

# Host Neutralizing Responses and Pathogenesis of Hepatitis C Virus Infection

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## Abstract

The recent development of novel model systems for the early steps of hepatitis C virus (HCV) infection has rapidly advanced our knowledge of antibody-mediated virus neutralization in patients with acute and chronic hepatitis C. This review summarizes our current understanding of host neutralizing responses during the course of HCV infection. It focuses on recent studies in HCV-infected patients investigating the role of antibody responses for viral clearance as well as the mechanisms of viral escape from virus neutralizing antibodies during progression into chronic infection. Moreover, the potential impact of virus neutralizing antibodies for the development of novel preventive and therapeutic antiviral strategies is discussed.

The hepatitis C virus (HCV) is a small enveloped positive-strand RNA virus that belongs to the genus *Hepacivirus* of the *Flaviviridae* family (1). Spontaneous viral clearance occurs in about 20-30% of acutely infected individuals and results in resolution of infection without sequelae. The majority of infected individuals however develops chronic hepatitis that may progress to liver cirrhosis and hepatocellular carcinoma (2).

Following infection, HCV starts to replicate in the host's hepatocytes and HCV RNA becomes detectable in the serum within 1-3 weeks (3). It is believed that the type and strength of the host's immune responses during the acute phase of HCV infection determines the outcome of infection. While the role of CD4 and CD8 T cells in clearing HCV infection is widely accepted, the role antibodies play in HCV clearance has long remained a matter of debate. Determining the relative contribution of antibodies to HCV clearance has long been difficult due to the absence of convenient *in vitro* model system for evaluating the neutralizing potential of anti-HCV antibodies. The recent development of sensitive and robust neutralization assays based on HCV pseudotyped particles (4-6) has enabled several investigators to study the role of neutralizing antibodies in acute and chronic HCV infection.

HCV pseudotyped particles (HCVpp) consist of unmodified HCV envelope glycoproteins assembled onto retroviral (6) or lentiviral (5) core particles. HCVpp infect human hepatoma cell lines and hepatocytes in an HCV envelope protein-dependent manner. The presence of a green fluorescent protein or luciferase marker gene packaged within these HCVpp allows reliable and fast determination of antibody-mediated neutralization of infection. Another recently developed approach to study virus neutralization consists of recombinant cell culture-derived HCV (HCVcc) infecting human hepatoma cell lines (7-9). Mechanisms of viral entry and neutralization *in vitro* appear to be similar between HCVpp and HCVcc infection (10-13). Interestingly, two studies demonstrated that *in vitro* neutralization in the HCVpp model system correlated with neutralization of serum-derived HCV (4, 14) and most recent results suggest that antibody-mediated neutralization of HCVpp appears to correlate

with neutralization of HCV in the human liver uPA-SCID mouse *in vivo* (T. Vanwolleghem and G. Leroux-Roels, personal communication 2007). Although differences in the export pathway of HCVpp and native HCV may result in consequences for the properties of produced viral envelope (15, 16), the HCVpp system has been used by most investigators since it is characterized by a high robustness and the ability to perform high-throughput assays allowing the quantification of virus neutralization of a large number of patient samples.

The vast majority of antibodies induced in the course of HCV infection have no antiviral activity: they may be elicited by intracellular, degraded or incompletely processed proteins released from dying cells or be directed against epitopes that do not play any role in the virus entry process (17, 18). Antibodies directed against the viral envelope proteins may prevent or control viral infection if they are directed against epitopes implicated in virus entry. These antibodies exhibit antiviral activity in neutralization assays *in vitro* and are thus termed “neutralizing antibodies”. Neutralization by antibodies may be mediated by a number of different mechanisms (17, 19). Neutralizing antibodies may directly block attachment of the virus to the host cell and thus inhibit dissemination of infection. In addition, neutralizing antibodies may also interfere with post-binding steps such as entry of the virus into the host cell as well as transcription of the viral genome. Moreover, neutralizing antibodies may act as opsonins in enhancing phagocytosis of virus particles thereby decreasing viral load. They may be classified as “isolate-specific” or “cross-neutralizing” depending on their ability to neutralize only the “autologous” virus (a defined viral strain present in the patient of interest) or “heterologous” viral strains (strains different from the autologous strain – usually obtained from individuals different from the patient of interest).

