Hepatitis C virus (HCV) is the major causative agent of chronic non-A, non-B hepatitis. The life cycle of HCV is largely unknown because a reliable culture system has not yet been established. HCV presumably binds to specific receptor(s) and enters cells through endocytosis, as do other members of Flaviviridae. The viral genome is translated into a precursor polyprotein after uncoating, and viral RNA is synthesized by a virus-encoded polymerase complex. Progeny viral particles are released into the luminal side of the endoplasmic reticulum and secreted from the cell after passage through the Golgi apparatus. Understanding the mechanisms of HCV infection is essential to the development of effective new therapies for chronic HCV infection. Several host membrane proteins have been identified as receptor candidates for HCV. Recent advances using pseudotype virus systems have provided information surrounding the initial steps of HCV infection. An HCV RNA replicon system has been useful for elucidating the replication mechanism of HCV. In this review, we summarize our current understanding of the mechanisms of HCV infection and discuss potential antiviral strategies against HCV infection.

Keywords: HCV, infection, receptor, pseudotype virus, replication, assembly, antiviral target
HCV has single-stranded, positive-sense RNA as a genome. The viral RNA acts as messenger RNA and encodes a large precursor polyprotein. The viral RNA does not possess cap structure in the 5’ end but has an internal ribosome entry site (IRES) sequence. Viral structural proteins core, E1 and E2 proteins and p7 are processed by signal peptidase (black arrow head); other non-structural proteins essential for replication are processed by NS2 and NS3 proteases.
HCV are trapped by glycosaminoglycans (GAGs) and then transferred to a cell surface receptor and/or coreceptor and internalized into cells through endocytosis. Acidification leads to conformational changes of the HCV envelope proteins into a fusion-competent state and induces the fusion of viral membranes with host membranes. Fusion allows the viral genome to be liberated into the cytosol. After uncoating, viral RNA is translated into a precursor polyprotein that is processed into each viral protein by cellular and viral proteases, and replication takes place by the viral polymerase complex on the ER membrane. Core protein binds to the viral sense RNA and forms the nucleocapsid. HCV particles seem to bud into the ER lumen after the interaction of the nucleocapsid with E1 and E2 proteins. It was suggested that the small, hydrophobic peptide p7 is important for viral budding. HCV particles are released from the plasma membrane through the secretory pathway.
was shown to be essential for virus budding (Harada et al., 2000). NS2 is a membrane-spanning protein with four transmembrane regions (Yamaga & Oo, 2002). No functional role for NS2 has yet been described, except for its autoprotease activity cleaving between the junction of NS2 and NS3. NS3 forms a complex with NS4A on the ER membrane. This interaction stabilizes NS3 and retains it on the ER (Wolk et al., 2000). NS3 has serine protease and RNA helicase activities assigned to the N-terminal one-third and remaining two-thirds of the protein, respectively (Dubuisson et al., 2002). Cleavage sites downstream of NS3 are processed by NS3 to yield mature nonstructural proteins (Bartenschlager, 1999; Grakoui et al., 1993; Tomei et al., 1993). NS4B is localized to the ER (Hugh et al., 2001) and was reported to induce membranous web formation together with other non-structural proteins (Egger et al., 2002). Although mutation of NS4B affected the hyperphosphorylation of NS5A (Koch & Bartenschlager, 1999), the function of NS4B in terms of viral replication remains to be defined. NS5A is anchored on the ER through its N-terminal 30 amino acids (Brass et al., 2002). NS5A was found to be a highly phosphorylated polypeptide that may be involved in resistance to the antiviral effects of interferon (IFN) alpha (Enomoto et al., 1995; Enomoto et al., 1996; Hale et al., 1998; Hale et al., 1997). NS5A plays an important role in viral replication, because mutation of the phosphorylation sites of NS5A results in enhanced replication of the HCV replicon in the Hu-7.5 cell line (Blight et al., 2000; Guo et al., 2001; Krieger et al., 2001; Lohmann et al., 2001). NS5B is also anchored in the ER through a C-terminal hydrophobic region, and the main body of the replication complex serves as an RNA-dependent RNA polymerase (Schmidt-Mende et al., 2001).

