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World J Gastroenterol 2007 September 28; 13(36): 4818-4823
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

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Interaction of hepatitis C virus with the type I interferon system

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Supported by the Deutsche Forschungsgemeinschaft
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Received: June 26, 2007 Revised: July 9, 2007

Abstract

Hepatitis C virus (HCV) needs to tightly manipulate host defences in order to establish infection. The innate immune response slows down viral replication by activating cytokines such as the type I interferons (IFN-α/β), which trigger the synthesis of antiviral proteins and modulate the adaptive immune system. HCV has therefore developed a number of countermeasures to stay ahead of the IFN system. Here, I will attempt to summarize the current state of research regarding IFN responses against HCV and the viral escape strategies. Particular emphasis will be put on the newly discovered mechanisms HCV employs to avoid the induction of IFN in infected cells.

Key words: Hepatitis C virus; Innate immunity; Interferon system; Escape mechanisms

Weber F. Interaction of hepatitis C virus with the type I interferon system. *World J Gastroenterol* 2007; 13(36): 4818-4823

http://www.wjgnet.com/1007-9327/13/4818.asp

INTRODUCTION

The type I interferon system which mainly involves IFN-α and -β is a powerful and universal intracellular defence system against viruses. Knockout mice which are unresponsive to IFN-α/β due to targeted deletions in the type I IFN receptor quickly succumb to viral infections although they have a regular adaptive immune system[1,2]. Likewise, humans with genetic defects in STAT-1, which is involved in the signaling cascade of the IFN system, die of viral disease at an early age[3].

INTERFERON INDUCTION

All nucleated cells of the mammalian body are able to synthesize and secrete type I IFNs in response to virus infection. Secreted IFNs are then recognized by neighboring cells and cause them to express potent antiviral proteins[4,5]. As a result, virus multiplication is slowed down or even stopped, and the organism buys time for the establishment of an adaptive immune response.

Type I IFNs are classified according to their amino acid sequence and comprise a large number (at least 13) of IFN-α subtypes and a single IFN-β[6], as well as some additional family members[7,8]. Expression patterns, i.e. which IFNs will be synthesized at which time point, mostly depend on the particular cell type.

Fibroblasts secrete mainly IFN-β as an initial response to infection but switch to IFN-α during the subsequent amplification phase of the IFN response[9]. By contrast, dendritic cells, which play an important role in immunosurveillance, directly secrete high levels of IFN-α subtypes[10,11].

Induction of IFN-β gene expression in fibroblasts occurs by the intracellular, so-called "classic pathway" (Figure 1). In infected cells, a signaling chain is activated by viral RNA molecules which are generated during genome transcription and replication[12]. Two intracellular RNA helicases, RIG-I[13] and MDA5[14], act as sentinels for viral RNA[15-17]. Then, a recently discovered adaptor protein binds to RIG-I and MDA5 and mediates the signal to downstream factors. It is called either Cardif for "CARD adaptor inducing IFN-β"[18], IPS-1 for "interferon-β-promoter stimulator 1"[19], MAVS for "mitochondrial antiviral signaling" molecule[20], or VISA for "virus-induced signaling adaptor"[21]. Cardif/IPS-1/MAVS/VISA activates two IκB kinase (IKK)-related kinases, IKKε and TANK-binding kinase-1 (TBK-1), which phosphorylate the transcription factor IRF-3[22,23]. IRF-3 is a member of the IFN regulatory factor (IRF) family and plays a central role in the activation of the IFN-β promoter[24]. Phosphorylated IRF-3 homo-dimerizes and moves into the nucleus where it recruits the transcriptional coactivators p300 and CREB-binding protein (CBP) to