#### **Impact of host neutralizing responses on control of HCV infection**

Anti-HCV antibodies become detectable a few weeks (4-14 weeks) after infection (3, 20) (Fig. 1). These antibodies target a wide variety of epitopes of both structural and non-

structural proteins. Early studies pointed to a role for antibodies in the control of HCV infection: HCV-infected patients with primary antibody deficiencies have been reported to have accelerated rates of disease progression (21, 22) – although alterations in T cell function may also have contributed to rapid disease progression in these patients (21, 22). In addition, passive protection against HCV has been demonstrated in different patient cohorts: patients undergoing liver transplantation for HCV- and hepatitis B virus (HBV)-related liver cirrhosis and receiving infusion of anti-HBs hyperimmune globulin containing anti-HCV antibodies (23) as well as patients that had been administered immunoglobulin preparations derived from HCV RNA-positive plasma but containing HCV-neutralizing antibodies (14). Despite these evidence, the role of antibodies in HCV clearance has long been questioned as studies reported cases of resolution of HCV infection in the absence of detectable anti-HCV antibodies in standard diagnostic testing (24) and anti-HCV antibodies present after an initial HCV infection did not prevent re-infection in polytransfused thalassaemic children (25). Other studies involving either patients with post-transfusion hepatitis C, health care workers or young intravenous drug users, failed to show any clear association between the presence of neutralizing antibodies in the acute phase of infection and viral clearance (20, 26, 27). Moreover, a recent study showed a higher frequency of neutralizing antibodies during the acute phase of infection in individuals who subsequently developed chronic HCV infection (28).

Overall, these studies suggest that viral clearance can occur in the absence of neutralizing antibodies. However, the heterogeneous patient cohorts, the absence of a well-defined viral inoculum as well as the fact that the viral surrogate ligands used for neutralization assays were derived from different isolates than the virus present in the infected individuals might have precluded the detection of isolate-specific neutralizing antibodies in HCV infected individuals analyzed in these studies.

Most recently, studies using a well defined viral inoculum and autologous surrogate ligands enabled the study of the role of isolate-specific neutralizing antibodies for control of HCV infection in humans. 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 (30). A study involving hemodialysis patients with nosocomial acquired HCV infection that were followed over 6 months, demonstrated evidence that viral load appears to correlate with the presence of neutralizing antibodies during the acute phase of infection: emergence of strong neutralizing responses correlated with decrease in viremia and control of HCV replication whereas absent neutralizing response associated with persistent high viremia and failure to control HCV infection (30). Moreover, in a well characterized single-source outbreak of hepatitis C in young, healthy pregnant women that have been accidentally exposed to HCV and followed-up for over 25 years, viral clearance was associated in the majority of patients with the rapid induction of high-titer and cross-neutralizing antibodies in the acute phase. In contrast, chronic HCV infection was characterized by a complete absence or reduced capacity to neutralize the transmitted virus as well as heterologous viruses in the early phase of infection (29). However, it is interesting to note that some patients (2 out of 17) were able to clear HCV infection without detection of neutralizing antibodies during the early phase of 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 but also underscore that viral clearance can occur in the absence of neutralizing antibodies. Both viral isolate as well as host-specific factors may have favoured the induction of a potent neutralizing response in this cohort. Interestingly, the association between high-titer neutralizing antibody responses in the acute phase of HCV infection and viral clearance has been confirmed most recently in another group of HCV-infected individuals with intravenous drug abuse where clearance subjects developed high-titer neutralizing antibodies during acute infection, whereas the majority of individuals developing chronic infection had low titer or absent neutralizing antibodies during acute infection (S. C. Ray, personal communication

2007). Taken together, these studies suggest that rapid induction of neutralizing responses may assist in control of HCV in the early phase of infection.

