Cytokine 74 (2015) 190–197

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Insights into antiviral innate immunity revealed by studying hepatitis C virus

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ARTICLE INFO

Article history:
Received 3 March 2015
Accepted 4 March 2015
Available online 25 March 2015

Keywords:
Hepatitis C virus
NS3/4A
MAVS
Interferon
Innate immunity

ABSTRACT

Experimental studies on the interactions of the positive strand RNA virus hepatitis C virus (HCV) with the host have contributed to several discoveries in the field of antiviral innate immunity. These include revealing the antiviral sensing pathways that lead to the induction of type I interferon (IFN) during HCV infection and also the importance of type III IFNs in the antiviral immune response to HCV. These studies on HCV/host interactions have contributed to our overall understanding of viral sensing and viral evasion of the antiviral intracellular innate immune response. In this review, I will highlight how these studies of HCV/host interactions have led to new insights into antiviral innate immunity. Overall, I hope to emphasize that studying antiviral immunity in the context of virus infection is necessary to fully understand antiviral immunity and how it controls the outcome of viral infection.

1. Introduction

HCV is a positive sense, single-stranded (ss) RNA virus of the genus Hepacivirus and family *Flaviviridae*. HCV infects and replicates in hepatocytes within the human liver. HCV infection can result in liver disease, including fibrosis and cirrhosis, can cause hepatocellular carcinoma, and is the leading indicator for liver transplantation [1]. There is no vaccine for HCV; however, recently developed, direct acting antiviral drugs (DAAs) are showing high efficacy towards HCV, although they are incredibly cost-prohibitive [2]. HCV isolates have been classified into 7 different genetic groups, referred to as genotypes, based on their sequences and display sequence diversity of greater than 30% [3,4]. The previous standard of care for hepatitis C was treatment with pegylated IFN-α plus ribavirin and resulted in cure rates of only 40–50% for the most difficult to treat HCV genotypes (1 and 4) [5]. However, the newest standards of care for HCV involve treatment with these newly developed DAAs, sometimes in IFN-free combinations, leading to cure rates of up to 95% in the controlled settings of clinical trials [2]. It is currently unknown if these high cure rates will be maintained following widespread usage of these DAAs. Further, how antiviral resistance will be managed under widespread usage is unknown and needs to be carefully considered.

HCV infection is sensed as foreign or non-self by the host through the antiviral innate immune response. This immune response is triggered shortly after infection in a cell-intrinsic manner by host proteins called pattern recognition receptors (PRRs) that detect specific pathogen-associated molecular patterns (PAMPs) in the virus to activate downstream signaling cascades that drive immunity, including expression of antiviral genes and various cytokines, such as the type I and III interferons and IL-1β. HCV is sensed by multiple PRRs, including members of the RIG-I (retinoic acid-inducible gene I)-like receptors (RLRs), the toll-like receptors (TLRs), and the nucleotide oligomerization domain-like receptors (NLRs) [6]. While the subsequent downstream antiviral response can be directly antiviral to limit virus replication and spread, it can also provide signals to the adaptive immune response for full induction of immunity to virus infection. As many viruses, including HCV, have developed effective countermeasures to inactivate this antiviral response, it is clear that the innate immune response plays an important role in determining the outcome of virus infection [7].

The HCV RNA genome is 9.6 kilobases in length and encodes for a single polyprotein that is processed by host and viral proteases into the 10 structural and non-structural proteins of the virus (Fig. 1). HCV was discovered in 1989 using modern molecular biology approaches and was found to be the causative agent of non-A non-B hepatitis, first described over ten years earlier [8]. Since the initial discovery of HCV, many aspects of the viral life cycle have now been revealed; some of which are now targets for DAAs [9].

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http://dx.doi.org/10.1016/j.cyto.2015.03.007
1043-4666/

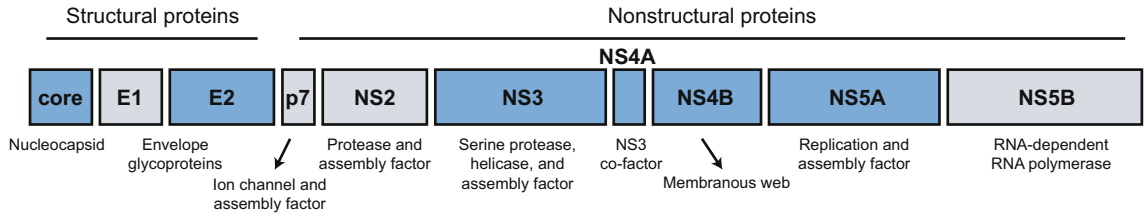

Fig. 1. The HCV proteins. The HCV polyprotein is processed into the structural and nonstructural proteins of the virus, as shown here. The HCV proteins that have been implicated in antiviral innate immune evasion, including core, E2, NS3–NS4A, NS4B, and NS5A, are highlighted in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The initial studies to define the virology of HCV took time to develop because HCV is very difficult to grow in cell culture. For example, it took 10 years after the discovery of HCV to be able to study replicating HCV RNA in a cell culture system [10], and a fully infectious clone of HCV to be used in cell culture was only developed within the last 10 years [11,12]. Current systems for studying HCV have expanded from studying the virus in Huh7 human hepatoma cell lines to using primary human hepatocytes, mice with a chimeric human liver, or mice engineered with various human factors that promote HCV infection [13,14]. Utilization of these systems, including emerging non-chimpanzee animal models for HCV infection [13], will expand our knowledge of the full complement of HCV/host interactions that dictate the outcome of infection.