Recent advances in the treatment of chronic HCV infection and antiviral agents and their targets were recently described in a review by Walker and colleagues (Walker et al., 2003). Here, we seek to summarize recent studies investigating candidates for HCV receptor or co-receptor and in vitro systems to facilitate our understanding of the life cycle of HCV.

**Initial stage of HCV infection**

Host surface molecules required for virus entry are classified as either receptors or co-receptors. Several viruses utilize only one molecule as a receptor for entry into host cells, while many viruses require a co-receptor that localizes near the receptors for their entry. Receptors are primarily involved in the attachment of virus to specific host cells and, in some cases, in viral entry. A longevity for a particular receptor may restrict host range, tropism and pathogenicity, although different viruses that have different or partially crossed pathogenicity may utilize the same receptor. Receptors need not be membrane proteins, as carbohydrates and lipids have been identified as receptors for different viruses. For HCV, several molecules have been reported to be candidates for receptor or co-receptor for viral entry. However, the critical determination of receptor or co-receptor requirements for HCV is quite difficult because a reliable in vitro cell culture system and a sufficient amount of viral particles are not currently available. Comparisons with other flaviviruses indicate that HCV E2 protein probably plays a role in receptor binding. To identify potential receptor molecules for HCV, soluble envelope protein was used as a probe to screen an expression library. This screen identified several candidates for an HCV receptor that exhibited direct binding to E2 protein and are known membrane proteins. A pseudotype system based on vesicular stomatitis virus (VSV) or retroviruses has also been used to identify putative receptors for difficult-to-culture viruses. This system is a powerful technique for examining binding to cells and also internalization mechanisms.

**Human CD81**

Following binding of a cell surface receptor, it is thought that HCV enters the cell through endocytosis. Pileri and colleagues (Pileri et al., 1998) first reported human CD81 (hCD81) as a candidate for the HCV receptor. Using a cDNA library derived from a human T cell lymphoma cell line, they used soluble HCV E2 protein as a probe. The soluble extracellular domain of E2 could bind to hCD81, a 25-kDa tetraspan membrane protein widely expressed in haematopoietic and epithelial cells. E2 was reported to lie between amino acids 384–746 in the polyprotein. The second extracellular loop of hCD81, EC2, is responsible for binding with the E2 protein (Pileri et al., 1998), while amino acids 480–493 and 544–551 of E2 were involved in the hCD81-binding site (Flint et al., 1999). In particular, amino acid 186 of hCD81 is critical for E2 binding and is one of three amino acids that differ from African green monkey CD81, which does not support E2 binding (Higginbottom et al., 2000). In contrast, E2 could bind to tamarin CD81 (the same amino acid at position 186 with hCD81) with higher affinity than hCD81, suggesting that species permissiveness to infection is not due to C8D1.

The most important question to address is whether expression of hCD81 alone is sufficient for HCV infection. Replacement of mouse CD81 with hCD81 did not confer susceptibility to HCV infection (Masciopinto et al., 2002). Human CD81 could bind to HCV E2 with a Kd value of 1.8 nM; however, it may simply serve as an attachment molecule because hCD81 does not efficiently internalize ligands (Petracca et al., 2000). HCV E2 and a pseudotype VSV expressing chimeric HCV E1 and E2 proteins also bound to the surface of the hepatoma cell line HepG2, a line that does not express detectable levels of hCD81 (Matsuura et al., 2001; Petracca et al., 2000). Recently, a
pseudotype retrovirus carrying authentic HCV envelope proteins was reported by Cosset and colleagues (Bartosch et al., 2003); they indicated that soluble recombinant hCD81 could neutralize the interaction of the pseudotype retrovirus with hepatoma cell line HuH-7. However, expression of hCD81 on nonpermissive cell lines did not confer susceptibility to either the pseudotype retrovirus or the pseudotype VSV (Bartosch et al., 2003). An assay, referred to as neutralization of binding (NOB), has been developed to assess the ability of antibody to inhibit the interaction between soluble truncated E2 and hCD81 (Rosa et al., 1996). The appearance and maintenance of high NOB antibody titres was correlated with clinical resolution of liver disease and viral clearance (Ishii et al., 1998). Thus, other molecules similar to hCD81 may contribute to binding and/or entry of HCV to host cells. Another possibility is that hCD81 is one component of the HCV receptor complex, as described below.

