

Hepatitis C virus: new lights on the horizon

Santiago Dueñas-Carrera, Alexis Musacchio

Hepatitis C Department, Vaccines Division, Center for Genetic Engineering and Biotechnology
Ave 31, Playa, PO Box 6162, ZP 10600, Havana, Cuba
Fax: (53-7) 271 4764

REPORT

Hepatitis C virus (HCV) is now a major health problem. More than 170 million people are infected with this pathogen worldwide, frequently causing cirrhosis and liver cancer. HCV is an enveloped positive-strand RNA virus whose genome encodes a single polyprotein precursor of 3000 amino acids, which is processed by cellular and viral proteases [1] to produce structural and non structural proteins (Figure 1). At present, there is no available preventive vaccine against HCV infection and the consensus therapeutic treatment, consisting of pegylated interferon-alpha (IFN-alpha) in combination with ribavirin, is poorly effective against certain viral genotypes [2].

The 11th International Symposium on Hepatitis C Virus and Related Viruses Molecular Virology, Pathogenesis & Antiviral Therapy was organized with the aim of discussing the most recent results in the HCV field. The meeting took place at Heidelberg, from October 3 to 7, 2004.

More than 500 known scientists from more than 20 countries attended this historical meeting. The event was organized in sessions according the following topics: Antiviral therapy and clinical virology, Replication, Translation, Nonstructural proteins, Assembly and virus entry, Structural proteins, Epidemiology, Dynamics and evolution, Innate immunity, Acquired immunity, Prophylaxis and diagnosis, Pathogenesis. A total of 98 lectures and more than 250 posters were presented.

At the antiviral therapy session, differences in gene response between patients resistant to interferon/ribavirin therapy and those showing early virus clearance were described by Milton Taylor (Department of Biology, Indiana University, USA). At the same time, the identification and characterization of several promising inhibitors of HCV NS5B polymerase or NS3/4A protease were also described. Particularly, BILN-2061 and VX-950, have shown important inhibitory actions on the HCV NS3/4A protease both *in vitro* and *in vivo*. However, the appearance of mutations conferring resistance to these and other HCV inhibitors is now a reality. This fact could hamper the effective application of the hundreds of small molecular inhibitors that are currently evaluated to treat HCV infection. To study different aspects of HCV infections and to evaluate the effectiveness of antiviral molecules like interferon and BILN-2061, the human liver-UPA-SCID mice model was successfully used by Philip Meuleman (Center for Vaccinology, Ghent University and Hospital, Belgium) and Norman Kneteman (KMT Hepatech, Inc., Canada). The HCV replicon-based system also arises as a powerful tool for the study of new anti-HCV compounds. This system seems to be very useful for the evaluation of polymerase candidates or protease inhibitors against a panel of HCV isolates, to study the role of resistant muta-

tions that developed during treatment in the support of clinical trials, and to reduce the time required to evaluate drug susceptibility of heterologous antigens from several months to weeks.

At the replication session, very interesting data about regulation of Hepatitis C viral RNA abundance by microRNA122 were presented by Catherine Jopling (Department of Microbiology and Immunology, Stanford University, USA). Other RNA elements and nuclear factors were also revealed to be important in HCV replication. Additionally, Petra Nedderman (Biochemistry IRBM, Italy) showed that compounds that selectively inhibit NS5A hyperphosphorylation could be employed to establish infection systems for HCV in cell culture and may be a starting point for the development of novel antiviral agents. Moreover, Doris Quinkert (Department of Molecular Virology, University Heidelberg, Germany) and specially, Benno Wolk (Center for the study of Hepatitis C, The Rockefeller University, NY, USA), charmed us with excellent pictures and videos regarding the quantitative analysis and a dynamic view of HCV replication complexes. The results suggest that large replication sites are relatively stable structures with only limited intracellular movement and exchange of viral non-structural proteins. In contrast, smaller structures are subject to fast, microtubule-dependent transport processes. Ongoing studies are investigating the role of these processes in the formation, maintenance, and turnover of HCV replication complexes.

