN-stimulated gene factor 3 (ISGF3) and STAT1 homodimers. IFN-γ (the only type II IFN) activates STAT1, but not ISGF3, and induces a partially overlapping but distinct set of genes. Adapted from Ref. [116], with permission from Elsevier.

HCV. The HCV RNA is translated at ribosomes into a long precursor polyprotein that is then cleaved by cellular and viral proteases into the mature viral proteins [8]. Inhibitors of the viral NS3/4A protease are used for therapy of HCV genotype 1 infections [9,10]. HCV replication occurs in the cytoplasm in specific membrane alterations named the membranous web [8]. Replication is very dynamic: an estimated 10^{12} virions are produced and cleared per day in an infected individual [11].

Innate immunity and interferons

Innate immune responses are the first line of defense against viral infections and interferons (IFNs) are the central cytokines responsible for the induction of an antiviral state in cells and for the activation and regulation of the cellular components of innate immunity such as natural killer (NK) cells [12]. Type I IFNs (comprising several IFN-α and one IFN-β) and type III IFNs (IFN-λ1, -λ2, and -λ3; also designated IL29, IL28A, and IL28B) are produced by cells infected with viruses and by key sentinel cells of the innate immune system: macrophages and dendritic cells (DCs). Type II IFN (IFN-γ) is produced by NK and natural killer T (NKT) cells as part of the innate immune response, and by antigen-specific T cells (both CD4+ Th1 and CD8+ cytotoxic T lymphocytes).

Two important pathways that detect viral genomes and induce type I and type III IFNs have been discovered and characterized during recent years: the toll-like receptor (TLR) dependent pathway [13,14] and the cytosolic pathway triggered by binding of viral RNA to the RNA helicases retinoic acid inducible gene-I (RIG-I) and melanoma differentiation antigen 5 (MDA5) [15,16]. TLRs are a family of transmembrane pattern recognition receptors that recognize microbial pathogen-associated molecular patterns (PAMPs) and activate the expression of genes involved in inflammatory and immune responses [14]. There are at least 10 human TLRs, and 3 of them are involved in the recognition of viral infections: TLR3, TLR7, and TLR9. TLRs are expressed on various immune cells such as macrophages, dendritic cells (DCs), B cells, but also on fibroblasts and epithelial cells. While TLRs involved in the recognition of bacterial components are expressed on the cell surface, TLR3, TLR7, and TLR9 are localized in intracellular compartments such as endosomes. TLR3 recognizes double-stranded RNA [17], TLR7 detects single-stranded RNA [18,19] and TLR9 interacts with unmethylated DNA with CpG motifs [20]. TLR3 activation induces signaling cascades that mainly involve adapter molecules Toll-IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF) and the kinase TBK1. TLR7 and TLR9 signal through MyD88 and the IRAK4-IRAK1-IKKα kinase cascade [21]. Both pathways converge on the activation of the key transcription factors NF-κB and interferon regulatory factor (IRF) 3 and 7. Importantly, macrophages and DCs do not have to be infected by viruses in order to produce IFNs. Instead, they constantly sample material from the outside, including virus containing remnants of apoptotic cells and intact viral particles. Degradation processes in the endosomes then expose viral nucleic acids to recognition by TLRs.

Viruses that avoid the endosomal entry pathway can be detected by the cytosolic pathways of type I and III IFN induction. These signaling pathways are initiated by the recognition of viral 5′ triphosphate RNA and double-stranded RNA by RIG-I and MDA5. Binding of viral RNA induces a conformational change of these sensors that results in the binding to mitochondrial antiviral signaling protein (MAVS, also designated Cardif, VISA,