A Summary of the 20th International Symposium on Hepatitis C Virus and Related Viruses

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Approximately 420 international researchers attended the 20th International Symposium on Hepatitis C Virus and Related Viruses in Melbourne, Australia, from October 6–10, 2013. Since the discovery of hepatitis C virus (HCV) in 1989, significant advances have been made in our understanding of the molecular biology and host response to this significant pathogen, many of which have been presented at this symposium. In the following report we give a brief summary of the highlights from the meeting.

Virus Entry

The repertoire of host entry factors seems to be expanding with 2 new host cell molecules reported to play a role in HCV entry. Liang et al showed that E-cadherin is essential for HCV entry and Tang et al described the role of cell-death inducing DFFA-like effector b (CIDEB) in the entry of cell culture derived HCV, but not HCV glycoprotein pseudotyped particles. The 3-dimensional structure of the major cellular attachment protein of HCV, glycoprotein E2, has eluded researchers for many years. A major breakthrough was reported by the group of Law et al, who presented the first 2.65-Å structure of a minimized form of E2 that retains the ability to bind neutralizing antibodies and CD81 and inhibits HCV pseudoparticle entry. The smaller size of glycoprotein E2, relative to glycoprotein E of the flaviviruses, is likely to be relevant to interpretation of the cryo-electron microscopy (EM) structures presented for cell-culture–derived infectious HCV particles by Meunier et al in the assembly session and recently published independently by Rice and colleagues.13 In these cryo-EM structures, there are far fewer glycoproteins embedded in the particles relative to the number of apolipoprotein (Apo)A, ApoB, and ApoE molecules. In addition, strong evidence emerged that ApoJ is also required for assembly of infectious HCV as presented by Chun-Chieh-Lin in the assembly session. Finally, a major advance to our understanding of HCV entry in polarized hepatocytes was presented by Randall et al. Previous studies on the role of each HCV entry receptors/factors has been studied in nonpolarized Huh 7.5 cells, far removed from the complex polarized architecture of hepatocytes in the liver. The Huh-7 polarized cells are permissive to HCV infection, allowing the complete replication cycle to be examined providing a much-needed system in which to reexamine the role of each of the identified entry receptors and cofactors. In this system, CD81 and SR-B1 are found on the surface of cells, and claudin 1 and occludin localize exclusively to tight junctions. DiD-labelled HCV particles co-traffic with early entry receptors in an actin-dependent process to tight junctions and can be blocked by antibodies to CD81. In contrast with previous reports, no alteration in tight junction integrity was observed during HCV infection.

Viral Replication

Alexander Khromykh delivered a keynote address highlighting unique aspects of HCV replication compared with other members of the Flaviviridae family. Kim et al presented work on a new genotype 3a infectious clone (S310) that required one adaptive mutation for efficient replication in vitro. Gouttenoire et al presented data confirming the amphipathic α-helical folding of AH1 in NS4B, and its involvement in RNA replication and virion assembly. Insertion of bacteriophage MS-2 binding stem-loops within 3′ untranslated region of the HCV genome, allowed Fiches et al to show using live imaging technology that HCV RNA and NS5A co-localize and co-traffic throughout the cell. The relative roles of these RNA-positive structures in either RNA replication or assembly remain to be determined, although
they suggest that motile NS5A HCV RNA positive foci may represent a trafficking between sites of replication and assembly. A number of presentations focused on NS5A: Ross-Thriepland et al showed that a NS5A peptide fragment spanning residues 221-240 was highly phosphorylated, and Eyre et al incorporated a 'SNAP' tag into domain III of NS5A to reveal that newly synthesized NS5A-positive foci were almost entirely distinct from and half the size of aged NS5A-positive foci. Using Förster resonance energy transfer assays, Issur et al demonstrated the existence of 2 functionally distinct homodimers of NS5A domain I in solution. Finally, Konan et al presented data indicating that 2 PIP4P effectors, ceramide transport protein and phosphatidylinositol-4-phosphate adapter protein 2, are essential components of the HCV replication complex, suggesting that HCV hijacks the host sphingolipid biosynthetic machinery to build its replication complex.

