Recombinant Virus like Particles comprising Hepatitis C Virus (HCV) structural proteins and HCV Replicon RNA

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Abstract

There is currently no effective system available for the study of the infection process by hepatitis C virus since virus from infectious sera grows very poorly, if at all, in cells *in vitro*. The development of replicon systems in recent years has enabled the study of virus replication within cells but has done little to facilitate research into the processes of virus particle assembly or the mechanism of infection. Even full-length replicons, which express all of the structural proteins in addition to the proteins involved in genome replication, do not seem to be capable of assembling infectious particles. Attempts have been made to address this problem by developing pseudotype systems in which the structural glycoproteins of HCV envelop the genomic components of a reporter virus, such as VSV or retroviruses. Although useful, these approaches are still not ideal and we have attempted to develop a completely homologous system in which the genome of HCV, as represented by a replicon, is encapsidated by HCV structural components.

In this approach Huh-7 cells bearing a HCV replicon were transduced with a baculovirus constructed to express the structural coding region (core-p7) of the HCV genome under the control of a CMV promoter. Cell supernatants and cell lysates were then examined for evidence of particle assembly and the ability to transfer replicons to fresh cells. Samples were examined by co-immunoprecipitation, western blotting and RT-PCR. Although HCV related material was not detected in cell growth media, both protein and RNA components were found in cell lysates. Anti-E2 monoclonal antibody co-immunoprecipitated E2, core proteins and RNA from both the structural coding region of the genome and the replicon. Lysates from replicon bearing cells transduced with baculoviruses expressing HCV structural proteins were able to transmit replicons to fresh Huh-7 cells whereas those from cells that were not transduced or were transduced with control baculovirus did not. Also, these components (iVLPs) were coincident in sucrose gradient fractions following centrifugation. Finally, EM showed that the morphology and the size of observed particles were consistent with previous reports of HCV particles. By using immunogold labeling, VLPs structures specifically labelled with anti-

E2 or anti-core antibodies were seen under electron microscopy. The immunolabelled VLPs structure were found to be located in the cytoplasm.

The production of iVLPs has the advantage that the replicon genomes are assembled within HCV related nucleocapsids and the route of particle assembly is similar of that of HCV. Therefore, they represent a valid model to investigate the early steps of HCV infection, as well as sero-neutralization. They afford a valid model to investigate the contribution of either cell-surface molecule in the early stages of HCV cell entry by performing receptor competition assays. Also, iVLPs afford the opportunity of evaluation as a vaccine candidate itself.