While we now know many of the important features of both the innate and adaptive immune response to HCV [15], many of these features were unknown when HCV/host interactions were first being studied. These early studies of HCV and antiviral innate immunity were limited by the viral tools and the knowledge of innate immunity available at the time. Even today, we still do not have a full understanding of the complex interactions that govern HCV interactions with the host innate immune response in the infected liver. This review will feature several key discoveries on antiviral innate immunity in the context of HCV to further illustrate how virology research can elucidate fundamental aspects of host cell biology and antiviral immunity [16].

2. Early studies on antiviral immunity to HCV focus on PKR

The first hepatitis C therapies utilized the well-known antiviral cytokine IFN-α, which along with IFN-β is a member of the type I IFN family. In cell culture, type I IFN effectively limits HCV replication, however as a therapy in patients, type I IFN-based therapies have varying levels of effectiveness [5]. Type I IFN signals through the IFN receptor (IFNAR1 and IFNAR2) to drive JAK/STAT signaling that activates the expression of hundreds of IFN-stimulated genes (ISGs) whose encoded proteins limit virus replication and spread. The antiviral mechanisms of action for many of these ISGs, including how they might be antiviral towards HCV, have not yet been fully described [17–19]. The first work demonstrating that HCV induces an innate immune response came from HCV-infection studies in chimpanzees, which found elevated ISGs in the infected chimpanzee livers [20]. Not long after that, the first studies suggesting that HCV might have a way to evade some aspects of this host innate immune system were published. These studies evaluating IFN treatment outcomes in Japanese patients infected with a genotype 1b virus found that sequence heterogeneity within the viral NS5A protein at the interferon sensitivity-determining region (ISDR) could predict IFN treatment outcomes [21,22]. While today we know that the NS5A protein plays diverse roles in the viral life cycle, including regulating HCV assembly versus replication [23], these studies on the NS5A ISDR were the first to reveal a virologic function for the NS5A protein. While it is not entirely clear how the sequence variation at the ISDR in NS5A contributes to IFN-based therapy responses amongst the different HCV genotypes or in human populations of different ancestries [24], these studies set the stage for the subsequent work that identified the mechanisms of how HCV antagonizes the antiviral response.

To identify how HCV antagonized the antiviral innate immune response, studies focused on the antiviral effector proteins that had been characterized to date, including the Mx proteins, 2′-5′ oligoadenylate synthestase, RNAseL, and the double-stranded (ds) RNA-activated protein kinase R (PKR). At the time, PKR was the most extensively studied of these antiviral effector proteins. The antiviral activity of PKR is activated by dsRNA, which stimulates its dimerization, autophosphorylation, and phosphorylation of eIF2α, resulting in a global block to cellular translation [25]. We now know that PKR-sensing of dsRNA also activates a kinase-independent function of PKR that induces the antiviral IFN response [26]. Even in the late 1990s, viral antagonizers of PKR had been described [27]. Therefore, because genetic variation within the HCV NS5A protein predicted the IFN-sensitivity of HCV, it seemed likely that HCV also encoded a viral antagonizer of PKR. The most probable candidate was the HCV NS5A protein. Indeed, the NS5A protein did interact with PKR to disrupt its dimerization and ability to catalyze eIF2α phosphorylation [28,29]. Subsequently, it was also shown that the HCV E2 protein also inhibited PKR activation by acting as a pseudosubstrate through its encoded PKR-eIF2α phosphorylation homology domain [30].

The fact that HCV encodes at least two strategies to restrict PKR function would seem to suggest that preventing the inhibition of translation by PKR would be required for effective HCV replication. However, PKR activation does not actually directly regulate HCV translation because translation of the HCV polyprotein is unaffected by eIF2α phosphorylation [31–34]. This is because HCV translation is directed by an internal ribosome entry site (IRES) within its 5′ untranslated region (UTR) that can use eIF2A for translation initiation instead of eIF2α [35]. While HCV RNA translation is unaffected by PKR activation and eIF2α phosphorylation, we know that during HCV infection the translation of ISGs and/or IFN is suppressed by PKR activation and the subsequent eIF2α phosphorylation [31,32]. Therefore, in the context of an activated IFN system, PKR activation by HCV may allow HCV to evade the antiviral function of ISGs and therefore be a positive regulator of HCV replication. Based on these findings, there appears to be an unexplained role for NS5A and E2 inhibition of PKR function during HCV infection. As HCV encodes these two PKR-antagonizers, PKR suppression must have some beneficial role in the virus life cycle. It is possible that at early times after infection, before a potent IFN signaling response has been activated, PKR inhibition by HCV proteins could relieve the translational suppression of critical host factors required to promote viral replication. However, we know that at later times after infection when the IFN signaling response is activated, PKR is no longer repressed by the E2 and NS5A proteins perhaps because they are involved in other aspects of the viral life