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Hepatitis E Virus Lifecycle and Identification of 3 Forms of the ORF2 Capsid Protein

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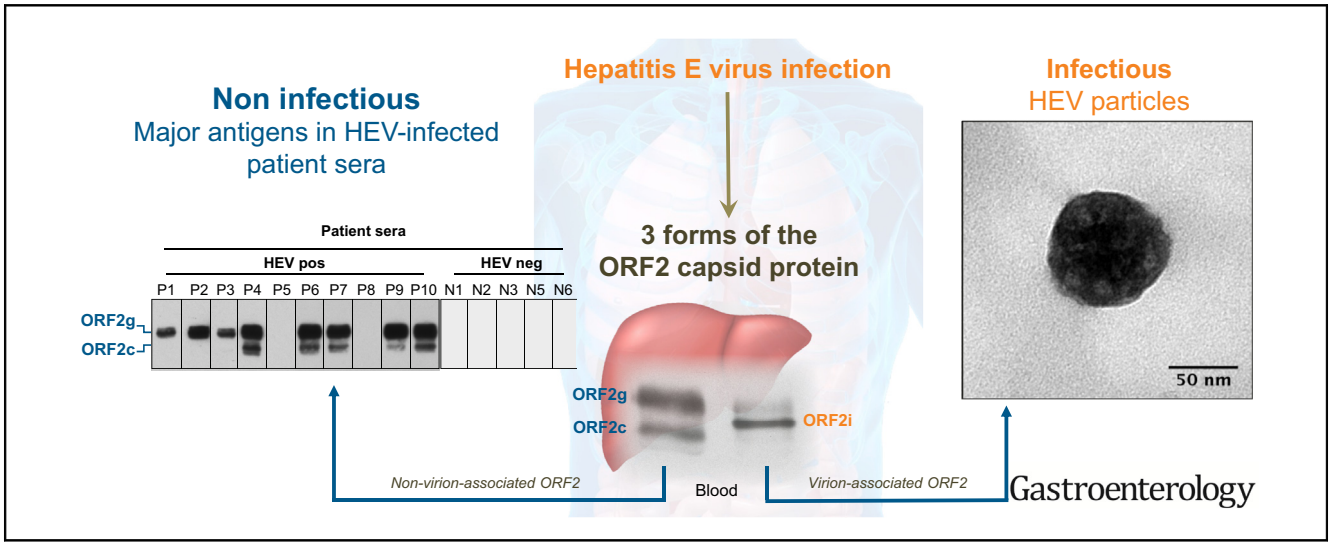

See Covering the Cover synopsis on page 7; see editorial on page 20.

BACKGROUND & AIMS: Hepatitis E virus (HEV) infection is a major cause of acute hepatitis worldwide. Approximately 2 billion people live in areas endemic for HEV and are at risk of infection. The HEV genome encodes 3 proteins, including the ORF2 capsid protein. Detailed analyses of the HEV life cycle has been hampered by the lack of an efficient viral culture system. **METHODS:** We performed studies with gt3 HEV cell culture–produced particles and patient blood and stool samples. Samples were fractionated on iodixanol gradients and cushions. Infectivity assays were performed in vitro and in human liver chimeric mice. Proteins were analyzed by biochemical and proteomic approaches. Infectious particles were analyzed by transmission electron microscopy. HEV antigen levels were measured with the Wantaï enzyme-linked immunosorbent assay. **RESULTS:** We developed an efficient cell culture system and isolated HEV particles that were infectious in vitro and in vivo. Using transmission electron microscopy, we defined the ultrastructure of HEV cell culture–produced particles and particles from patient sera and stool samples. We also identified the precise sequence of the infectious particle-associated ORF2 capsid protein. In cultured cells and in samples from patients, HEV produced 3 forms of the ORF2 capsid protein: infectious/intracellular ORF2 (ORF2i), glycosylated ORF2 (ORF2g), and cleaved ORF2 (ORF2c). The ORF2i protein associated with infectious particles, whereas the ORF2g and ORF2c proteins were massively secreted glycoproteins not associated with infectious particles. ORF2g and ORF2c were the most abundant antigens detected in sera from patients. **CONCLUSIONS:** We developed a cell culture system and characterized HEV particles; we identified 3 ORF2 capsid proteins (ORF2i, ORF2g, and ORFc). These findings will advance our understanding of the HEV life cycle and improve diagnosis.

EDITOR'S NOTES

BACKGROUND AND CONTEXT

Although Hepatitis E virus (HEV) is the major leading cause of enterically transmitted viral hepatitis worldwide, particles and HEV life cycle have been poorly studied and characterized up to now.

NEW FINDINGS

The ultrastructure of HEV cell culture-produced particles and particles from infected patients was defined by electron microscopy. The precise sequence and three forms of the infectious particle-associated ORF2 capsid protein was identified.

LIMITATIONS

This study was limited to HEV genotype 3.