HCV-specific T-lymphocytes appear 5 to 9 weeks after infection with the virus (Fig. 1) and play an important role in both viral control and liver injury. CD4<sup>+</sup> T-cells have major regulatory functions as they help CD8<sup>+</sup> T-cells to eliminate infected cells and B-cells to generate antibodies. Similar to studies showing an association between strong neutralizing responses during the first months post contamination and control of infection (29, 30), it has been demonstrated that patients who spontaneously clear HCV infection mount vigorous multi-epitope-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses (31-34). In line with these results, two recent studies point to a prominent role of CD4<sup>+</sup> T cells in the control of human HCV infection (28, 35). It is interesting to note that the cellular immune responses persist for many years after elimination of the virus (36) whereas neutralizing antibody responses become weak or are lost after viral clearance (29, 36) (Fig. 1). However, waning neutralizing antibody responses may not necessarily enhance susceptibility to new infection since recall responses may exist as shown for host responses to other viral infections (18).

Taken together, these studies suggest that clearance of HCV may be mediated by an orchestrated interplay of cellular and neutralizing immune responses. This is in line with recent studies demonstrating that immune control of other poorly cytopathic viruses, such as lymphocytic choriomeningitis virus or simian immunodeficiency virus/human immunodeficiency virus (HIV) requires a collaboration of both neutralizing antibodies and antiviral cellular responses (18, 37).

### **Viral escape from host neutralizing responses during HCV infection**

In the majority of individuals, the immune system fails to eliminate HCV during the first 6 months after contamination and HCV infection persists. HCV replication, estimated by HCV RNA peripheral blood levels, seems to remain relatively stable in these individuals (Fig. 1).

Persistent HCV infection is characterized by the induction of HCV-specific antibodies directed against both structural and non-structural proteins and able to cross-neutralize heterologous viruses or quasispecies that arise in the course of infection (4, 26, 27, 29, 36, 38, 39). A kinetic study of neutralizing antibody responses against the viral inoculum in a single-source outbreak of HCV infection showed that isolate-specific as well as cross-neutralizing antibodies emerged during the chronic phase of infection (29). Paradoxically, these “neutralizing” antibodies induced during chronic HCV infection are not able to clear the virus. Additionally, an alternative kinetic study of acute mono-infected patients revealed that high-density lipoprotein (HDL) is a serum factor that impairs the efficiency of the cross-neutralizing antibodies that are present during the acute phase of the disease (30, 40). Altogether, these results indicate that HCV has evolved mechanism(s) that counteract the impact of the humoral response during both the acute and the chronic phase of the disease.

In line with these data, it has previously been shown that HCV genome complexity was associated with the inability to clear HCV infection and development of chronic disease: whereas resolving hepatitis C was associated with a relative stable pool of viral variants, progression into chronic infection correlated with diversification of the quasispecies population (41-43). Taken together, these studies demonstrate that the host neutralizing responses are not able to control the circulating pool of viruses during chronic infection.

Using various HCV model systems (for review see (44)) rapid progress has been made in understanding viral escape from antibody-mediated neutralization. A prerequisite of the understanding of these mechanisms has been the identification of various host entry factors mediating the first steps of viral infection (for review see (44, 45)): binding and entry of HCV is believed to be a multistep process involving several host cell surface molecules such as CD81 (46), scavenger receptor class B type I (SR-BI) (47), the glycosaminoglycan heparan sulfate (48, 49), claudin-1 (CLDN1) (50) and the low-density lipoprotein (LDL) receptor (51) (Fig. 2). Most recently, it has been shown that in addition to CLDN1, two other