**LDL receptor**

The low-density lipoprotein (LDL) receptor was reported as a candidate for the HCV receptor. Serum fraction composed of HCV with LDL, or very low-density lipoprotein (VLDL), was involved in binding to the LDL receptor (Agnello et al., 1999). It has been suggested that other members of the Flavivirus family also utilize the LDL receptor for viral entry (Agnello et al., 1999). A polipoprotein B and apolipoprotein E integrated in LDL or VLDL are essential for binding to the LDL receptor, because antibody against apolipoprotein B or apolipoprotein E could inhibit the interaction between HCV and LDL receptor (Agnello et al., 1999). Interestingly, the interaction between HCV particles and the LDL receptor was not dependent on the presence of the E2 envelope protein (Wunschmann et al., 2000). A low-density fraction containing HCV genome has high infectivity. Viral particles containing apolipoprotein B, HCV core and viral RNA could bind to a hepatoma cell line in an apolipoprotein-dependent manner (Ngadre et al., 2002). However, HCV-like particles prepared from insect cells infected with a recombinant baculovirus exhibited binding to a hepatoma cell line through a pathway independent of the LDL receptor and hCD81 (Triyatniet al., 2002). In addition, VLDL and LDL could not inhibit the interaction between the hepatoma cell line and the pseudotype retrovirus composed of authentic HCV envelope proteins (Bartosch et al., 2003; Hsu et al., 2003b). The role of the LDL receptor in HCV pathogenesis remains obscure.

**DC-SIGN and homologues**

Dendritic cell-specific intracellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), a C-type lectin, is reported to serve as a receptor for human cytomegalovirus (Halay et al., 2002), ebola virus (Alvarez et al., 2002; Colmenares et al., 2002), HIV-type 1 (Geijtenbeek et al., 2000), Mycobacterium tuberculosis (Gijtenbeek et al., 2003), and Leishmania amastigotes (Colmenares et al., 2002). Two pathogens interact with DC-SIGN through high mannose oligosaccharides. Two papers reported that DC-SIGN and related molecules L-SIGN and DC-SIGNR are utilized as HCV receptors (Gardner et al., 2003; Pohlmann et al., 2003). Soluble E2 protein and a pseudotype HIV bearing chimeric HCV envelope proteins fused with the transmembrane region of sindbis virus envelope protein, could bind to DC-SIGN and DC-SIGNR (Pohlmann et al., 2003). Their interactions were inhibited by mannose molecules and antibodies against the receptor candidates, suggesting that DC-SIGN and its homologues recognize the oligosaccharides of HCV envelope proteins (Pohlmann et al., 2003). DC-SIGN and DC-SIGNR are expressed in liver sinusoidal endothelial cells but not hepatocytes (Pohlmann et al., 2003). These receptors may play an important role in hepatocyte infection with HCV through sinusoidal endothelial cells. Interestingly, dengue fever virus also utilizes DC-SIGN as a receptor in dendritic cells (Tassaneetrithep et al., 2003). Although chimeric HCV E2 envelope protein and pseudotype HIV bearing the chimeric HCV envelope proteins could bind to cells expressing DC-SIGN or DC-SIGNR (Pohlmann et al., 2003), the pseudotype HIV carrying authentic HCV envelope proteins was not internalized (Hsu et al., 2003b). DC-SIGN and DC-SIGNR may serve as attachment proteins, but it does not appear that either is the entry receptor for HCV.