Viral Assembly and Egress

Numerous host factors and viral nonstructural proteins are essential to the processes of HCV assembly and secretion. In the opening plenary talk, Thomas Pietschmann gave a detailed overview of the HCV assembly and secretion pathways. Yi et al reported that delayed but effective processing of the E2-p7 junction is essential to infectious virus production via enhancement of the localization of NS2 to putative assembly sites and enhanced NS2-NS3 interaction. Also on the topic of p7, Montero dos Santos Perin et al identified several ion channel inhibitors, including quinidine, that specifically inhibited virion assembly. Gawlik et al identified 4 basic residues in HCV core domain I that are essential to its interaction with NS5A and infectious virus assembly. Einav et al screened a library of 30 ESCRT proteins for interactions with viral proteins involved in assembly (core, E1, E2, NS2, and NS5A) and demonstrated that the HRS protein interacted with all of the HCV proteins above and was essential for infectious virus production. Finally, Meunier et al used direct immunocapture EM to demonstrate the extraordinary heterogeneity and irregular appearance of HCV lipoviral particles. Taken together, it is clear that many viral and host proteins contribute to the complex and finely tuned processes of HCV assembly and secretion.

Innate Immunity

In the keynote lecture, Michael Gale summarized how HCV interferes with innate immune regulation and introduced the concept of targeting retinoic acid inducible gene I (RIG-I) as a novel drug target to modulate the innate immune response. Grabski et al presented that co-culture of pDCs with Huh7.5 cells infected with HCV Jc1 induced higher interferon (IFN)-α responses than HCV JFH1 that was attributed to domain 2 of HCV core. Meurs et al showed that in JFH-1 infection interleukin-29 is induced earlier and more strongly than IFN-β and induces the IKK-related kinase IKKe, a noncanonical IKK known to impair nuclear factor-κB activation. Honda et al demonstrated using laser capture and immunohistochemistry that there is impaired immune cell infiltration, reduced chemokine expression, while ISG expression was increased in livers from patients with the nonresponder IFNL3 genotype. Lamarre et al described that the WNT/CTNNB1 pathway negatively regulates antiviral responses in HCV-infected hepatocytes and promotes HCV replication. Their data also confirmed regulation of the innate antiviral response by a canonical-like WNT/CTNNB1 pathway upon viral infections of primary human hepatocytes.

Adaptive Immunity

The adaptive immunity session was opened by a keynote address from Steven Foung, who reviewed the features of the broadly neutralizing antibody response detected in humans with HCV and the mechanism of immune escape. Barrett et al described that during antiviral therapy with (IFN-free) sofosbuvir and ribavirin patients with a sustained virologic response had improved HCV specific T-cell responses at the end of treatment and 24 weeks post treatment compared with the majority of relapsers. This suggests that immune responses may play a role in viral clearance in the context of direct antiviral agent (DAA) therapy. Shin et al presented data that HCV attenuates IFN-induced MHC class I expression to circumvent CD8+ T-cell responses. In terms of macrophage polarization, Szabo et al showed that in chronic hepatitis C, circulating monocytes are polarized toward an M2 phenotype that exhibit anti-inflammatory and profibrogenic characteristics. In vitro culture of monocytes with JFH-1 infected Huh7.5 cells also induced an M2 phenotype in a Toll-like receptor 8-dependent manner and could activate stellate cells.

Virus Evolution

Using well-characterized patients acutely infected with HCV, Bull et al revealed that the average half-life of HCV founder viruses was 61 days with natural clearance associated with greater magnitude and sustained cytotoxic T lymphocyte responses; in contrast, emergence of escape variants and persistence was associated with decline and temporary loss of detectable cytotoxic T lymphocyte responses. Sacks-Davis et al presented new data from the International Collaboration of Incident HIV and Hepatitis C in Injecting Cohorts Study (InC3). They studied the risk of HCV reinfection after spontaneous clearance of primary HCV infection and observed a shorter time to spontaneous clearance of HCV re-infection, suggesting partial protective immunity after clearance of primary infection. These 2 abstracts highlight the importance of the innate and adaptive immune responses for fighting HCV. Dahari et al performed a detailed study of viral kinetics during the anhepatic phase of 5 patients undergoing liver transplantation. In a novel finding, virus levels in blood remained static during the anhepatic phase of 3 patients, before declining in the early post-reperfusion phase, consistent with a central role for the liver in viral clearance as well as viral production. These data also raise the possibility of extrahepatic HCV production.

