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## Host Neutralizing Responses in Hepatitis C Virus Infection

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**Key words:** hepatitis C virus; virus-host cell interaction; viral entry; neutralizing antibodies

## Abstract

Hepatitis C virus (HCV) is a major cause of hepatitis world-wide. The majority of infected individuals develop chronic hepatitis which can then progress to liver cirrhosis and hepatocellular carcinoma. Spontaneous viral clearance occurs in about 20-30% of acutely infected individuals and results in resolution of infection without sequelae. Both viral and host factors appear to play an important role for resolution of acute infection. A large body of evidence suggests that a strong, multispecific and long-lasting cellular immune response appears to be important for control of viral infection in acute hepatitis C. Due too the lack of convenient neutralization assays, the impact of neutralizing responses for control of viral infection had been less defined. In recent years, the development of robust tissue culture model systems for HCV entry and infection has finally allowed to study antibody-mediated neutralization and to gain further insights into viral targets of host neutralizing responses. In addition, detailed analyzes of antibody-mediated neutralization in individual patients as well as cohorts with well defined viral isolates has enabled to study neutralizing responses in the course of HCV infection and characterize the impact of neutralizing antibodies for control of viral infection. This review will summarize recent progress in the understanding of the molecular mechanisms of antibody-mediated neutralization and its impact for HCV pathogenesis.

## Introduction

With an estimated 170 million infected individuals, hepatitis C virus (HCV) has a major impact on public health (1). HCV is a major cause of hepatitis world-wide. The majority of infected individuals develop chronic hepatitis which can then progress to liver cirrhosis and hepatocellular carcinoma. Treatment options for chronic HCV infection are limited and a vaccine to prevent HCV infection is not available.

HCV is a small enveloped positive-strand RNA virus that belongs to the genus *Hepacivirus* of the *Flaviviridae* family. This virus exhibits high genetic heterogeneity and has been classified into six genotypes and several subtypes. The HCV genome encodes a single precursor polyprotein of about 3,000 amino acids that is cleaved co- and post-translationally by host and viral proteases into functional structural and non-structural proteins. The virion is composed of three different structural proteins: the core protein forming the viral nucleocapsid and two envelope glycoproteins, E1 and E2.

*In vivo*, HCV infects only humans and chimpanzees (2). Each individual is infected with a mixture of distinct but closely related HCV genomes, termed quasispecies. The liver is the primary target organ of HCV, and the hepatocyte is its primary target cell. Replication of the HCV genome has been demonstrated *in vivo* and *in vitro* in liver hepatocytes, and hematopoietic cells including dendritic cells and B lymphocytes (3, 4). HCV establishes persistent infection in the majority of infected individuals despite the fact that it is recognized and targeted by the host's immune system (5).

Viral proteins are recognized as non-self by the host's immune system and induce the production of antibodies. During the natural course of infection, a large number of antibodies targeting epitopes of both structural and non-structural proteins are produced. The vast majority of antibodies induced have no antiviral activity, either because they are elicited by intracellular, degraded or incompletely processed proteins released from dying cells or

because they are directed against epitopes that do not play any role in the virus entry process (6, 7). A small proportion of antibodies termed “neutralizing antibodies” are able to target exposed epitopes of the viral structural proteins and neutralize the infectious virus by preventing or controlling viral infection. This review will summarize the current knowledge about host neutralizing responses in HCV infection. It starts with a brief description of the current model systems allowing the study of neutralizing responses, followed by viral targets of neutralizing antibodies. Finally, neutralizing responses in the course of HCV infection and the impact of neutralizing antibodies for HCV pathogenesis are discussed.