**Human scavenger receptor, class B type I**

Scarselli and colleagues reported another putative receptor using cross-linkers (Scarselli et al., 2002). E2 molecules of HCV types 1a and 1b bind to a hepatoma cell line independently of hCD81. They tried to isolate the protein that binds to soluble E2 protein in the hepatoma cell line HepG2 (Scarselli et al., 2002). Using cross-linkers, soluble E2 was found to associate with lipid raft-located proteins and, based on expected molecular weights, the scavenger receptor class B type I (SR-B1) was identified as a potential binding partner for HCV E2. E2 of type 1a strain H 77 could bind to human SR-B1, but not mouse SR-R1, through its hyper variable region 1 (HVR1). Human SR-B1 is expressed on the hepatoma cell line HepG2, which does not express hCD81, suggesting that human SR-B1 may be involved in the binding of HCV to cells in a hCD81-independent manner. However, HVR1 is not essential; an HCV cDNA clone lacking HVR1 was found to infect chimpanzees (Forns et al., 2000). Overall, the involvement of human SR-B1 in HCV infection is not clear.
Pseudotype viruses for characterization of HCV infection

VSV has a non-segmented 11 kb genome of negative stranded RNA. The VSV genome is transcribed in the cytoplasm and codes five structural proteins. Recombinant VSV, in which native envelope protein G is replaced with other membrane proteins, could contribute to the study of viruses that inefficiently replicate in experimental systems. Additionally, such pseudotype viruses could lead to the induction of cellular and humoral host immunity (Schnell et al., 1996). Several reports describe the characterization of VSV recombinants bearing HCV envelope proteins. HCV E1 and E2 proteins are retained in ER by C-terminal retention signals (Cocquerel et al., 1999; Cocquerel et al., 1998).

In the pseudotype VSV system, envelope proteins should be expressed on cell surface because VSV buds from the plasma membrane. Pseudotype VSVs bearing chimeric envelope proteins, comprised of the ectodomains of HCV envelope proteins with the signal sequence, transmembrane and cytoplasmic regions of VSV G envelope protein, were constructed (Matsuura et al., 2001). The chimeric E1 and E2 proteins were translocated onto the cell surface and incorporated into the released VSV particles, which infect human, but not mouse, rat or hamster, cell lines. CHO cells expressing chimeric HCV envelope proteins could induce membrane fusion with HepG2 cells in a pH-dependent manner (Takikawa et al., 2000). This finding was confirmed by pseudotype HIV carrying authentic HCV envelope proteins (Bartosch et al., 2003; Hsu et al., 2003b). Fab fragments obtained from a chronic HCV-infected patient can neutralize the ability of pseudotype VSV to infect HepG2 cells, suggesting that infection of the VSV pseudotype mimics natural HCV infection (Burioni et al., 2002). However, a recombinant VSV possessing chimeric HCV envelope proteins described by Rose and colleague was not infectious (Buonocore et al., 2002). The molecular size of the chimeric E1 protein incorporated into the pseudotype VSV is larger than that of the recombinant VSV (Buonocore et al., 2002). Different glycosylation patterns or other modifications of the chimeric E1 proteins may affect the infectivity of VSV bearing chimeric HCV envelope proteins.

HCV envelope proteins are statically retained in the ER (Cocquerel et al., 1999). However, Bartosch and colleagues reported that the native form of HCV envelope proteins were partially expressed on cell surface of 293T cells and encapsulated retrovirus nucleocapsid, resulting in the production of infectious particles (Bartosch et al., 2003). This pseudotype retrovirus exhibits the highest infectivity against the hepatoma cell line Huh-7 among the cell lines tested. The glycoproteins E1 and E2 are necessary for the highest degree of infectivity, although the pseudotype bearing either E1 or E2 could infect Huh-7 at two hundred times lower infectivity than those bearing both envelope proteins (Bartosch et al., 2003). Although infection by pseudotype retrovirus was inhibited by the addition of anti-hCD81 antibody or a soluble form of hCD81, expression of hCD81 alone failed to confer mouse NIH 3T3 cells susceptible to infection by retrovirus pseudotype (Bartosch et al., 2003). A similar study suggested that pseudotype HIV bearing native forms of HCV envelope proteins infects the Huh-7.5 cell line in a pH-dependent manner and, furthermore, that the expression of one or all of the candidate receptor molecules (hCD81, LDL receptor, human SB-R1 and DC-SIGN) failed to confer permissivity to pseudotype infection (Hsu et al., 2003b).