Only three lectures comprised Translation session. A tissue-wide screening of Hepatitis C virus and poliovirus IRES activity in transgenic mice was presented by Willy Spaan (LUMC, Department Medical Microbiology, Leiden, The Netherlands). Eric Gowans (Macfarlane Burnet Institute, Melbourne, Australia) explained differential effects on the HCV IRES by vitamin B12 and the HCV core protein. Finally, data obtained by Niki Vasilaki (Molecular Vi-

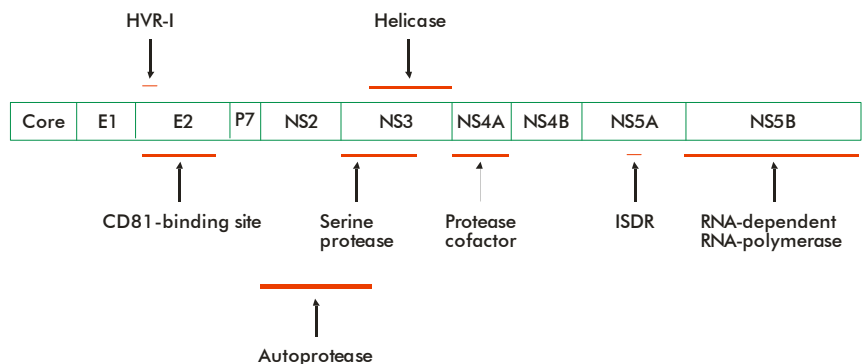


Figure 1. Schematic representation of HCV encoded polyprotein

rology Laboratory, Hellenic Pasteur Institute, Athens, Greece) indicate that the short form of core+1 protein is expressed independently from the HCV polyprotein and is negatively regulated by the expression of the HCV core protein.

An in-deep look at HCV non-structural proteins was certainly achieved at the meeting. The definition of NS5A as a Zinc metalloprotein, including the absolute requirement of Zinc for NS5A function in the hepatitis C replicase, was clearly demonstrated by Timothy Tellinghuisen (Center for the Study of Hepatitis C, The Rockefeller University, NY, USA). Moreover, Nicole Appel (Department of Molecular Virology, University of Heidelberg, Germany) established that inactivating NS5A mutations localized in the amino terminal amphipathic helix can not be complemented in trans and that the minimal sequence required for trans-complementation of lethal NS5A mutations is NS3 to NS5A, whereas NS5A alone does not restore HCV replication. Other studies provided further insights into the functional architecture of the HCV replication complex and the bases for understanding the resistance of NS5B to allosteric inhibitors.

Particularly relevant were the sessions related with assembly and virus entry, and structural proteins. Some years ago, the development of subgenomic replicons enabled the study of HCV replication [3, 4]. However, other steps of the viral life cycle, such as viral particle formation, release and infection have been poorly understood due to the lack of an efficient experimental system. A long-awaited full-length HCV replicon producing viral particles was presented by Takaji Wakita (Department of Microbiology, Tokyo Metropolitan Institute for Neuroscience, Japan). A full-length JFH1 RNA was transfected into Huh-7 cells and efficient RNA replication was observed. Expression of HCV proteins was also confirmed. Full-length JFH1 RNA replicating cells were passaged and continuously cultured. Recombinant HCV particles seem to be formed and secreted into the culture medium. Furthermore, according to results presented by Thomas Pietschmann (Department of Molecular Virology, University of Heidelberg, Germany), the cell culture-adaptive mutations, known to enhance RNA replication *in vitro* but blocking infectivity *in vivo*, inhibit particle formation. Moreover, virus assembly and release requires functional expression of HCV glycoproteins, since a deletion of E1 and E2 or point mutations in the transmembrane domain of E1 inhibited particle formation. Pietschmann also described a chimeric genome allowing the generation of viral particles. Remarkably, the infectivity of the released viral particles could be neutralized by antibodies directed to CD81, indicating the role of this cell surface molecule in the HCV particles entry. Furthermore, the recent development of infectious, the retroviral pseudotypes bearing HCV envelope proteins provides an additional opportunity to study viral entry and the characteristics of functional HCV E1 and E2 glycoproteins. All these analytical variants supply a powerful tool, not only to study viral particle formation, release and infection steps in viral life cycle, but also to construct anti-viral strategies and develop effective vaccines.

The generation of an effective HCV vaccine is exceptionally challenging due to viral heterogeneity and

the absence of adequate animal models or reliable tissue-culture systems for the analysis and propagation of this pathogen. Therapeutic vaccination with envelope protein E1, carried out by Innogenetics, is the most advanced strategy, reaching Phase II in humans. Immunization with this vaccine candidate is well tolerated; it elicits a significant de novo E1-specific T-cell response and increases the anti-E1 antibody levels in HCV chronically infected individuals. Improvement in liver histology has been also detected in these individuals, although no significant reduction in viral load has been observed [5].