IMPACT

This study leads to major advances into the understanding of molecular mechanisms of the HEV lifecycle that have also a significant impact on HEV diagnosis.

Keywords: Hepatitis E; PLC/PRF/5 Cells; Infectious Particles; ORF2 Products.

Hepatitis E virus (HEV) is the leading cause of enterically transmitted viral hepatitis globally, and is responsible for 20 million infections and 70,000 deaths every year.[1] Although HEV infection is usually self-resolving, severe forms or chronic infections have been described, mainly in immunocompromised patients. A high rate of mortality has also been reported among pregnant women. HEV infection has also been associated with extrahepatic disorders, including renal and neurological disorders.[2] Four genotypes (gt) are pathogenic in humans. Gt1 and gt2 exclusively infect humans, whereas gt3 and gt4 are zoonotic and mainly infect animals that may transmit the virus to humans. Recently, gt3 infections have been emerging in the Western world, likely due to contaminated blood transfusions and the consumption of contaminated food.[1] The diagnosis of hepatitis E is based on the detection of anti-HEV antibodies and/or viral RNA in patient serum.[3] Recently, a new assay based on detecting the HEV capsid protein antigen was developed (Wantaï Biologicals, Beijing, China), especially for laboratories with no molecular diagnosis facilities.

HEV is a quasi-enveloped, positive-sense RNA virus expressing 3 open reading frames (ORFs): ORF1, ORF2, and ORF3.[1] ORF1 encodes the ORF1 nonstructural polyprotein, which contains several functional domains essential for viral replication. ORF2 encodes the ORF2 viral capsid protein, which is involved in particle assembly, binding to host cells and eliciting neutralizing antibodies. ORF3 encodes a small multifunctional phosphoprotein involved in virion morphogenesis and egress. Although HEV is a nonenveloped virus in bile and feces, patient serum and cell culture–produced particles have been described to be associated with cellular lipids, as for hepatitis A virus,[4] and display the ORF3 protein at their surface.[5]

The growth of HEV in cell culture has been proven to be very difficult.[5] However, several HEV strains have been adapted to cell culture, including the gt3 Kernow C-1 strain, which contains an insertion of a 58-amino acid (aa) human S17 ribosomal protein.[6] Although these systems have led to increased understanding of the HEV life cycle, they still produce low infectious titers, limiting direct biochemical analysis of viral proteins and infectious material. Notably, the exact sequence of infectious particle–associated ORF2 protein is unknown. In addition, the ultrastructure of particles has never been robustly studied by immune electron microscopy.

In our study, we describe an HEV cell culture system that leads to early and massive expression of viral proteins and infectious particles, permitting their direct biochemical analysis. For the first time, we define the ultrastructure of HEV particles by electron microscopy and identify the precise sequence of the infectious particle–associated ORF2 capsid protein. We demonstrate that in infected cell culture and patients, at least 3 forms of the ORF2 capsid protein are produced. The 2 major ORF2 proteins are not associated with infectious particles, despite being the major antigens present in HEV-infected patient sera.

Materials and Methods

Chemicals and Cell Cultures

PLC/PRF/5 (CRL-8024), PLC1, PLC3, and A549 (CCL-185) cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% inactivated fetal calf serum (DMEM/fetal calf serum) at 37°C. Transfected cells were maintained at 32°C in a medium containing DMEM/M199 (1 vol:1 vol), 1 mg/mL of lipid-rich albumin (Albumax I) and 40 nM Na_2SeO_3.

Plasmids and Transfection

Plasmids expressing the cell culture adapted gt3 Kernow C-1 strain (HEV-p6, GenBank accession number JQ679013) or the replicon expressing the *Gaussia* luciferase gene (HEV-p6GLuc) were provided by S.U. Emerson.[6] The replication-deficient replicon HEV-p6GLucGAD was generated by mutating the GDD motif into the RNA-dependent RNA polymerase gene.[7]

***Authors share co-first authorship.**

Abbreviations used in this paper: **aa, amino acid; Ag, antigen; BFA, brefeldin A; DMEM, Dulbecco's modified Eagle's medium; DT, sodium deoxycholate and trypsin treatment; ER, endoplasmic reticulum; ffu, focus forming unit; GLuc, *Gaussia* luciferase; GNA, *Galanthus nivalis* agglutinin; gt, genotype; HCV, hepatitis C virus; HEV, hepatitis E virus; HEVcc, cell culture–produced HEV particles; HEVser, HEV particles from patient serum; IC, immunocapture; Neura, neuraminidase; O-Gly, O-glycosidase; ORFs, open reading frames; PBS, phosphate buffered saline; p.e., post-electroporation; PHH, primary human hepatocytes; RT, room temperature; real time-qPCR, real-time-quantitative polymerase chain reaction; SP, signal peptide; TEM, transmission electron microscopy; WB, Western blotting.**

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