RNA replicon system

The use of in vitro studies of HCV proteins has led to the accumulation of knowledge regarding the pathogenesis of this disease, as well as providing insights into potential therapies. However, the assessment of antiviral drug candidates for HCV patients has been hampered by the lack of a robust and reliable cell culture system and the absence of a small animal model. Viral proteins whose biological activities are already known (such as envelope proteins, helicase and protease, and RNA-dependent RNA polymerase) could be targets for the development of antiviral drugs. Bartenschlager and colleagues reported that an RNA replicon comprised of the 5′-UTR, neo gene EMCV IRES (the gene encoding HCV genotype 1b N52 or N53 up to N55B and 3′-UTR) could replicate autonomously in Huh-7 cell line (Lohmann et al., 1999). Stable replication with the highest amount of RNA replication was obtained from an RNA replicon containing the NS3 to NS5B genes. Detection of positive and negative strands of RNA and processed nonstructural proteins in cells indicate that the replicon system partially mimics the HCV replication cycle. Studies of the replication cycle of HCV have been advanced by improvements of the replicon system. Sequences upstream of HCV IRES in the 5′-UTR are essential for RNA replication (Fribe et al., 2001) and the 3′-UTR plays an important role in the initiation of minus-strand RNA synthesis (Fribe & Bartenschlager, 2002). Replication of replicons was stable for at least 1 year (Pletschmann et al., 2001). Efficient replication of subgenomic replicons carrying type 1b infectious strain Con1 generally required several adaptive mutations. Once RNA replicons acquired adaptive mutations, the efficiency of colony formation was increased by an unknown mechanism (Blight et al., 2000; Lohmann et al., 1999). A daptive mutations were generally detected in the genes encoding NS3, NS5A and NS5B proteins (Blight et al., 2000; Lohmann et al., 2003; Lohmann et al., 2001). A daptive mutations with in NS5A were found to confer efficient replication in vitro.
independently of IFN sensitivity (Blight et al., 2000; Guo et al., 2001). Although a full-length HCV replicon released substantial amounts of nuclease-resistant HCV RNA-containing particles, comparable amounts of such RNA-containing particles were detected in the supernatant of cells carrying the subgenomic replicons. These results indicate that the RNA-containing particles are released independently of the presence of HCV structural proteins and that Huh-7 cells may lack host cell factors essential for HCV assembly and release (Pietschmann et al., 2001).

It is not known why adaptive mutation is necessary for the replication of HCV replicons in Huh-7 cells, while viral particles produced under similar conditions are infectious to chimpanzees. Bukh and colleagues demonstrated that adaptive mutations are not necessary for in vivo infectivity (Bukh et al., 2002). A full-length clone bearing three adaptive mutations was not infectious to chimpanzees, while a clone bearing one adaptive mutation could infect but the mutation reverted back to wild-type (Bukh et al., 2002). In contrast to the Con1 strain, an RNA replicon construct based on the genome of type 1b infectious clone HCV-N could stably replicate, conserving the wild-type sequence of the polyprotein-coding region (Ikeda et al., 2002). The HCV replicons described above are constructed with genomes of type 1b strains (Con1 and HCV-N). Recently, two groups reported efficient replication of replicons carrying genotype 1a strain H77 (Blight et al., 2003; Gu et al., 2003). Subgenomic and full-length replicons based on type 1a sequences could replicate in Huh-7.5 (Blight et al., 2003; Gu et al., 2003) and exhibited lower sensitivity to IFN alpha than type 1b replicons (Gu et al., 2003). Adaptive mutations appeared in NS3, NS5A, and NS5B (Blight et al., 2003; Gu et al., 2003). The HCV replicon system provides a useful tool to assess antiviral compounds targeting the HCV replication complex.