members of the claudin family, CLDN6 and CLDN9, may also function as HCV co-receptors (52). Kinetic studies have recently demonstrated that heparan sulfate predominantly plays a role during HCV attachment (53) whereas CD81 (53-55), SR-BI (56-58) and CLDN1 (50) promote HCV entry into target cells during post-binding steps. HCV is most certainly internalized in a clathrin-dependent manner (59-61) and HCV genome delivery into the host cell cytosol prior to HCV replication is pH dependent (Fig. 2) (59, 62). By analogy to other viral infections, antibodies neutralizing HCV may render virions non-infectious by interfering with different steps of the viral life cycle such as binding, entry or fusion. Several viral epitopes targeted by neutralizing antibodies elicited by HCV infected individuals have already been identified (reviewed in (63)). However, the mechanisms of antibody-mediated HCV neutralization remain elusive. Most recently, using a panel of monoclonal anti-HCV envelope antibodies, it could be shown that antibodies may neutralize HCV during various steps of HCV entry including binding and post binding events. Moreover, polyclonal anti-HCV immune globulin purified from HCV-infected patients was able to target different steps of viral entry including membrane fusion (A. Haberstroh, E. K. Schnober, M. B. Zeisel, F.-L. Cosset, T. F. Baumert 2007, unpublished results).

Viral escape from antibody-mediated neutralization has been shown to occur on several levels. These include (i) the high variability of the HCV genome and limited induction of cross-neutralization antibodies, (ii) induction of antibodies interfering with neutralizing antibodies, (iii) the association of HCV with serum factors such as LDL and very low density lipoproteins (VLDL), (iv) the interplay of HCV glycoproteins with HDL, (v) the shielding of neutralizing epitopes by glycosylation of defined amino acids of envelope glycoproteins, and (vi) direct cell-to-cell transfer of the virus. Thus, a combination of different mechanisms, e. g. masking of neutralizing epitopes or receptor binding sites by mutational variation or by glycosylation, as described for other viruses such as HIV (64-66) may also apply to HCV.

As HCV replicates through an error-prone viral replicase, this virus exists as a pool of constantly changing, distinct but related genomic variants (a quasispecies) in infected individuals. The immune system is believed to exert constant pressure on these viral variants favouring the emergence of T- and B-cell escape mutants. Recently, the detailed follow-up of the well-characterized chronic HCV patient H, an individual who was infected with HCV in 1977 and was the source of prototype infectious HCV strains H and H77, provided new insights into the time-course of induction of neutralizing antibodies and viral escape from humoral responses (38). By using HCVpp bearing envelope glycoproteins of HCV variants present at different time points during acute and chronic infection, von Hahn and colleagues could demonstrate that neutralization of heterologous strains does not reflect neutralization of the viral variants present in the patient's serum at the time of blood sampling (38). In fact, at a given time point, the "neutralizing" antibodies of this patient were able to neutralize previous HCV strains that had been present several months or years before but not the present or future viral variants of the patient. Several amino-acid changes located in defined B cell epitopes in the HCV envelope glycoprotein sequences could be identified and may explain escape from neutralizing antibody responses (38). This continuous escape from neutralizing antibodies in the course of HCV infection has been recently confirmed by another study investigating evolution of neutralizing antibody responses in acute HCV infection (S. C. Ray, personal communication 2007).

Most recently, it has been shown that anti-HCV antibody containing immunoglobulin preparations comprise competing and/or interfering antibodies in addition to neutralizing antibodies (67). Thorough analysis of epitopes targeted by these immunoglobulins revealed two distinct HCV envelope glycoprotein epitopes but only one epitope was involved in HCV neutralization. In contrast, binding of antibodies to the other epitope abolished viral neutralization by masking the neutralizing epitope (67). These data suggest that HCV induces both neutralizing and non-neutralizing antibodies, the latter being able to interfere

with the function of neutralizing antibodies thereby allowing escape from the host's humoral responses.

