Genetic heterogeneity and properties of hepatitis C virus

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Abstract

Hepatitis C virus (HCV) is a member of the Flaviviridae family. Its genome is a positive single-stranded RNA molecule which comprises three distinct regions : a 5' non coding region, a long open reading frame encoding both the structural and non structural viral proteins, and a 3' non coding region. HCV circulates in infected individuals as complex mixtures of genetically distinct but closely related variants referred to as "quasispecies". The quasispecies nature of HCV genomes appears to play a major role in viral persistence, cell tropism of viral variants, pathogenicity and resistance to antiviral therapy. (Acta gastroenterol. belg., **1998**, 61, **189-191**).

Key words : hepatitis C virus, genome, quasispecies, viral persistence, interferon alpha.

Hepatitis C virus (HCV) is a member of the Flaviviridae family (genus Hepacivirus). The viral particle is 55 to 65 nm in diameter and comprises : (i) a single-stranded linear RNA genome, (ii) a cubic capsid, and (iii) a lipidic envelope in which two envelope proteins (E1 and E2, respectively) are anchored as heterodimers (1). HCV replicates mainly in the liver and it remains unknown whether or not it could also replicate in extra-hepatic sites, such as peripheral blood mononuclear cells (PBMC). HCV circulates in infected individuals as complex mixtures of genetically distinct but closely related variants referred to as "quasispecies" (2). The quasispecies nature of HCV genomes plays a major role in many aspects of the disease, including viral persistence, cell tropism of viral variants, pathogenicity and resistance to antiviral therapy.

I. Structure and function of HCV genome

Structure of HCV genome

HCV genome is a positive single-stranded RNA molecule, about 10,000 nucleotides long. It can be subdivided into three distinct regions, from the 5' end to the 3' end, respectively (Fig. 1) (1).

- The 5' non coding region is highly conserved among HCV strains and contains regulatory sequences. Among them, a stable and highly conserved stem-loop structure is located immediately upstream of the open reading frame and functions as an internal ribosome entry site (IRES), allowing initiation of viral polyprotein translation through a cap-independent process.
- The long open reading frame (ORF) encodes the structural (C, E1 and E2) and non structural (NS2, NS3, NS4A, NS4B, NS5A, NS5B) HCV proteins.
- The short 3' non coding region contains a variable region, followed by a poly-uridyl stretch of variable length and a highly conserved 98 nucleotides stem-loop structure. This structure seems to play an important role in transcription of the complementary RNA strand subsequently used as an intermediate of replication in cell cytoplasm.

Translation of the ORF leads to the generation of a precursor polyprotein which is secondarily cleaved by cellular and viral proteases (1). The structural proteins, including a capsid protein (C or p21) and two envelope proteins (E1 or gp 31, and E2 or gp 70) are liberated by the action of cellular signal peptidases, as is the small p7 protein, the function of which remains unknown (Fig. 1) (1). NS2 is a zinc-dependent protease which mediates autoproteolytic cleavage at the NS2/NS3 site. The NS3 protein has a serine protease function. When complexed with NS4A, NS3 is responsible for cleavage of downstream proteins, ie NS3/NS4A, NS4A/NS4B, NS4B/NS5A, NS5A/NS5B (Fig. 1). NS3 also bears helicase and NTPase functions. The functions of NS4B and NS5A proteins are unknown, but they appear to be integrated into the polymerase complex during replication and could therefore play an important role in HCV replication. Finally, NS5B is the viral RNA-dependent RNA polymerase (1).

II. Quasispecies distribution of HCV genomes

HCV quasispecies

HCV, like other RNA viruses, exists within its hosts as pools of related genetic variants referred to as quasispecies (2). This confers a significant survival advantage because the simultaneous presence of multiple variant genomes and the high rate of generation of new variants allows for the rapid selection of the mutants with better fitness for any new environmental condition (3). The genetic heterogeneity within the HCV quasispecies population results from a high