### **Model systems for the study of antibody-mediated virus neutralization**

For many years, studies of host neutralizing responses against HCV had been hampered by the lack of a convenient tissue culture system for HCV entry and infection. In recent years, several *in vitro* models have been developed to study defined aspects of HCV host cell interaction and antibody-mediated virus neutralization: These include recombinant HCV envelope glycoproteins (8, 9), HCV-like particles (10), HCV pseudotyped particles (11-13), and, more recently, cell-culture derived infectious HCV (14-16). Recombinant HCV envelope glycoproteins have been successfully used as a surrogate model to study virus-host cell interaction leading to the identification of putative HCV receptor candidates including CD81 (8), scavenger receptor class B type I (SR-BI) (9) and heparan sulfate (17) as well as antibodies inhibiting cellular binding of envelope glycoproteins (18). HCV-like particles (HCV-LP) generated by self-assembly of the HCV structural proteins in insect cells have been shown to exhibit morphologic, biophysical, and antigenic properties similar to putative virions isolated from HCV-infected patients (10). In contrast to individually expressed envelope glycoproteins E1 and E2, E1/E2 heterodimers of HCV-LPs are presumably presented in a native, virion-like conformation. HCV-LPs have been shown to bind and enter human hepatoma cells as well as primary hepatocytes and dendritic cells in a receptor-mediated manner, therefore representing a useful model system for the study of HCV-host cell

interaction (17, 19-24) including the characterization of antibodies interfering with cellular binding of particles (22, 25).

Retroviral HCV pseudotyped particles (HCVpp) represent a convenient and elegant approach to study viral entry and antibody-mediated neutralization (11, 12). Infectious HCVpp consist of unmodified HCV envelope glycoproteins E1 and E2 assembled onto retroviral or lentiviral core particles (11, 12). HCVpp are produced by transfecting cells with expression vectors encoding the full-length E1/E2 polyprotein, retroviral or lentiviral core proteins, and a packaging-competent retro- or lentiviral genome carrying a marker gene. The presence of a green fluorescent protein or luciferase reporter gene packaged within these HCVpp allows reliable and fast determination of infectivity mediated by the envelope glycoproteins. HCVpp are infectious for certain cell lines of hepatocyte origin, principally Huh-7 cells, as well as for human primary hepatocytes (11, 12). This system has been extremely useful in identifying neutralizing antibodies as well as characterization of the molecular mechanisms of antibody-mediated neutralization (12, 25-30). Vesicular stomatitis viruses (VSV)/HCV pseudotypes expressing HCV E1 or E2 chimeric proteins containing transmembrane and cytoplasmic domains of the VSV G glycoprotein (13, 31) have been developed as another HCV pseudotype model system to study HCV entry and antibody-mediated neutralization. VSV/HCV pseudotypes infect human hepatoma cell lines and sera from HCV-infected chimpanzees or humans neutralize the pseudotype virus infectivity (13, 32). In contrast to retroviral HCV pseudotypes demonstrating strong tropism for liver-derived cell lines, VSV/HCV pseudotypes are generated in relatively lower titer and can infect a broad range of mammalian cell lines, including cell lines not derived from the liver.

Most recently, several laboratories succeeded in establishing the efficient production of infectious HCV particles using a unique clone derived from a viral isolate of a Japanese patient with fulminant hepatitis C (JFH-1) (14-16). Successful infection of naïve Huh-7 and Huh-7-derived hepatoma cells with cell-culture derived HCV (HCVcc) was demonstrated by

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detection of viral proteins and a highly reproducible time-dependent increase of viral RNA in infected cells (14-16). Virus production in Huh-7 cells was dependent on an active viral polymerase and expression of a functional viral envelope containing the HCV envelope glycoproteins E1 and E2 (14-16). Inoculation of naïve chimpanzees with JFH-1 or chimeric J6/JFH-1-derived HCV particles synthesized *in vitro* resulted in viral infection *in vivo* (14, 33) demonstrating the biological significance of this model system. The ability to generate infectious HCVcc of different genotypes – such as the development of chimeric HCVcc (34) or HCVcc derived from HCV genotype prototype 1a strain H77 (35) - certainly improves the scope of the cell culture system for HCV infection. Infection of HCVcc has been shown to be efficiently neutralized by anti-HCV antibodies derived from human sera (14) as well as polyclonal anti-envelope antibodies (34).

The chimpanzee remains the only natural occurring animal model for the study of HCV infection *in vivo*. The clinical course of infection is usually milder in chimpanzees than in humans. However, these animals have provided unique opportunities to study adaptive immune responses to HCV (36). Using the chimpanzee model, antibodies with neutralizing properties have first been described (37, 38). These antibodies were directed against epitopes in the envelope glycoprotein E2 hypervariable region 1 (HVR-1) of HCV and appeared to be isolate-specific. The chimpanzee model has also been used to study protective immunity against re-exposure. Vaccination studies (39) and passive immunization with rabbit anti-sera (38) have shown some protection but infection of chimpanzees with HCV does not provide complete protective immunity against re-infection with homologous or heterologous virus (40-44).