Escape from antibody-mediated virus neutralization may also occur by masking of epitopes targeted by neutralizing antibodies through the association of HCV with lipoproteins. It has been demonstrated that HCV exists in heterogeneous forms in human serum and may be associated with VLDL, LDL and HDL (68-71). These viral particles may enter human hepatoma cells through their associated lipoprotein moieties in an HCV envelope glycoprotein-independent manner (72, 73). The association of HCV with these lipoproteins may thus shield the virus from neutralizing antibodies targeting the HCV envelope glycoproteins. Further studies using the recombinant infectious HCVcc associated with lipoproteins (15) may allow to study these mechanisms in the near future.

In contrast to LDL and VLDL, HDL was identified as a serum factor enhancing *in vitro* HCV infectivity (40, 74). This effect has been shown to be mediated by apolipoprotein C1 (26, 75), that resides in HDL, and by the lipid-transfer properties of SR-BI (40, 74), an HDL receptor. Furthermore, the interplay of HCV glycoproteins with HDL and SR-BI is able to mediate protection from cross-neutralizing antibodies present in sera of acute and chronic HCV-infected patients (10, 40, 76). The hypervariable region 1 (HVR1) of the HCV envelope glycoprotein E2, whose variability has been associated with inability to clear the virus (42), appeared to play a major role in this process (77). In fact, infectivity of HCVpp harbouring HVR1-deleted E1E2 glycoproteins was not increased by HDL (40). Deletion of this region in HCVpp that were not efficiently neutralized or cross-neutralized by serum from HCV-infected individuals was able to restore the HCV neutralizing ability of antibodies. Moreover, more antibodies directed against various epitopes of HCV envelope glycoprotein E2 were required to neutralize HCVpp in the presence of HDL whereas this resistance to antibody-mediated neutralization was abolished in HCVpp bearing HVR1 mutations or in conditions where the lipid-transfer activity of SR-BI was abolished. These results suggest that the interaction

between the viral HVR1, HDL and SR-BI provides a mechanism to protect HCV from neutralization by antibodies targeted outside the HVR1 (10, 40, 76).

An additional strategy of HCV to evade the host's neutralizing responses is shielding of epitopes that might be targeted by neutralizing antibodies through glycosylation. In fact, HCV envelope glycoproteins are highly N-glycosylated and this may modulate their immunogenicity. Two recent studies have shown that removal of several glycans from HCV envelope glycoproteins increases antibody-mediated neutralization of HCV entry (78, 79). Helle and colleagues showed that at least 3 specific glycans on HCV envelope protein E2 reduced the sensitivity of HCVpp to antibody-mediated neutralization (79). These glycans also hindered access of CD81 to its binding site. Mutations of these glycosylation sites rendered HCVpp more sensitive to neutralization by serum from HCV-infected patients. This also enhanced interaction of HCVpp with CD81. Similar results have been obtained by Falskowska and colleagues: at least 4 glycans located in the N-terminus of E2 reduced HCVpp sensitivity to neutralization (78). Two out of these 4 glycans also influence CD81 interaction (78). These data suggest that several amino acids located near the CD81 binding domain represent a major target for neutralizing antibodies. Glycosylation of these sites tends to diminish the impact of neutralizing antibodies targeted to these epitopes although the virus also needs to conserve the ability to bind to CD81 in order to enter target cells. This may explain why, unlike what has been shown for HIV, these glycosylation sites on HCV are highly conserved (79). N-linked glycosylations may also limit antibody response to the HCV envelope glycoprotein E1 which is a naturally poor immunogen. Different studies indicated that immunogenicity of this viral protein can be increased by mutating different glycosylation sites (80, 81). Taken together, these studies show that N-glycosylation of HCV envelope glycoproteins can provide protection against virus-neutralizing antibodies.