**Virus assembly**

HCV core protein is processed by signal peptidase and host intramembrane protease (McLauchlan et al., 2002) and is localized to the ER and lipid droplets (Hope & McLauchlan, 2000; McLauchlan et al., 2002). The intramembrane processing of HCV core protein is inhibited by the chemical compound (Z-LL)₂ keton (McLauchlan et al., 2002). Signal peptidase peptidase (SPP) was identified as the intramembrane protease that is inhibited by (Z-LL)₂ keton (Weihofen et al., 2002). Prolactin, HLA-E and other host proteins are known substrates of SPP (Lemberg et al., 2001). HCV core protein is cleaved by (Z-LL)₂ keton-sensitive intramembrane protease, resulting in localization of the processed HCV core protein on lipid droplets (Lemberg & M artoglio, 2002; McLauchlan et al., 2002; Weihofen et al., 2003). McLauchlan and colleague suggested that Domain II of HCV core protein, a hydrophobic region upstream of the C-terminal transmembrane region, is necessary for stability of HCV core protein and localization to the ER or lipid droplets (Hope & McLauchlan, 2000). There are several homologues to SPP in humans (Grigorenko et al., 2002; Weihofen et al., 2002); these may cleave the transmembrane region of HCV core protein. HCV core protein is a highly basic protein corresponding to the capsid protein found in other members of Flaviviridae (Grakoui et al., 1999), suggesting that HCV core protein forms a nucleocapsid with the RNA genome. HCV core protein binds most efficiently to loop IIId and, to a lesser extend, loop I and the region from nt 23–41 (Tanaka et al., 2000).

The putative envelope glycoproteins of HCV are the E1 and E2 proteins, which contain five or six and 11 N-linked glycosylation sites, respectively (M iyamura & Matsuura, 1993). H ost signal peptidase processes the polyprotein into core, E1, E2 and p7 (Figure 1). E1 and E2 proteins lie from amino acid 193–383 and 384–746, respectively (Matsuura et al., 1992; Mizushima et al., 1994) and penetrate the ER membrane via C-terminal transmembrane domains, which may covalently interact with each other (Dubuisson & Rice, 1996; Matsuura et al., 1992; Ralston et al., 1993). The heterodimer is most likely the prebudding form of the functional complex (Deleersnyder et al., 1997).

The structure and function of HCV envelope proteins have been predicted from studies on truncated soluble forms of both envelope proteins (Matsuura et al., 1992; M Ichalak et al., 1992) and on chimeric proteins in which the wild-type transmembrane domains have been replaced with the transmembrane domains of other proteins (Cocquerel et al., 1998; Flint et al., 1999). E2 protein appears to attach to the ER membrane through its C-terminus, because the deletion of at least 31 amino acids from C-terminus of E2 leads to its secretion (M Ichalak et al., 1997; Mizushima et al., 1994; Selby et al., 1993). Anchoring of E1 on the ER membrane may depend on a longer C-terminal region of at least 62 amino acids (M Ichalak et al., 1997), while our data suggest that the hydrophobic domain between positions 262 and 290 is important for anchoring to the ER membrane (Matsuura et al., 1992). Cocquerel and colleagues (Cocquerel et al., 1999) presented the hypothesis that the transmembrane domains of E1 and E2 proteins adopt a transient hairpin structure with both their N- and C-termini facing the ER lumen and subsequently reorient themselves. This data,
taken together with glycosylation studies (Muenier et al.,
1999), suggest that E1 is a type I transmembrane protein. Howev-
er, a physical interaction between the core protein and E1 protein has also been suggested (Lo et al., 1996). The overall topology of E1 and its relation to core protein remains unresolved.