### **Targets of host neutralizing responses**

In recent years, rapid progress has been made in the understanding of the molecular mechanisms of HCV life cycle (Figure 1). Attachment of the virus to the target cell is mediated through binding of HCV envelope glycoproteins to binding factors present on the

host cell surface, such as the glycosaminoglycan heparan sulfate (17, 25). Binding and entry of HCV is believed to be a multistep process involving several attachment and entry factors, such as CD81, SR-BI and claudin-1 (45-47). HCV is most certainly internalized in a clathrin-dependent manner (48-50) and HCV genome delivery into the host cell cytosol prior to HCV replication is pH dependent (48, 51). In analogy to other viral infection, antibodies neutralizing HCV may render virions non-infectious by interfering with different steps of the viral life cycle (52, 53). Binding of the antibodies to the virus may directly block attachment of the virus with the host cell and thus inhibit dissemination of infection. Neutralizing antibodies may also interfere with post-binding steps such as interaction of the virus with host entry factors. If endocytosis is an obligate replicative step, internalization of the virus into the host cell by endocytosis may also cause neutralization. Neutralization of viruses by antibodies may also take place during fusion at the cell surface or in endosomes: neutralizing antibodies may directly interfere with the fusogenic protein, hinder conformational changes necessary for the fusion process or simply obstruct contact between cellular and viral membranes. In addition, neutralizing antibodies may also interfere with viral uncoating or the first steps necessary for viral replication (Fig. 1). The identification and characterization of antibodies targeting distinct steps of viral entry is thus an important strategy for the understanding of the molecular mechanisms of antibody-mediated neutralization.

Using the above described model systems, it could be demonstrated that envelope glycoproteins E1 and E2 are critical for host cell entry and thus represent important targets for virus neutralization. Monoclonal or polyclonal antibodies targeting both linear and conformational epitopes of envelope glycoprotein E2 have been shown to inhibit cellular binding of HCV-LP binding, entry of HCVpp and infection of HCVcc (Table 1) (10-12, 14-16, 19, 20). Several viral epitopes targeted by neutralizing antibodies have already been identified: epitopes of the E2 HVR-1 region (aa 384-410) (12, 18, 27), two epitopes adjacent to the N-terminal region HVR-1 region (aa 408-422 and aa 412-419) (22, 54, 55), the E2 CD81 binding region (aa 474-494 and aa 522-551) (12, 54, 56, 57) and conformational



epitopes within glycoprotein E2 (58-61). These epitopes may represent potential candidate targets for antibodies in passive immunoprophylaxis. Indeed, two studies have demonstrated that monoclonal antibodies directed against conformational epitopes (60) or epitope aa 412-423 exhibited broad cross-neutralizing activity among all major genotypes of HCVpp entry (54) as well as HCVcc infectivity (62). Most recently, at least three epitopes (aa 270-284, 416-430, 600-620) playing a role in membrane fusion processes have been identified in the envelope glycoproteins E1 and E2 (63). Since one epitope (aa 416-430) has been shown to represent a target for monoclonal antibodies efficiently neutralizing HCV infection (54), it is conceivable that membrane fusion may represent another target for anti-HCV antibodies with neutralizing properties.

