Hepatitis E Genome Organization

Subjects: Virology

Contributor: J. Calvin Kouokam, Milena Mazalovska

Hepatitis E virus (HEV), a pathogen that causes acute viral hepatitis, is a small icosahedral, quasi-enveloped, positive ssRNA virus. Its genome has three open reading frames (ORFs), with ORF1 and ORF3 encoding for nonstructural and regulatory proteins, respectively, while ORF2 is translated into the structural, capsid protein. ORF2 is most widely used for vaccine development in viral hepatitis. Hepatitis E virus-like particles (VLPs) are potential vaccine candidates against HEV infection. VLPs are composed of capsid subunits mimicking the natural configuration of the native virus but lack the genetic material needed for replication. As a result, VLPs are unable to replicate and cause disease, constituting safe vaccine platforms. Currently, the recombinant VLP-based vaccine Hecolin® against HEV is only licensed in China.

Keywords: Hepatitis E virus ; ORF2 capsid protein ; HEV VLPs ; vaccine

1. Introduction

Hepatitis E virus (HEV) is an enterically transmitted pathogen and a major cause of acute hepatitis in many developing countries within Africa and Asia [1]. Approximately one third of the world population live in areas in which HEV is endemic and thus are at risk of infection [2]. Unlike other viruses causing hepatitis, HEV-related disease is a zoonotic infection with pigs, wild boars and certain other species such as deer and rabbits being considered as reservoirs for the virus [3][4]. Although the fatality rate during epidemics is low, i.e., between 0.2–5% [5], the mortality rate in pregnant women is as high as 25%, possibly due to altered hormone status and decreased immunity [6][7][8]. Even though HEV infection is considered self-limiting or asymptomatic in healthy individuals, it can lead to severe disease in patients with preexisting liver conditions, with high morbidity and mortality [9][10]. Chronic infection could develop in immunocompromised patients such as organ transplant recipients [11], individuals administered immunosuppressants [12], patients on chemotherapy for hematological malignancies [13], HIV-infected patients [14] and cases of superinfection with other hepatitis viruses [15]. In 10% of chronically infected patients, HEV leads to rapid progression to liver cirrhosis in less than 3 years [16]. In addition, it has become evident in recent years that HEV infections can be associated with neurological manifestations [17][18], renal aliments [19], hematological disorders [20] and acute pancreatitis [21]. Furthermore, recent data indicate a link between HEV infection and progression to hepatocellular carcinoma in patients infected with hepatitis B virus (HBV) or hepatitis C virus (HCV) [22][23]. Atsama et al. [22] reported significantly higher prevalence of anti-HEV IgG in hepatocellular carcinoma (HCC) patients infected with either HBV or HCV compared with HBV/HCV-infected patients with chronic liver disease but not suffering from HCC [22]. This finding suggests that infection with HEV could worsen liver inflammation and increase the severity of other infections. Another study also reported that HEV superinfection accelerates the progression of chronic HBV infection and increases 1-year mortality [23].

Traditional approaches for the development of an HEV vaccine have been ruled out because the manufacturing of either live attenuated or inactivated vaccine would be impossible due to the complexity and low yield of viral culture. Even though culturing the virus has been difficult in the past, a few strains have been adapted to cell culture, leading to a better understanding of the HEV life cycle [24].

Presently, significant progress has been made in the development of HEV vaccines based on the ORF2 capsid protein as either a subunit or virus-like particle (VLP) [25]. VPLs represent one of the most attractive systems for vaccine development due to their safety, immunogenic properties and ease of production [26]. VLPs are generated from one or more viral capsid proteins that self-assemble into high-molecular-weight structures that resemble the native virions but lack the viral genome [27]. As a result, VLPs are replication- and infection-incompetent, making them a safe alternative to attenuated or inactivated viruses in vaccine development. Since they are structurally similar to the native virus, they can induce stronger B and T cell responses than traditional small subunit vaccines [28]. Additionally, VLPs can be better taken up by professional antigen-presenting cells (APCs) as exogenous and endogenous antigens for processing and presentation by MHC class II and I molecules, respectively. Cross-presentation by MHC class molecules activates CD4+ and CD8+ T cells that elicit specific cytotoxic T lymphocyte (CTL) responses resulting in infection control [29]. Furthermore,

VLPs can be assembled not only from proteins from a single virus, but also from proteins of distinct viruses or various other pathogens, e.g., bacteria and protozoa [30]. To date, several VLPs have been produced for protection against infectious diseases in prokaryotic or eukaryotic expression systems [31], and in some cases assembled in cell-free conditions [32]. Some of these products have been licensed, including Engerix® (Hepatitis B virus) [33], Cervarix® (human papilloma virus) [34], Recombivax HB® (HBV) [35] and Gardasil® (HPV) [36], while others are still under pre-clinical and clinical evaluation [37][38].