Establishment of persistent infection

It is well-known that 80% of patients have persistent HCV infection, while cure occurs in the remaining patients. Presumably, HCV has the ability to escape from the host immune system and/or to alter or suppress the host immune response. Most patients fail to resolve HCV, despite generating both cellular and humoral immune responses. It is possible that the high mutation rate of HCV allows for escape from the host immune response. The RNA viral genome is highly efficient at mutating and is comprised of several genomes called quasispecies. Different host environments, including the immune response, act as selective pressures on the generation of new viral particles. Sequence diversity of HCV genomes results from the lack of proofreading activity within the viral polymerase (Bukh et al., 1995a; Bukh et al., 1995b).

The role of the host antibody response in protecting the host from the establishment of chronic infection is unclear. For example, antibodies against HCV proteins do not hinder reinfection (Farci et al., 1992; Lai et al., 1994), and humoral responses are decreased in many people who have recovered from HCV infection (Takaki et al., 2000). In contrast, NOB antibody titres are observed in naturally resolved cases of chronic HCV, as described above (Ishii et al., 1998). Whether humoral immune responses against HCV proteins are necessary for protection and recovery has not been clarified.

Chimpanzees, which are known to be the most faithful model for HCV infection (Shimizu et al., 1990), develop persistent infection and hepatocellular carcinoma after infection with HCV (Muchmore et al., 1988). HCV-specific T-cell responses were observed in the blood of patients (Ferrari et al., 1994; Luchner et al., 2000; Thimme et al., 2001) and in the livers of chimpanzees (Cooper et al., 1999; Erickson et al., 2001) during acute HCV infection and are maintained for decades after recovery. Strong responses of intrahepatic cytotoxic T lymphocyte (CTL) were detected in two chimpanzees who resolved acute HCV infection despite a poor antibody response, although weak responses of CTL were found in four chimpanzees who suffered from chronic infection (Cooper et al., 1999). These data suggest that a CTL response, rather than a humoral response, plays an important role in resolving HCV infection during the acute phase, at least. Mutation of dominant epitopes recognized by CTL appeared in three persistently infected chimpanzees during the acute phase of infection, but most dominant epitopes were sustained in HCV-resolved chimpanzees (Erickson et al., 2001). It appears that once HCV escapes the CTL response during the acute phase, chronic infection may be established.

The molecular mechanism of immunosuppression induced by HCV has not been elucidated. Several lines of evidence suggest that HCV proteins have immunomodulatory function. Ahn and colleagues reported that HCV core protein binds to the gC1q receptor (Kittlesen et al., 2000). T-cell proliferation was decreased by the interaction of HCV core with the gC1q receptor (Kittlesen et al., 2000), which inhibits the phosphorylation of ERK/MEK (Yao et al., 2001). Thus, inhibition of ERK/MEK may lead to inhibition of interleukin (IL)-2 and IL-2R production (Yao et al., 2001). Envelope protein E2 was shown to bind to PKR via the phosphorylation site of E2 that is homologous to that of eIF2 alpha. This sequence is conserved in the E2 proteins of type 1a strains and results in the suppression of eIF2 alpha phosphorylation (Taylor et al., 1999). Furthermore, inhibition of natural killer cell activation, cytokine production and proliferation through cross-linking of hCD81 by E2 protein suggests that HCV can alter innate immunity via inhibition of cytokine production by natural killer cells; this inhibition facilitates the establishment of a persistent infection (Crotta et al., 2002; Tseng & Kliment, 2002). N53/4A complex also has the ability to inhibit phosphorylation of IRF-3 (Foy et al., 2003). Viral infection induces the phosphorylation of IRF-3 by viral-activated kinase, leading to the transcription of type I IFN and cytokines (Foy et al., 2003). Expression of the N53/4A complex or HCV polyprotein inhibits the phosphorylation of IRF and transcription of type I IFN through N53 protease activity (Foy et al., 2003).