Indeed, although some promising results obtained with different vaccine candidates were described at the meeting, there is still a long way before an effective vaccine can reach the market. Particularly, a T-cell based HCV vaccine candidate developed by Antonella Folgori (IRBM P. Angeletti, Pomezia, Roma, Italy) and colleagues. This vaccine is capable of blunting acute viremia and protecting from acute and chronic diseases induced by heterologous virus challenge in chimpanzees. In fact, immunization consists of a prime/boost strategy based on the alternate administration of recombinant adenovirus and DNA constructs expressing HCV non-structural antigens.

The induction of potent and sustained, humoral and cellular immune responses might be necessary to prevent or clarify HCV infection [6-8]. However, insufficient information is currently available on immune parameters during HCV infection. A very interesting study presented here on the characterization of humoral responses in a cohort of acute phase patients infected by a single-source HCV, demonstrated for the first time the presence of neutralizing antibodies during the acute phase of HCV infection that correlates inversely with viral kinetics. Other papers evidenced that cross-reactive neutralizing antibodies appear late in persistently infected individuals, suggesting that a selective mechanism may operate to prevent their appearance during acute infection. Additionally, the early appearance of cytokine producing CD4 and effectors memory CD8 cells in the incubation phase of Hepatitis C prior to the ALT peak seems to correlate with the outcome of HCV infection. However, a comprehensive study carried out by Georg Lauer (Partners AIDS Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA) in a large Brazilian cohort with acute HCV infection indicates that the pathways to resolution and persistence in acute HCV infection are more diverse than previously thought. Astonishingly, differences in the dynamics of the immune response between individuals having similar courses and outcomes were observed.

An interesting hypothesis was presented during the analysis of HCV epidemiology, dynamics and evolution. It expressed that T cell response to the circulating virus may be suppressed in a sequence-specific manner in patients with persistent HCV infection and that, despite their ability to clear one HCV strain, patients may be re-infected with a heterologous strain that can persist. This implies that patients might be exposed to multiple subtypes, adding a new point of view into the host-virus interaction in HCV infection.

The detection of genome-scale ordered RNA structure (GORS) in the HCV genome as a factor in viral

persistence was discussed by Peter Simmonds (Centre for Infectious Diseases, University of Edinburgh, UK). GORS might be related with the modulation of innate intracellular defense mechanisms triggered by double-stranded RNA. Irrespective of the function, the observed evolutionary conservation of GORS in HCV imposes a considerable constraint on their genome plasticity and is closely involved in the adaptation to the host. In this regard, one surprising discovery was the existence of human blood components that facilitate HCV infection, confirmed by several independent researchers.

Finally, intensive ongoing efforts are now directed towards a detailed dissection of the HCV pathogenic mechanism. Particularly, HCV core is assumed to be involved in HCV immunopathogenesis [9, 10]. More specifically, it has been proposed that core protein inhibits the activation of T-cells by interaction with gC1qR [9]. Other reports indicate that HCV core can modulate the interferon regulatory factor, Jak-Stat, and inducible nitric oxide synthase pathways [10]. Additionally, recent studies suggest that the interaction between E2 and CD81 can induce the aggregation of lymphoid cells and inhibit B-cell proliferation [11]. Besides, Tseng and coworkers reported that the binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer cell functions [12].

At the meeting, the newly described pathogenic effects in HCV infection were focused on HCV core,

NS5A and NS5B. Chromosomal instability, altered lipid regulation and inhibition of apoptosis were some of the most important features at the cell level caused by HCV infection.

The meeting was also ideal for exchanging ideas, establishing collaborations and proposing future guidelines in different topics. Particularly pleasant and useful was the space opened for presenting the different HCV databases (European, Japanese and American databases).

After more than 40 hours of concentrated analysis, the concluding remarks were given by the president of the Organizing Committee, Dr. Ralf Bartenschlager (Department of Molecular Virology, University of Heidelberg, Germany). Grateful words were also given by different participants in order to acknowledge the excellent organization and scientific quality of the meeting.

Finally, it was announced that the next XII International Symposium on Hepatitis C Virus and Related Viruses, Molecular Virology, Pathogenesis & Antiviral Therapy will take place in October 2005, in Montreal, Canada. Knowledge on HCV is increasing very rapidly and useful analytical tools are also being developed. In the near future, some of the vaccine candidates or antiviral compounds that are currently under evaluation might be components of an effective system to prevent or clarify HCV infection. Nevertheless, important challenges must first be faced. New lights are visible, but they are still far away on the horizon.