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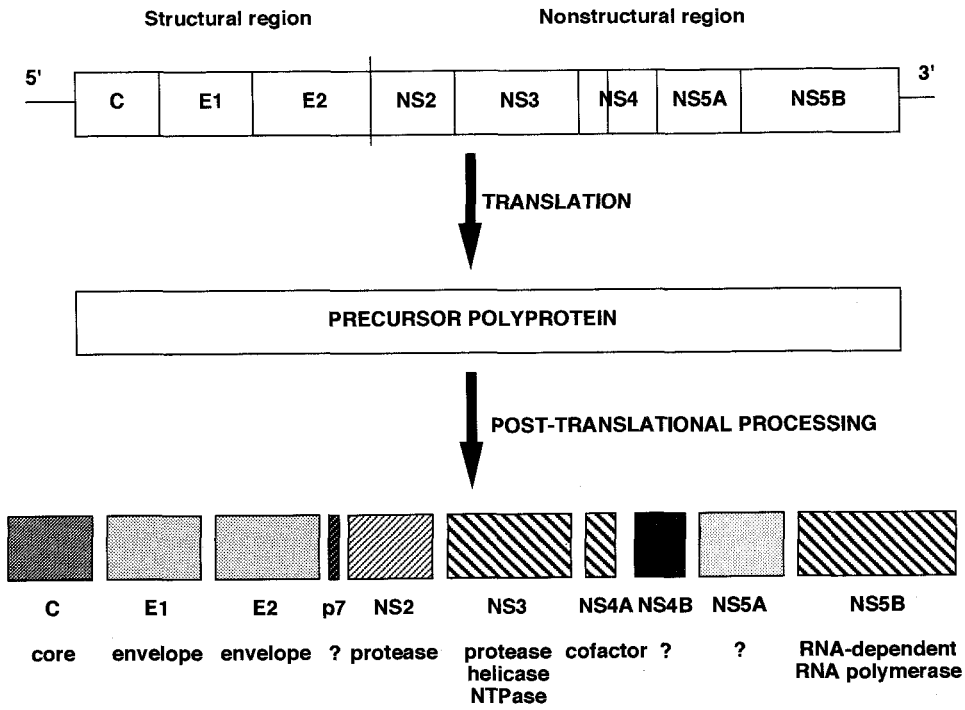

Fig. 1. — Structure of hepatitis C virus genome. Traduction of the open reading frame and viral polyprotein processing.

RNA-dependent RNA polymerase error rate (with misincorporation frequencies averaging about 10^{-4} to 10^{-5} per base site), and the apparent absence of any error correction of proofreading mechanism. Most mutant viral particles cannot replicate, but the remainder can transmit new genetic information to their progeny. The fittest infectious particles are selected by their replication capacities and especially by the selective pressure exerted by host cells and the immune response (3). Immune selective pressures act on regions encoding cytotoxic and neutralizing epitopes. The hypervariable region 1 (HVR1) of the genome is located at the 5' end of the E2 envelope gene and encodes a 27 amino acid stretch which is a target for the anti-HCV neutralizing response (4). It is commonly used to maximize the detection of quasispecies variants.

Clinical implications of the quasispecies distribution of HCV genomes

Viral persistence occurs in about 85% of infected patients. This high rate of chronicity could at least partly be explained by the capacity of viral quasispecies to continuously generate variants escaping neutralizing and cytotoxic responses (4). Escape from the action of host cellular proteins involved in viral elimination could also play a role.

Different compositions of HCV quasispecies have been reported in the liver, peripheral blood, and PBMCs from the same patients, respectively (5). HCV is mainly produced in the liver, but it is possible that different quasispecies variant kinetics lead to the differences observed between the liver and peripheral blood. In addition, recent data suggest that a minority of quasispecies variants could be lymphotropic (6). Whether these variants accumulate without replicating or can replicate in PBMC remains debated.

It is unknown whether more aggressive HCV quasispecies variants might exist. To date, it has not been possible to address this important issue in the absence of efficient cell-culture system. Given the pathogenesis of liver lesions in chronic hepatitis C (liver injury seems to be principally immune-mediated), it is possible that quasispecies variants can be more aggressive than others through their specific interactions with the immune responses of the host instead of being intrinsically more virulent.

The quasispecies nature of HCV plays a major role in HCV resistance to interferon treatment. Indeed, it has been shown that a high genetic complexity of the hypervariable region 1 is associated with a low rate of sustained HCV clearance after 6 to 12 months of treatment at the dose of 3 megaunits three times per week (7). We recently confirmed the association between a low genetic complexity and viral elimination in a genomic region (NS5A) which genetic heterogeneity can be considered representative of the overall genetic heterogeneity of the HCV strain (8). This suggested that the genomic mutations associated with resistance to a standard dosage of interferon alpha would be numerous, related, and probably located at various positions throughout the viral genome. These mutations could allow fitter minor variants to escape the specific immune response of the host. In patients who do not clear HCV,

interferon treatment induces profound changes in HVR1 quasispecies in about 80% of the cases (Pawlotsky *et al.*, manuscript in preparation), as well as in NS5A quasispecies (8). In these patients, the consequences of interferon-induced quasispecies changes on subsequent evolution of liver disease remain to be elucidated.

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