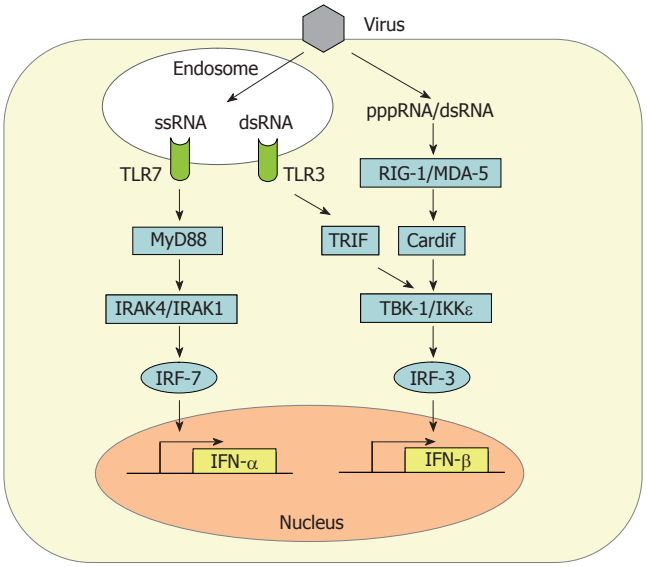

Figure 1 Type I IFN gene expression. Detection of viral ssRNA and dsRNA leads to transactivation of IFN-α and IFN-β promotors by IRF-7 and IRF-3. IRF-3 is phosphorylated by the kinases IKKε and TBK-1 which in turn are activated by the intracellular RNA-sensor proteins RIG-I and MDA5. RIG-I preferentially senses 5'triphosphorylated ssRNAs (pppRNA) whereas MDA-5 recognizes dsRNA. Cardif (also termed IPS-1/MAVS/VISA) serves as an adaptor protein connecting RNA sensing and IRF-3 phosphorylation. A second dsRNA signaling pathway involves endosomal TLR-3 and the adaptor protein TRIF which also activates IKKε and TBK-1. The endosomal ssRNA receptor TLR7 utilizes the adaptor protein MyD88 to stimulate IFN-α synthesis via the kinases IRAK4 and IRAK1 and the transcription factor IRF-7.

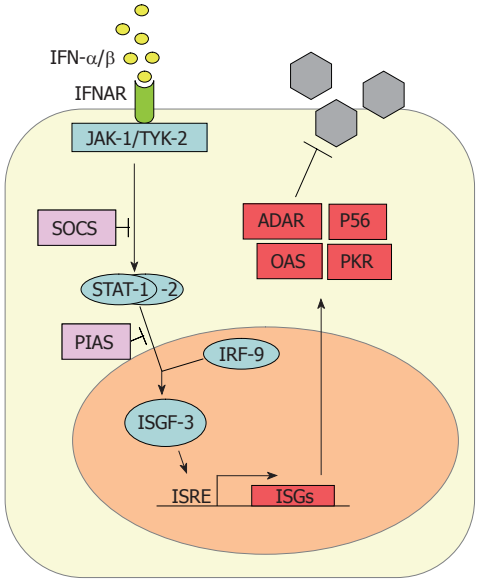

Figure 2 Cellular response to IFNs. Newly synthesized IFN-α/β binds to its cognate receptor (IFNAR) and activates the expression of numerous IFN-stimulated genes (ISGs) *via* the JAK/STAT pathway. ADAR, P56, OAS and PKR are IFN-stimulated gene products with antiviral properties against HCV. The SOCS and PIAS proteins negatively regulate the IFN-induced signaling pathway at different stages.

initiate IFN-β mRNA synthesis[24,25]. This first-wave IFN triggers expression of a related factor, IRF-7, which in fibroblasts is only present in low amounts[26]. IRF-7 can be activated the same way as IRF-3[27-29], leading to a positive-feedback loop that initiates the synthesis of several IFN-α subtypes as the second-wave IFNs[9,30]. In addition, NF-κB and AP-1 are recruited in a dsRNA-dependent way[31,32]. Together these transcription factors strongly upregulate IFN-β gene expression.

Until very recently, it was assumed that the main trigger of intracellular cytokine induction by all viruses is double-stranded RNA (dsRNA) which supposedly forms as a by-product of genome replication. However, we have recently found that some viruses do not produce substantial amounts of dsRNA[33]. Instead, ssRNA containing a 5' triphosphate group is much more potent than dsRNA in activating RIG-I-dependent IFN induction[34-36].

