

Hepatitis C Virus

Marian Major, PhD



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The HCV particle is approximately 50nm in diameter and consists of an envelope derived from host membranes into which are inserted the virally-encoded glycoproteins (E1 and E2) surrounding a nucleocapsid and a positive-sense, single-stranded RNA genome of approximately 9,500 nucleotides. The genome contains highly conserved untranslated regions (UTRs) at both the 5' and 3' termini which flank a single open reading frame encoding a polyprotein of ~3,000 amino acids. This is processed co- and posttranslationally by cellular and viral proteases to produce the specific viral gene products. The structural proteins, core, E1 and E2, are located in the N-terminal quarter with the non- structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) in the remaining portion of the polyprotein. HCV isolates are classified based on sequence homology into 6 distinct clades. Clinical and virologic data have not uncovered significant phenotypic differences between the groups. It appears that patients infected with types 1a and 1b respond somewhat less well to interferon treatment and are associated with more rapid progression to chronic liver disease than those infected with other genotypes. It must be remembered however that all types so far isolated have the potential to cause serious liver disease.

All natural isolates of HCV exist as quasispecies with variation throughout parts of the genome. An unusually high degree of amino acid variation has been observed in the N terminus of the E2 protein. This hypervariable region, referred to as HVR1, is located between aa 384-410 of the polyprotein (aa1-27 of E2). The HVR 1 varies within anyone isolate and between different genotypes. It should be stressed that no one HVR1 sequence pattern has been associated with any particular genotype nor with any particular outcome of disease, therefore, this region cannot be used to make distinctions at this level.

The NS2 region forms part of a protease that also includes the N terminus of NS3. This mediates autoproteolytic cleavage at the NS2/NS3 junction of the polyprotein. The NS3 also encodes serine protease, nucleotide triphosphatase and RNA helicase activities. The serine protease activity has been particularly well characterized and probably plays an essential role in HCV processing, making it a desirable target for antiviral drugs. The NS4 region encodes NS4A and NS4B. The NS4A protein functions as a cofactor in NS3 activity. It is essential for the NS3/4A and NS4B/5A cleavage events and enhances cleavage at the NS4A/4B and NS5A/5B sites. The function of NS4B, a very hydrophobic protein, is unknown although it is required for the phosphorylation of NS5A together with NS4A and NS3. The primary function of NS5A is also unknown although from comparative studies with other viruses it is predicted to play a role in RNA replication. NS5B forms the C-terminal protein of the HCV polyprotein and is thought to represent the viral RNA polymerase involved in RNA replication (1).

One of the major hurdles in testing both neutralizing antibodies and antiviral drugs to combat HCV infection is the lack of an effective cell culture system for the virus. The data regarding the functions of HCV genes and replication of the virus have been derived using established mammalian cell expression systems or the chimpanzee animal model. There have been several reports of mammalian cells able to support the growth of HCV. These systems rely on the use of reverse transcriptase-PCR (RT-PCR) for the detection of virus and in particular strand specific RT-PCR as evidence of virus replication (1).

Alternative approaches to studying virus assembly or the role of viral antigens in replication have involved the production of virus-like particles or pseudo- type VSV particles containing E1 or E2. A subgenomic replicon containing most of the nonstructural proteins of HCV can replicate to high levels in a hepatoma cell line and this systems may provide the best means yet for defining functional HCV replication units and testing of antiviral drugs (2, 3).

The chimpanzee (*Pan troglodyte*) is the only proven animal model for HCV infection and disease. Evidence for a lack of protective immunity against HCV, the role of HVR 1, and insights into possible vaccine strategies has been gained from chimpanzee studies. In most aspects, the virologic and clinical presentation of acute HCV infection is similar in humans and chimpanzees. This includes onset of disease, level of viremia and timing of serologic response and liver damage, as indicated by ALT elevation. There are, however, some differences. Only about 50% of chimpanzees develop persistent HCV

infections compared to as many as 80% of humans.

HCV-specific antibodies are generally detectable 7 to 31 weeks after infection. The humoral immune response is usually multispecific and targeted against epitopes within the HCV core, NS3 and NS4 proteins. Very little antibody appears targeted to the envelope proteins during acute infection. A major problem in assessing the relevance of antibody responses is the lack of a convenient neutralization assay for HCV. As in other viral infections, antibody binding could interfere with HCV entry into host cells or viral replication and it could opsonize virions for elimination by macrophages. Evidence for a protective role of antibodies stems from limited studies in which chimpanzee or tissue culture infectious HCV was neutralized *in vitro* by incubation with envelope-specific antibody (1, 3).