New antiviral therapy against HCV

RNA interference (RNAi) is a recently described phenomenon that leads to the post-transcriptional regulation of protein expression (Fire et al., 1998; Paddison & Hannon, 2002). In lower organisms such as Caenorhabditis elegans, RNAi is composed of two distinct steps. A double-stranded RNA is processed into small interfering RNA (siRNA) of 21–23 nucleotides by Dicer, an RNAase-III-family nuclease (Zamore et al., 2000). The siRNA is then incorporated into the RNA-induced silencing complex (RISC), which leads to the specific destruction of a target mRNA recognized by the antisense strand of the siRNA (Hammond et al., 2000). Potential therapeutic uses for RNAi have been proposed for HIV (Jacque et al., 2002; Lee et al., 2002; Nivina et al., 2002), poliovirus (Gitlin, et al., 2002) and hepatitis B virus (McAffrey et al., 2003).
IFN-alpha/ribavirin combination therapy leads to remission of disease in only 40% of patients with chronic HCV; furthermore, this therapy causes significant side effects (Wedemeyer et al., 1998). Thus, it is very important to develop an alternative therapy for chronic HCV infection. McCaffrey and colleagues reported that siRNA specific to NS5B inhibit the translation of NS5B-fused luciferase in mice (McCaffrey et al., 2002), although this experiment did not address the replication step of HCV.

Several recent studies demonstrated that RNAi suppresses gene expression and RNA replication of HCV in the replicon system (Kapadia et al., 2003; McCaffrey et al., 2002; Randall et al., 2003; Wilson et al., 2003; Yokota et al., 2003). The application of RNAi to chronic HCV therapy, possibly in conjunction with IFN/ribavirin, holds great promise but needs to be further investigated.

Richardson and colleagues reported an interesting approach to reduce the number of HCV-infected cells using a modified pro-apoptotic protein (Hu et al., 2003a). The Bid family member Bid becomes pro-apoptotic following caspase-8 activation in the liver (Li et al., 1998; Yin et al., 1999). A denovirus encoding recombinant Bid, in which the caspase cleavage site was replaced with an HCV NS3 cleavage site, suppressed HCV replication not only in the RNA replicon system in vitro, but also in mice implanted with human livers infected with HCV without any cytotoxicity of uninfected human liver cells (Hu et al., 2003a).

Core protein has also become a target for antiviral therapy. SPP is a presenilin-type protease. The primary accumulation of the primary target of presenilin, beta-amyloid precursor protein, is the cause of Alzheimer’s disease. Several compounds developed for presenilin can also inhibit the activity of SPP in addition to (z-LL)2-ketone. In order to be effective for SPP, a compound must pass through the plasma membrane as SPP is retained in the ER membrane by ER-retrieval signal (Weihofen et al., 2002). The development of such compounds could prove useful for the treatment of chronic HCV infection.

Conclusions

As described above, several putative cell surface receptors for HCV envelope proteins have been identified by different groups. The report of cell surface expression of wild-type HCV envelope proteins by Bartosh et al. (Bartosh et al., 2003; Pohlmann et al., 2003) was extremely surprising, given that HCV envelope proteins are believed to have ER retention signals (Cocquerel et al., 1999; Cocquerel et al., 2002). It is hoped that researchers will be able to identify the near future if both envelope proteins expressed on the cell surface sustain their native forms and the ability to interact with their cognate receptor(s). Replicon systems are useful tools for the study of HCV replication and for the development of new drugs for HCV therapy. Huh-7 cells can acquire the ability to allow the HCV genome to replicate, but they are deficient in the assembly and/or release of HCV particles. If the Huh-7 cell line was reconstituted by the introduction of the true HCV receptor, co-receptor, and other host factors essential for viral assembly and release of infectious particles, the cell line would provide a breakthrough in HCV study. However, an adaptive mutation of type 1b strain reverted back to authentic amino acid, and most adaptively mutated clones could not establish infection when inoculated into chimpanzees (Bukh et al., 2002). Environmental conditions surrounding the HCV RNA replicon system may hold a unique position in Huh-7 cell line. The establishment of a robust culture system for HCV replication is the most important issue that needs to be resolved in order to better understand the pathogenicity of HCV and develop an effective measure for the treatment of chronic HCV infection.

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Mechanisms of HCV


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