Development and Characterization of Subgenomic and Full-length Genome replicons based on the sequence of HCV AD78 Strain

Abstract:
Despite significant progresses observed in the field of HCV research during the current decade, a protective and therapeutic vaccine for HCV is so far not available. However, our understanding of HCV replication and cell entry mechanisms has been reinforced with the development of a cell culture system for HCV (Lohmann et al., 1999; Wakita et al., 2005). So far, a certain number of HCV sequences have been successfully used for generating both subgenomic and full-length replicon of HCV. Unfortunately, their use for the creation of chimeric molecules of HCV in order to study different aspects of HCV biology such as the influence of sequences evolution on HCV replication remains difficult to implement. That limitation might be due to the incompatibility of the back-bone sequence with the heterologous sequences deriving from unrelated isolates or strains of HCV. To overcome such a limitation, beneficial would be to use sequences of closely related isolates of HCV for generation of the replicon and subsequent genes exchanges. The major aim of this thesis was the development of a novel subgenomic and full-length genomic replicon system based on the sequence of HCV AD78 strain, which caused a single-source outbreak in several thousands women in Germany following a prophylactic administration of virus-contaminated anti-D immunoglobulin. Since the consensus sequence of HCV-AD78 strain established from the contaminated anti-D immunoglobulin failed to replicate, HCV sequences amplified from sera of patients infected with this strain were used to sequentially substitute their homologous in the well characterized prototype subgenomic replicon Con1. As a result, a set of functional Con1/AD78 chimeric HCV replicons molecules including a replicon completely based on the sequence of HCV-AD78 strain were generated. Huh7 cell lines persistently bearing these replicons were established and characterized. Moreover, the obtained results demonstrated that a viability of the chimeric replicons very much depends on the genetic context of the back-bone sequence, and that interactions between non-structural proteins may represent a critical determinant of replication competence. These data demonstrate the existence of a very complex interplay between different non-structural HCV proteins and regulatory elements in a particular cellular environment. Availability of serial blood samples from the AD78 cohort together with the generated AD78-based replicons would allow developing and implementing a series of new experimental approaches to study several important aspects of HCV research,
including the HCV evolution and role of HCV-specific humoral and cellular immune responses in resolution of HCV infection.