Hepatitis C : virology, clinical aspects and the relation to cryoglobulinemia

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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 : a single-stranded linear RNA genome, a cubic capsid, and a lipidic envelope in which two envelope proteins (E1 and E2, respectively) are anchored as heterodimers. HCV replicates mainly in the liver and might also replicate at lower levels in extra-hepatic sites, such as peripheral blood mononuclear cells (PBMCs). The 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 (reviewed in 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 serves as an "internal ribosome entry site" (IRES), allowing the initiation of the viral polyprotein translation through a cap-independent process.
- The unique open reading frame (ORF) encodes the structural (C, El and E2) and non structural (p7, 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 the transcription of the complementary RNA strand, subsequently used as an intermediate of replication in cell cytoplasm.

The translation of the ORF leads to the generation of a precursor polyprotein which is secondarily cleaved by cellular and viral proteases (reviewed in 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. NS2 is a zinc-dependent protease which mediates the autoproteolytic cleavage at the NS2/NS3 site. The NS3 protein has a serine protease function. When complexed with NS4A, NS3 is responsible for the cleavage of downstream proteins, ie NS3/NS4A, NS4A/NS4B, NS4B/NS5A, NS5A/NS5B. NS3 also bears helicase and NTPase functions. The functions of the NS4B and NS5A proteins are unknown, but they appear to be integrated into the polymerase complex and could therefore play an important role in the regulation of HCV replication. Finally, NS5B is the viral RNA-dependent RNA polymerase.

HCV, like other RNA viruses, exists within its hosts as pools of related genetic variants referred to as quasi-species (2). This confers a significant survival advantage, as the simultaneous presence of multiple variant genomes and the high rate at which new variants are generated allows rapid selection of mutants better suited to new environmental conditions (reviewed in 3). The genetic heterogeneity within the HCV quasispecies population results from a high 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. The quasispecies nature of HCV genomes plays a major role in many aspects of the disease, including viral persistence (4), cell tropism of viral variants (5,6), pathogenicity and resistance to antiviral therapy (7,8).

The progression of hepatitis C virus (HCV)-related liver disease appears to be greatly influenced by epidemiological parameters and cofactors, such as the age at infection, disease duration, alcohol consumption or viral coinfections. The role of HCV replication in disease progression is complex and debated. Infection is characterized by the penetration of an heterogeneous quasispecies into the host's blood. Some particles can enter target cells, mainly hepatocytes, and replicate. HCV replication leads to the progressively increasing production of large amounts of virions, the majority of which is released in the general circulation. The presence of viral components in the body triggers both non specific and HCV-specific host defences, in particular humoral and cellular immune responses. These defences are not able to eliminate the virus in most cases, leading to viral persistence (reviewed in 9). Thus, when the infection becomes chronic, a steady- state is reached, with an estimated half-life of free HCV virions on the order of 3 hours and a daily production of approximately 10^{12} virions/day (10). The high levels of replication are responsible for the continuous appearance of mutations on the HCV genome, due to the lack of fidelity of the RNA-dependent RNA polymerase. These mutations

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are continuously selected by the environment in which the virus replicates, leading to the generation of more and more complex and diverse quasispecies. Random genetic drift is usually responsible for the spontaneous genetic evolution of HCV quasispecies. Nevertheless, the emergence of a variant bearing a significant survival advantage or any dramatic change in the environment in which the virus replicates conferring a significant survival advantage to already present variants is followed by a shift in the viral population, with the positively selected variant(s) becoming major. Overall, the natural history of HCV infection is characterized by a progressive genetic drift, with successive shifts of virus populations. In chronic hepatitis C, the continuous antigenic stimulation related to viral replication is likely responsible for the liver lesions, which appear to result of a direct cytotoxic effect of CD8-positive T- cells and of the action of various cytokines secreted locally by both CD4-positive T-helper cells and CD8-positive T-cells, leading to hepatic cell death through apoptosis (reviewed in 9 and 11). Shifts in the populations of viruses may induce shifts in epitope immunodominance that alter the local immune response and may result in more or less inflammation in the liver. The accumulation of such events during the natural history of the disease is likely at least partly responsible for its progression towards the aggravation of fibrosis and the appearance of complications such as cirrhosis and hepatocellular carcinoma.

In addition, 35% to 55% of the patients with chronic hepatitis C have detectable cryoglobulins, which may induce more or less severe manifestations of cryoglobulin-associated vasculitis and membrano- proliferative glomerulo-nephritis (reviewed in 12). HCV-associated cryoglobulins appear to be made of immune complexes including HCV virions, specific anti-HCV IgG and IgM pentamers with a rheumatoid factor activity. The proliferation and the clonal expansion of B-lymphocytes producing rheumatoid factors with high affinity for HCV-IgG complexes might result of persistent antigenic stimulation related to viral replication. Two lines of evidence suggest such a role for continuous HCV antigenic stimulation in monoclonal B cell expansion : (i) the recent demonstration that the HCV second envelope protein binds to tetraspanin CD81, a molecule that associates with CD 19 and CD21 at the surface of B lymphocytes, and that this binding delivers an exogenous stimulatory signal to B cells, which appears to be independent of putative intracellular HCV replication (13) ; (ii) the detection of nonsynonymous somatic mutations and intraclonal diversity in the CDR3 region of the expressed V_H gene of monoclonal IgM kappa rheumatoid factor secreted by CD5-negative B-cells in HCV-associated type II MC (14). This could explain that HCV infection is occasionally associated with B-cell non-Hodgkin's lymphomas, often of the lymphoplasmocytoid lymphoma/immunocytoma type, a histotype frequently associated with type II mixed cryoglobulinemias (15, 16).

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