Virus–Host Interactions

The virus–host interaction presentations were split across 2 sessions. A keynote address from Gyongi Szabo
introduced the pro- and anti-inflammatory cytokine networks that play a role in the context of the HCV infected liver. Joyce et al described the ability of HCV to hijack the host nuclear pore complex and delocalize nuclear pore complex components to the membranous web during HCV infection, presumably to cargo components from the cytosol to the membranous web. Lin et al described the ability of ApoJ to stabilize and interact with the HCV core and NS5A proteins at the surface of lipid droplets and is essential for virus assembly and release. Finally, Ramage et al identified approximately 75 new pro-HCV host factors as part of a large-scale, proteomics-based screen to identify host interacting partners of the 10 genotype 2a HCV viral proteins.

In the second session, Szabo et al presented compelling data that exosomes isolated from HCV-positive patient plasma and HCV-infected cells in culture contained HCV RNA, miR-122, Ago2, and HSP90 and could transmit HCV RNA to naive primary hepatocytes and Huh-7 cells. This observation raises the possibility that HCV may be transmitted independent of HCV virions, thereby evading the immune system. Extrahepatic replication of HCV remains controversial and new evidence was provided by Machida et al, who showed that a chimeric lymphotropic (SB) strain and JFH1 HCV could infect RIG-I deficient Raji cells expressing miR-122, and B cells in a humanized mouse model. Finally, McLauchlan et al showed data suggesting a novel role for ubiquitin in HCV replication. Clearly, we have only uncovered the tip of the iceberg when it comes to HCV–host interactions and their impact on the HCV lifecycle and pathogenesis.

Pathogenesis and HCC

Stanley Lemon, in the keynote presentation, outlined the novel regulation of HCV replication by endogenous lipid peroxidation. The work presented showed how HCV-mediated regulation of endogenous miR-27a, a regulator of the lipid synthetic transcription factor RXRs, as well as lipid metabolism associated SREBP1, SREBP2, PPARα, PPARγ, and FASN. Novel pathogenic pathways were a further theme of the work presented by Awan et al, who used SILAC and mass spectrometry to identify the calcytenin-1 protein was associated with early endosome transport of HCV. Shirasaki et al showed that TGF-β impairs interferon signaling by c-JUN–mediated Foxo3a activation, which in turn activates SOCS3. Studies of the mechanisms underlying host responses that may explain the hepatocellular carcinoma risk in HCV were also presented by Machida et al and Lerat et al. In summary, this session showed novel pathways of HCV pathogenesis and the interplay of complex host pathways and the virus, which drive disease progression; however, we still have a long way to go in explaining the molecular mechanisms that underpin HCC development in the context of HCV infection.

Host Genetics and Response

Thomas O’Brien presented a keynote lecture summarizing the research of his team that led to the discovery of a dinucleotide polymorphism (ss469415590, ΔG/TT) between IFNL3 and IFNL2 that creates an open reading frame in a new gene, IFNL4, that is more strongly associated with HCV clearance, particularly in individuals of African ancestry. Supporting the specific role for a gene product of IFNL4, Mihm et al demonstrated IFNL4 mRNA expression in liver, particularly in patients with hepatitis C, and Murakawa et al used favorable or unfavorable IFNL3 promoter-reporter plasmids in vitro to demonstrate that IFNL3 promoter activities were significantly lower for the nonresponder alleles. Lucas et al used high-resolution HLA typing in individuals with genotype 1a or 3a HCV infection to demonstrate HLA class II-restricted associations with viral polymorphisms in NS2-NS5B. There was no overlap between associations for the different genotypes, suggesting divergent immune pressure. Further, 2 of the associations fell within known CD4+ T-cell epitopes suggesting novel CD4+ T-cell targets within the HCV genome. In another study, Klennerman et al reported evidence that HLA class I and II genes combine independently with IFI and KIR genotypes to impact additively, but not interactively, to the outcome of chronic hepatitis C in Irish women from a single source outbreak of chronic hepatitis C (CHC).