The cellular immune response is thought to play a particularly important role in the host's defense against HCV: Most analyses, performed so far, have concentrated on the induction, effector function and maintenance of HCV-specific CD4⁺T helper cells and CD8⁺ cytotoxic T cells. However, the induction of T cells targeting the liver introduces the problem of immune mediated liver disease. These cells, that are estimated to represent approximately 1 % of the total body lymphocytes, are continuously recruited from the peripheral blood and do not undergo clonal expansion in the liver. The mechanisms that regulate T cell induced viral clearance versus T cell induced liver injury are not completely understood. Liver infiltrating, HCV specific T cells are Th1-dominant and have been shown to produce IFN-gamma and TNF-alpha. These cytokines can suppress replication and gene expression of other viruses such as HBV without provoking liver disease. Thus, it is possible that the hepatitis C virus is less susceptible to these cytokines so that destruction of infected hepatocytes plays the predominant role and promotes chronic inflammatory liver disease (1).

The mechanisms whereby HCV circumvents the immune response, persists and causes chronic liver disease are currently undefined. One hypothesis is that the HCV-specific immune response is too weak to clear HCV from all infected hepatocytes once a persistent infection is established. Recent reports indicate that individual HCV proteins may actively suppress the immune response of the host specifically, core, E2, and NS5A. Numerous regulatory roles of HCV core that affect signal transduction, expression of viral and cellular genes, cell growth and proliferation are known. In addition, E2 and NS5A have been shown to interfere with the antiviral actions of interferon-alpha. A third explanation for HCV persistence could be the high genetic variability of the virus. Recent studies suggest that circulating HCV has a half-life of only about 3hr indicating relatively efficient virus replication and release, at least in patients with high levels of viral load. This dynamic process, capable of continuously generating viral variants, allows genetic variation to be an important strategy for the establishment and maintenance of persistent infection in order to adapt quickly to humoral or cellular immune selection pressure.

HCV presents a nearly perfect target for an antiviral drug approach to therapy. Hepatitis C is a chronic disease that progresses slowly over many years. Therefore there is a long period of time in which antiviral therapy could be initiated as well as a long treatment window when therapy might be effective. The viral genome codes for several enzymatic activities vital for replication and which are quite distinct from human analogues. These include proteases, a helicase and the RNA dependent RNA polymerase which all could be targeted by inhibitors. One of the most promising approaches to anti-hepatitis C virus drug discovery is the development of inhibitors of the virally encoded protease, NS3. This chymotrypsin-like serine protease is essential for the maturation of the viral polyprotein, and efficient enzymatic activity requires complex formation between NS3 and its cofactor NS4A.

Prevention of hepatitis C at present is based on prevention of exposure to contaminated blood by screening of blood and plasma donors, identification of carriers by testing high-risk individuals and public health measures designed to prevent spread. The common tests used for diagnosis of HCV infection are based on detection of serum antibody to various HCV antigens. Acute HCV infections are relatively rare amongst blood donors but the antibody tests often fail to detect these persons in the window period between the time of infection until the time of appearance of antibody detectable by the assay. Therefore, the antibody assays also have limited utility for diagnosis of patients with acute HCV infections. Tests for HCV RNA genome detection based on the polymerase chain reaction (PCR) or other highly sensitive RNA detection systems have been developed for the diagnosis of acute hepatitis.

No effective vaccine has been developed to prevent HCV infections as the virus itself presents numerous problems. The lack of a suitable *in vitro* culture system makes production of vaccine quantities of whole virus impractical. The genetic diversity of

the virus also complicates vaccine development. A vaccine based on the EI and E2 glycoproteins was tested in chimpanzees. The vaccine produced an antibody response to EI/E2 but it was short lived and required frequent boosting (4). It is possible that a vaccine that only prevents chronic infections would be useful. Such a vaccine would prevent the most serious problems associated with chronic HCV and would greatly reduce the chance of transmitting the virus to others.

The challenges to the development of an effective HCV vaccine may require non-classical approaches. A strong T cell response including both T helper cells and cytotoxic T lymphocytes have been shown to be active for both clearing and preventing infection in chimpanzees. It has been suggested that specifically inducing CTL responses to well conserved epitopes included in non-structural proteins, may be important for a prophylactic vaccine. Several laboratories are now studying this possibility in the chimpanzee model although a prophylactic vaccine of this design is still a long way from being available for humans.

References

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