### **Impact of Virus Neutralizing Antibodies for Pathogenesis of Infection**

HCV RNA is detectable already one week following infection. Despite the rapid onset of viral replication, there is a delay in the appearance of HCV-specific T<sup>+</sup>-lymphocytes and HCV-specific antibodies which only appear several weeks after infection. Patients who spontaneously clear HCV infection have been described to mount a vigorous multi-epitope-specific CD4 and CD8 T-cell responses (64, 65). Antibody-mediated neutralization occurs during HCV infection *in vivo* but the role of antibodies for the control of HCV infection has been difficult to study. Antibody-mediated neutralization has been suggested by study of patients undergoing liver transplantation for HCV- and hepatitis B virus (HBV)-related liver cirrhosis. Infusion of anti-HBs hyperimmune globulin containing anti-HCV appeared to reduce HCV infection in the transplanted liver (66). In addition, HCV-infected patients with primary antibody deficiencies have been reported to have accelerated rates of disease progression (67, 68). Moreover, passive protection against HCV has been demonstrated in a cohort of patients that had been administered immunoglobulin preparations derived from HCV RNA-positive plasma but containing HCV-neutralizing antibodies (69). However, in the majority of patients, HCV infection is established despite the induction of an humoral immune response that targets various epitopes of the HCV envelope glycoproteins (22, 26, 28, 70, 71).

Until recently, functional studies analyzing the neutralizing antibody response during acute and chronic HCV infection using HCV model systems demonstrated a lack of neutralizing antibodies in the majority of patients with acute HCV infection (22, 26, 70, 72). These studies were limited by the fact that the viral surrogate ligand was derived from a different isolate than the virus present in the infected patient thus precluding the detection of isolate-specific antibodies. Most recently, studies using well defined nosocomial or single-source HCV outbreaks with a defined inoculum enabled to study the role of isolate-specific neutralizing antibodies for control of HCV infection in humans. Using the HCVpp model system, two studies have demonstrated that neutralizing antibodies are induced in the early phase of infection by patients who subsequently clear the virus (29) or control viral infection (73). In a well characterized single-source outbreak of hepatitis C, viral clearance was associated with a rapid induction of neutralizing antibodies in the early phase of infection. In contrast, chronic HCV infection was characterized by absent or low-titer neutralizing antibodies in the early phase of infection (29). In addition, patients with resolution of infection were shown to exhibit a broader cross-neutralizing activity of antibodies in the early phase of infection. An impaired ability to cross-neutralize viral variants rapidly emerging during acute infection may thus contribute to viral evasion from neutralizing responses in persistent HCV infection (29). These results suggest that a strong early broad neutralizing antibody response may contribute to control of HCV in the acute phase of infection and assist cellular immune responses in viral clearance. This conclusion is further supported by recent findings for HIV demonstrating that neutralizing antibodies act in concert with antiviral cellular responses for control of HIV infection (74-76). Furthermore, experimental data obtained in animal models have demonstrated that immune control of poorly cytopathic viruses, such as lymphocytic choriomeningitis virus (LCMV) or simian immunodeficiency virus requires a collaboration of both the cellular and humoral arms of the immune system (7, 77). Indeed, gene-targeted mice expressing the immunoglobulin heavy chain of virus-neutralizing antibodies exhibit an accelerated LCMV clearance (75). Applying these findings to HCV infection - another prototype of persistent-prone non-cytopathic viruses - it is conceivable, that both cellular (5,

64, 65, 78, 79) and neutralizing responses (29, 73) may contribute to control of HCV infection during the very early phase of viral infection.

Patients who do not clear the virus develop high-titer and even cross-neutralizing antibodies during the chronic phase of infection (14, 22, 26, 29, 70, 80). Paradoxically, these antibodies are not able to control HCV infection. Viral escape from antibody-mediated neutralization in these patients may occur on several levels: (i) HCV exists as a quasispecies with distinct viral variants in infected individuals changing constantly over time and this variability has been shown to represent a mechanism of escape from antibody-mediated neutralization in the chimpanzee model (26); (ii) the interplay of HCV glycoproteins with high-density lipoprotein and SR-BI has been shown to mediate protection from neutralizing antibodies present in sera of acute and chronic HCV-infected patients (27, 74); and (iii) as shown for other viruses such as human immunodeficiency virus (HIV), escape from neutralizing antibodies may occur through a combination of different mechanisms, for instance point mutations, insertions/deletions or changes in glycosylation patterns of the viral envelope (30, 81) or conformational masking of receptor binding sites following envelope-antibody interaction (82) preventing neutralizing antibody binding (84).