Finally, HCV may also escape sensing from neutralizing antibodies by infecting surrounding cells by direct cell-to-cell passage. In the majority of cases, viruses infect

surrounding cells after the formation of viral particles that are released from infected cells (cell-free virus) and enter naïve cells by a receptor-dependent mechanism. These viral particles are accessible to neutralization by antibodies. Alternatively, viruses may also use cell-to-cell transfer to infect neighbouring cells (82) thereby escaping potential interaction with neutralizing antibodies. This phenomenon has been described for various viruses, e. g. vaccinia virus, human T cell leukaemia virus type 1, HIV and herpes viruses (reviewed in (82)). A direct cell-to-cell transmission for HCV has most recently been suggested by two different reports (83) (J. Witteveldt and A. H. Patel, personal communication 2007). In one study, direct cell to cell transfer was assessed by co-culture of HCV infected cells with fluorescently labelled naïve cells in the presence of neutralizing antibodies. A higher proportion of fluorescent HCV-infected cells could be demonstrated by flow cytometry when producer cells and target cells were allowed to grow in direct contact as compared to cells grown in distinct compartments separated by a cell-impermeable membrane (83). Experiments using human 293T cells as well as 293T cells transfected with a CLDN1 expression construct suggest that HCV cell-to-cell transport is CLDN1 dependent (83). Moreover, as anti-CD81 antibodies partly inhibited cell to cell transfer, direct cell-to-cell transport may in part also be mediated by CD81 (83). Interestingly, CD81-negative HepG2 human hepatoma cells that are resistant to infection by cell-free HCV were sensitive to HCV infection in this co-culture model system in the presence of virus-neutralizing antibodies suggesting that there are also CD81-independent routes of cell-to-cell transport (83). CD81-independent cell-to-cell transfer was also demonstrated by the second study showing spreading and replication of CD81-binding deficient HCVcc mutants that are unable to enter target cells from the extracellular milieu (J. Witteveldt and A. H. Patel, personal communication 2007). Taken together, these studies demonstrate that HCV might spread through direct cell-to-cell transport by at least two different routes, i. e. CD81-dependent (83) and CD81-independent (83) (J. Witteveldt and A. H. Patel, personal communication 2007), thereby escaping extracellular neutralizing antibodies.

## **Perspectives for preventive and therapeutic strategies based on neutralizing antibodies**

The elucidation of escape mechanisms from adaptive immune responses 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. Moreover, identifying successful immune responses against HCV as those naturally occurring in individuals spontaneously clearing infection might guide efforts to elicit such immune responses by appropriate vaccination. Data from acute and chronic HCV-infected patients as well as studies in chimpanzees suggest that a vaccine should be able to induce vigorous and multispecific B- and T-cell responses in order to control viral infection and prevent progression to chronicity (84).

In recent years, the study of re-exposure to HCV in intravenous drug users who previously cleared HCV infection suggested that at least partial protection from HCV re-infection might exist in humans (85). Even if sterilizing immunity against various viral strains might be difficult to achieve, progression into chronic HCV infection and thus development of cirrhosis and hepatocellular carcinoma may be avoided by a preventive or therapeutic vaccine. Moreover, chimpanzee studies have shown that a broad protective immunity against HCV can be induced as chimpanzees that had previously controlled a first infection were able to clear HCV following re-challenge with homologous or heterologous viruses (86-88). Vaccination studies in chimpanzees showed that different formulations are able to induce at least partial protection of the host against different viral strains and significantly reduced persistent infection (89, 90). Interestingly, it appears that formulations inducing both cellular and humoral immune responses might be more efficient (reviewed in (84)). In addition, deletion of N-glycosylation sites on HCV envelope glycoproteins leads to stronger and broader cellular and humoral immune response in mouse vaccination studies (80, 81, 91) underscoring the importance of unravelling HCV escape mechanisms to aid vaccine design.

Most recently, human monoclonal cross-neutralizing antibodies directed against conserved epitopes of HCV envelope glycoprotein E1 or E2 were identified (11-13, 92). These antibodies were able to efficiently block infection with heterologous HCV in the HCVpp and HCVcc model systems and two of them were able to protect from challenge with heterologous HCV *in vivo* using the human liver-chimeric Alb-uPA/SCID mouse model (92). These results further indicate that a prophylactic vaccine against HCV may be achievable (92).