Antivirals and Clinical

This symposium has long been an important forum for the presentation of studies identifying novel host- and HCV-targeted antiviral agents. Only 2 years into the era of DAA therapy, a significant shift has occurred in the treatment arena for chronic hepatitis C. One viral target that has received considerable attention is the HCV NS5A protein, and several NS5A DAAs are in phase II and III development, such as daclatasvir and ledipasvir. However, because NS5A has no known enzymatic function, it is unclear as to the mode of action of these inhibitors. Stan Lemon et al elegantly analyzed the mechanism of action of NS5A DAAs and revealed 2 phases of inhibition: A primary phase (<2 hours) affecting virus release, followed by a secondary slower phase that affects viral RNA synthesis. Qi et al demonstrated that the majority of drug–protein interactions are clustered at the surface of the NS5A dimer. Using an in vitro microscale thermophoresis assay and multi-angle light scattering, Ascher et al demonstrated that NS5A forms 2 types of dimers in solution. Moving away from NS5A, Suhy et al introduced the possible role of adenovirus delivery of short hairpin RNAs targeting HCV. Their lead therapeutic, TT-034, could produce predictive, efficacious short hairpin RNA molecules in a nonhuman primate model, in the absence of liver and warrants further study. There were a number of other presentations on host-targeted antivirals in early clinical development. However, as raised during discussion periods, given the availability of a large number of viral-targeted DAAs that are in mid- to late-stage clinical development with high potency and favorable side effect profiles, it remains to be seen whether host-targeted antivirals will have a place in the HCV arena moving forward.
Vaccine Development

The vaccine session was evenly divided between presentations that focused on approaches to generate neutralizing antibody and cell-mediated immunity. The likelihood is that both will be necessary in a successful vaccine. In the keynote address, Heidi Drummer at the Burnet Institute in Melbourne summarized the role of antibody in HCV infection and vaccines and presented details of vaccination studies with a subunit vaccine based on an E2 protein lacking 3 variable regions. The theme of neutralizing antibody was then taken up by Law et al. They reported that vaccination of humans with a recombinant E1/E2 not only elicited neutralizing antibody with the ability to neutralize multiple genotypes, but also elicited strong T helper cell responses. Whether therapeutic vaccines will have a place in the treatment of HCV in the light of new DAAs is not clear. However, Sallberg et al presented the results of a phase II clinical trial in which HCV patients were vaccinated with DNA encoding HCV NS3/4A that was delivered via the intradermal route by electroporation followed by 12 weeks of pegylated-IFN/ribavirin therapy. Patients who receive the DNA vaccine followed by the standard of care showed reduced viral loads compared with the pegylated-IFN/ribavirin standard of care alone. These results suggest that augmenting the T-cell response to HCV may further enhance the efficacy of antiviral therapy. Grubor-Bauk et al described a novel therapeutic strategy using autologous, NS3-positive, necrotic dendritic cells with the rationale being that necrotic cells are highly immunogenic. Although the strategy is unlikely to gain widespread acceptance, it may have a place in the therapy of infections resistant to DAA.

After the vaccine session, a roundtable discussion was held to discuss the obstacles and opportunities to HCV vaccine development. At the table were distinguished vaccine researchers and immunologists Ian Frazer, Paul Klenerman, Steven Foung, and Philip Meuleman, as well as field researcher Peter Higgs. Opening up the discussion, the question of the ideal HCV vaccine was discussed by Paul Klenerman and Steven Foung and the need for robust broadly reactive T- and B-cell responses was advocated. Discussion also came from audience members, who commented on the impact of patents on vaccine development and the need for standardized assays for measurement of T- and B-cell responses. The impact of DAAs on the HCV vaccine landscape cannot be ignored and there is a growing perception that DAAs have the capacity to rid the developed world of HCV. However, this is dependent on a steep reduction in costs associated with such therapies and their widespread use to treat HCV-infected people. In addition, our perception that HCV is only a disease relevant to the developed world must change. Increases in life expectancy in developing countries that harbor the greatest burden of disease will make liver disease a major problem in the future. Finally, it should be remembered that those who have successfully cleared HCV through treatment remain at risk of acquiring HCV if exposed. A vaccine that induces protective immunity used in combination with DAAs would be the most effective means to eradicate HCV.

In summary, HCV2014 was a great success and this symposium is essential in bringing together the international HCV research community to share and discuss the latest advances in HCV research. Although we are in a new phase of HCV therapy with the eminent roll out of effective DAA (since HCV2013 the oral nucleotide inhibitor of the HCV polymerase, sofosbuvir, has been licensed in both the United States and Europe) with few side effects, there remain significant gaps in our knowledge regarding the HCV life cycle, pathogenesis of disease, and the development of an effective vaccine. As such, the HCV research momentum must be maintained. The 21st International Symposium on Hepatitis C Virus and Related Viruses will be held from September 7 to 11 in Banff, Canada.

References

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