Among the cells of the lymphatic system, myeloid dendritic cells (mDCs)[11] and, most prominently, plasmacytoid dendritic cells (pDCs)[10] are the main IFN producers. In addition to the classical, intracellular pathway of IFN induction described above, pDCs sense the presence of viruses by the extracytoplasmic toll-like receptors (TLRs)[37-39]. It is thought that TLRs serve as sensors for viral infection of phagocytosed cells[40]. Human pDCs mostly express TLR7 and TLR9 which recognize viral single-stranded(ss) RNA and dsDNA, respectively[41], whereas mDCs express TLR3 which responds to dsRNA[42]. Upon activation, TLRs signal through different intracellular adaptor molecules such as MyD88 (TLR7 and 9) or TRIF (TLR3) to induce IFN transcription[41]. Interestingly, DCs already contain high levels of IRF-7[43,44], thus explaining their ability to rapidly produce high amounts of alpha-IFNs. Furthermore, TLR7 and TLR9 are retained in the endosomes of pDCs to allow prolonged IFN induction signaling[45].

INTERFERON SIGNALING

IFN-α/β subtypes all bind to and activate a common type I IFN receptor. It consists of two subunits (IFNAR-1 and IFNAR-2) and is present on virtually all host cells[5,6]. Binding of IFN-α/β leads to heterodimerization of the IFNAR subunits and to conformational changes in the intracellular parts of the receptor which activate the so-called JAK-STAT signaling pathway (Figure 2). The signal transducer and activator of transcription (STAT) proteins are latent cytoplasmic transcription factors which become phosphorylated by the Janus kinase (JAK) family members JAK-1 and TYK-2[46]. Phosphorylated STAT-1 and STAT-2 recruit a third factor, IRF-9 (also called p48), to form a complex known as IFN stimulated gene factor 3 (ISGF-3). The ISGF-3 heterotrimer translocates to the nucleus and binds to IFN-stimulated response elements (ISRE) in the promoter regions of IFN-stimulated genes (ISGs), thereby inducing their transcription.

Several specialized proteins serve as negative regulators and inhibitors of the JAK-STAT pathway. For example, the suppressor of cytokine signaling (SOCS) proteins specifically prevent STAT activation by binding to activated cytokine receptors, inhibiting the activity of JAKs, and targeting bound signaling proteins for proteasomal degradation[47]. Also, the protein inhibitor of activated STAT (PIAS) family members function as small

ubiquitin-like modifier (SUMO) E3 ligases and inhibit the transcriptional activity of STATs[48].

INTERFERON EFFECTOR PROTEINS WITH ANTIVIRAL ACTIVITY AGAINST HCV

IFN-α combined with ribavirin is the standard treatment for HCV infection, and its effect can be potentiated by co-adminitration of IFN-γ[49,50]. IFN-α/β activates the expression of more than 300 IFN-stimulated genes (ISGs) which have antiviral, antiproliferative, and immunomodulatory functions[51,52]. IFN-induced proteins include enzymes, transcription factors, cell surface glycoproteins, cytokines, chemokines and a large number of factors that need to be further characterized. Up to now, only a few antiviral proteins have been characterized in detail. Type I IFNs are known to be effective against HCV replicon systems[53,54], and several IFN-induced proteins have documented anti-HCV activity, namely protein kinase R (PKR)[55], the RNA-specific adenosine deaminase 1 (ADAR 1)[56], the 2'-5' oligoadenylate synthetases (2-5 OAS) / RNaseL system[57], and P56[58].

PKR, ADAR1, and 2-5 OAS are constitutively expressed in normal cells in a latent, inactive form. Basal mRNA levels are upregulated by IFN-α/β and these enzymes need to be activated by viral dsRNA. PKR is a serine-threonine kinase that phosphorylates the alpha subunit of the eukaryotic translation initiation factor eIF2[59]. As a consequence, translation of cellular and viral mRNAs is blocked. ADAR 1 catalyzes the deamination of adenosine on target dsRNAs to yield inosine. As a result the secondary structure is destabilized due to a change from an AU base pair to the less stable IU base pair and mutations accumulate within the viral genome[5]. The 2-5 OAS catalyzes the synthesis of short 2'-5' oligoadenylates that activate the latent endoribonuclease RNaseL[60]. RNaseL, in turn, then degrades both viral and cellular RNAs, leading to viral inhibition[61]. P56 binds the eukaryotic initiation factor 3e (eIF3e) subunit of the eukaryotic translation initiation factor eIF3. It functions as an inhibitor of translation initiation at the level of eIF3 ternary complex formation and is likely to suppress viral RNA translation[62,63].