Developing efficient passive immunization in combination with current or future antiviral therapeutics is another important goal to achieve post-exposure prophylaxis for health care workers and to prevent HCV re-infection during liver transplantation. Chronic hepatitis C is currently a leading indication for liver transplantation. Unfortunately, liver transplantation in HCV infected patients is invariably followed by re-infection of the graft. In addition, recurrent hepatitis C in transplant patients is challenging to treat. The fact that cases of HCV infection occurred through the administration of immunoglobulin preparations derived from HCV contaminated plasma from which anti-HCV antibodies had been excluded, suggest that passively transferred anti-HCV antibodies may prevent viral infection in humans (14). Moreover, Krawczynski and colleagues demonstrated the utility of post-exposure prophylaxis in the chimpanzee model: an immunoglobulin preparation derived from the plasma of HCV-infected patients allowed rapid clearance of viremia and prevented development of acute infection in the treated animals (93). Post-transplant immunotherapy with anti-HCV neutralizing antibodies might thus represent an efficient strategy to prevent liver graft re-infection. This approach is already successfully used to prevent re-infection of liver grafts in HBV-infected patients (94). Unfortunately, studies using anti-HCV antibodies in liver transplant patients have not been successful to date (reviewed in (95)). It has been suggested that this might have been due to insufficient antibody doses or frequency of administration (95). Moreover, it has been suggested that the presence of non-neutralizing antibodies in some anti-HCV antibody containing immunoglobulin preparations may mask

epitopes for neutralizing antibodies thereby rendering these preparation less efficient (see escape mechanisms described above) (67). Enrichment of anti-HCV antibody containing immunoglobulin preparations with efficient neutralizing antibodies by depletion of interfering antibodies might allow to improve the efficiency of immunotherapy in HCV-infected patients (67). Finally, the recent production of human monoclonal antibodies efficiently cross-neutralizing HCV may represent an important step for the development of immunopreventive strategies against HCV infection (11-13, 92).

Taken together, substantial progress has been made in studying neutralizing responses in the course of HCV infection and recent studies underscore the importance of neutralizing antibodies in modulating HCV pathogenesis. Advancing the understanding of the mechanisms of antibody-mediated virus neutralization should ultimately aid the development of new preventive and therapeutic antiviral strategies against HCV infection.

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## Figure Legends

**Figure 1.** Neutralizing humoral and cellular immune responses in resolving and chronic HCV infection. Resolving HCV infection is associated with rapid induction of virus-neutralizing antibodies as well as strong multispecific T-cell responses. However, elimination of HCV has also been documented in the absence of neutralizing antibodies. Chronic HCV infection is characterized by absent or low-titer neutralizing antibodies as well as weak and narrow T-cell responses during the early phase of infection and high-titer cross-neutralizing antibodies unable to control circulating isolates arise during the chronic phase of infection.

**Figure 2.** Mechanisms of viral escape from antibody-mediated neutralization during HCV entry. HCV binding and entry is a multistep process: following attachment of viral envelope glycoproteins E1 and E2 to host cell surface molecules, HCV is internalized into the host cell via clathrin-mediated endocytosis. Decreasing pH in the endosome leads to fusion between viral and endosomal membranes and to the release of the viral genome into the cytoplasm (adapted from reference (96)). Viral escape from antibody-mediated neutralization has been shown to occur amongst others including (1) the interference of non-neutralizing antibodies blocking binding of neutralizing antibodies to viral epitopes, (2) association of HCV with lipoproteins including low density lipoprotein (LDL) and very low density lipoprotein (VLDL) that may mask neutralizing epitopes; (3) the interplay of HCV with high density lipoprotein (HDL) and SR-BI that is able to mediate protection from neutralizing antibodies; (4) the shielding of neutralizing epitopes by glycosylation of amino acids of HCV envelope glycoproteins; and (5) direct cell-to-cell transfer of the virus. HS - heparan sulfate, LDLR - LDL receptor, SR-BI - scavenger receptor class B type I, CLDN1 - claudin-1, X – not yet identified host factor(s) for HCV binding and entry.