Most recently, it has been shown for a chronic HCV patient who has been meticulously followed-up for 30 years that HCV continuously escapes the host's immune system by repeated mutational changes resulting in loss of recognition of the HCV envelope glycoproteins by antibodies (80). In fact, neutralization of heterologous strains does not reflect neutralization of the viral variants present in the patient's serum at the time of sampling (80). These data suggest that the neutralizing antibody response of the host lags behind the rapidly evolving HCV envelope glycoprotein sequences of the quasispecies population. The fact that envelope glycoprotein sequences and neutralizing antibody specificity change over time suggest that neutralizing antibodies exert selective pressure on HCV evolution. In line with this hypothesis, it has been shown that HCV quasispecies

complexity is associated with the inability to clear HCV infection and development of chronic disease (83).

### **Conclusions and perspectives**

The development of robust tissue culture model systems for HCV infection within recent years has finally allowed to study antibody-mediated neutralization in HCV infection. Rapid progress has since then been made in determining the kinetic and targets of host neutralizing responses in the course of HCV infection. The novel model systems and patient cohorts with well defined viral isolates will now allow the identification of the molecular mechanisms of antibody-mediated neutralization as well as mechanisms of viral escape from host neutralizing responses. The elucidation of these mechanisms will be crucial for the understanding of HCV pathogenesis as well as the development of novel preventive and therapeutic strategies for control of HCV infection.

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**Table 1: HCV epitopes targeted by antibodies interfering with cellular HCV envelope glycoprotein binding, viral entry or infection.** Envelope glycoprotein epitope (position amino acid within the HCV polyprotein) and its potential function with the viral entry process is shown. The experimental system for antibody-mediated inhibition of viral binding, entry or infection is listed together with the respective reference. Abbreviations: CD-conformation-dependent; E1, E2 – envelope glycoproteins E1 and E2; HCV-LP - HCV-like particles; HVR-1 - hypervariable region-1; HCVpp - HCV pseudotyped particles; HCVcc- cell-culture derived HCV; SR-BI – scavenger receptor class B type I

Envelope glycoprotein	Epitope (amino acids)	Potential function	Model system
E1	192 - 226	Membrane fusion	HCV-LP binding <sup>[85]</sup>
	197 – 207		HCV-LP binding <sup>[24]</sup>
	270 - 284		HCVpp entry <sup>[63]</sup>
	313 - 332		HCV-LP binding <sup>[85]</sup>
E2	HVR-1	SR-BI / heparan sulfate binding	Chimpanzee <sup>[38]</sup>
	HVR-1	SR-BI / heparan sulfate binding	E2 binding, HCVpp entry <sup>[18, 27]</sup>
	396 – 407	Membrane fusion	HCVpp entry <sup>[12]</sup>
	408 – 422		HCV-LP binding <sup>[22]</sup>
	412 - 419		HCVpp entry <sup>[55]</sup>
	412 – 423		HCVpp entry, HCVcc infection <sup>[12, 57]</sup>
	416 – 430	Membrane fusion	HCVpp entry <sup>[63]</sup>
	432 – 443		HCVpp entry <sup>[12]</sup>
	436 – 447		HCVpp entry <sup>[12]</sup>
	474 – 494	CD81 binding	E2 binding, HCVpp entry <sup>[56, 86]</sup>
	522 – 551	CD81 binding	E2 binding, HCVpp entry <sup>[56, 86]</sup>
	600 – 620	Membrane fusion	HCVpp entry <sup>[63]</sup>
	640 – 653		HCV-LP binding <sup>[24]</sup>
	644 – 655		HCVpp entry <sup>[12]</sup>
	CD		E2 binding, HCVpp entry <sup>[58-61]</sup>



## Figure Legend

**Figure 1. Potential targets for neutralizing antibodies within the HCV life cycle.** A model of HCV infection with potential targets for virus neutralizing antibodies is shown. Antibodies can potentially interfere with the viral life cycle at different steps: (i) by blocking virus-target cell interaction inhibiting the binding of the virus to putative receptors on the cell surface, (ii) by interfering with post-binding events preventing conformational changes of the virus envelope that are required for subsequent steps in the virus life cycle, and (iii) by preventing the virus uncoating after entry. Abbreviations: ER – endoplasmic reticulum; HS – heparan sulfate; LDL-R – LDL receptor; SR-BI – scavenger receptor class B type I

Figure 1