INTERACTION WITH INNATE IMMUNE RESPONSES

Several recent studies have clarified that the RNA of HCV is a potent trigger of IFN induction, leading to the establishment of an antiviral state. Therefore, in order to establish infection and to persist in the human host, HCV has been forced to evolve efficient counterstrategies. Intracellular IFN induction by HCV appears to be mostly mediated by RIG-I binding to viral RNA[64]. Extracellularly, no specific TLR has been identified yet, but by deduction from data on related flaviviruses, TLR3 and TLR7 would be the most obvious candidates. The dsRNA-binding TLR3 was shown to be activated by West Nile virus[65], and the ssRNA-binding TLR7 is activated by Dengue virus[66]. Moreover, TLR7 can elicit HCV immunity, and a synthetic TLR7 agonist reduced HCV mRNA and protein levels in HuH-7 hepatocytes[67]. It is important to note that TRL7 is expressed in hepatocytes of normal as well as HCV-infected people[67]. Thus, TRL7 may indeed play a role during natural infection.

On the other hand, HCV is capable of disturbing the IFN response at multiple levels[68,69]. With respect to IFN induction, it was recently discovered that the NS3/4A protease specifically cleaves Cardif[18] as well as TRIF[70,71]. Since both these adaptor proteins are important for IFN induction via the classical intracellular pathway (Cardif) and the TLR3-driven endosomal pathway (TRIF), NS3/4A is the key factor of HCV to disturb IRF-3 activation[72] which would otherwise result in IFN gene transcription. In addition, NS3 directly interacts with TBK1 to inhibit its association with IRF-3 and its activation[73].

With respect to the IFN response, it was shown that expression of the full-length virus genome or the core protein suppresses IFN signal transduction[74,75]. Most likely, this is due to an up-regulation of protein phosphatase 2A by ER stress[76], resulting in association of STAT1 with its inhibitor PIAS1[77]. Moreover, for the core protein it was shown that it interferes with the JAK/STAT pathway[78] and is able to activate the JAK-STAT signaling inhibitor SOCS-3[79], further contributing to the HCV-induced block of IFN signaling.

HCV also directly counteracts the antiviral IFN response. The NS5A protein, which confers a multitude of functions in virus replication[80], also plays a key role in escape from the antiviral action of IFN. A stretch of 40 amino acids on NS5A, termed the IFN sensitivity region (ISDR), was correlated with responsiveness to IFN therapy[81-83]. Moreover, NS5A was shown to directly bind to and repress PKR, and this interaction involved the ISDR[84]. However, other groups did not find a connection between viral IFN susceptibility and a particular ISDR sequence[85-87], and PKR activity was not affected by expression of the HCV genome[88] or NS5A[89], although NS5A clearly reduced the antiviral effects of IFN[89]. A possible solution for this discrepancy could be that ISDR sequence variations affect the efficiency of HCV replication[90,91]. Thus, the correlation between particular ISDR sequences and IFN sensitivity could be caused by differences in HCV replication strength. In addition, NS5A induces IL-8 (also termed CXCL-8), a chemokine which inhibits the antiviral actions of IFN[92]. Elevated IL-8 levels were indeed detected in the sera of IFN non-responders[93]. Moreover, in cell culture CXCL-8 protein levels are positively associated with chronic HCV replication and CXCL-8 removal inhibits HCV replication[94]. Interestingly, CXCL-8 cannot only be induced by NS5A, but also by the HCV RNA-sensitive RIG-I pathway[95].

NS5A also interferes with the 2-5 OAS/RNaseL pathway by binding to 2-5 OAS[96]. Furthermore, the HCV genome sequences of IFN-resistant strains have fewer RNase L recognition sites than those of more IFN-sensitive ones[97], thus allowing escape from nucleolytic cleavage[97]. PKR activity is also modified by the internal ribosome entry site (IRES) of HCV[98] and the E2 protein[99].

The multiple countermeasures of HCV to avoid a

fully-fledged IFN response appear to be quite efficient, since 85% of the HCV-infected patients develop a chronic infection, and up to 60% of those patients do not respond to IFN therapy or experience a relapse when therapy is stopped[100]. Our rapidly increasing knowledge about HCV immune escape will certainly lead to a significant improvement in both prevention and therapy for hepatitis C.

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