2. Hepatitis E Genome Organization

Previously known as non-A non B hepatitis, HEV is currently classified in the *Hepeviridae* family with the two genera *Orthohepeviruses* and *Pischihepeviruses* [39]. The *Orthohepevirus* A genus includes genotypes 1 and 2 isolated from humans, genotypes 3 and 4 from both humans and animals, the newly proposed genotypes 5 and 6 from wild boars and genotype 7 from dromedary camels [40][41].

HEV is a quasi-enveloped, icosahedral, single-stranded positive-sense RNA virus that was molecularly characterized for the first time in 1990 [42]. Its genome is around 7.2 kb with features of a eukaryotic mRNA, including a 5′ cap and 3′ poly A tail, 5′ and 3′ untranslated regions (UTRs), and three open reading frames, including *ORF1*, *ORF2*, and *ORF3* [43]. During HEV genome replication two viral RNA species are generated, i.e., the full-length genomic RNA and a subgenomic RNA [44]. The subgenomic RNA allows the expression of *ORF2* and *ORF3* (Figure 1).

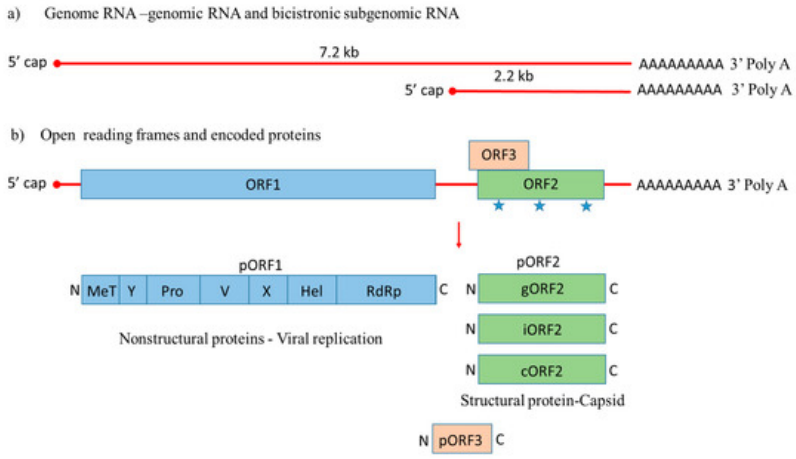

Figure 1. Genome organization of Hepatitis E virus. (**a**) Hepatitis E-Virus (HEV) genome generates the full-length genomic RNA and subgenomic RNA with 5′ cap, 3′ Poly A tail, 5′ UTR and 3′ UTR. (**b**) The genomic RNA has three open reading frames: *ORF1*, *ORF2*, and *ORF3*. *ORF1* encodes the nonstructural proteins for viral replication; *ORF2* is translated into the capsid protein with three potential glycosylation sites (★), with a small multifunctional protein encoded by *ORF3*. Three different capsid proteins have been discovered in vitro during infection, i.e., gORF2-glycosylated, iORF2-infectious and cORF2-cleaved ORF2.

ORF1 encodes nonstructural proteins involved in viral replication [45][46]. A small multifunctional 13 kDa protein is expressed from *ORF3*, which facilitates HEV transport throughout the cell and acts as viroporin for the release of the infectious virus from the host cell [47][48]. *ORF2* encodes the 72 kDa capsid protein comprising 660 amino acids that contains a hydrophobic stretch of 14–34 amino acids at the N-terminus, which functions as a signal sequence for its secretion [49]. ORF2 is involved in virion assembly, attachment to the host cell and immunogenicity [50][51][52]. Additionally, the capsid protein has three potential glycosylation sites (Asn 132, 310 and 562) [53].

Native HEV particles are round non-enveloped with spikes covering the surface [54][55]. It is considered that 180 copies of the ORF2 protein form the HEV virion giving it T = 3 icosahedral symmetry [56]. Recently, a few strains have been adapted for replication in cell culture, providing novel insights into the HEV cycle. Even though HEV particles present in the bile and feces are non-enveloped, it was demonstrated that in patient serum and cell cultures, HEV particles are partially associated with lipids and the ORF3 protein [57]. Moreover, recent studies have identified different forms of ORF2 in cultured cells. Large ORF2 protein amounts are released from HEV-infected cells in vitro and found in serum from HEV-infected patients. This secreted protein (ORF2s) was shown to be glycosylated form of the capsid protein that is not associated with the HEV virion. The other intracellular protein (ORF2c), a translation product of the same gene starting with the second AUG codon, is involved in HEV assembly [58]. Montpellier et al. reported iORF2 (infectious), gORF2 (glycosylated) and additional ORF2 truncated protein (ORF2c) are not involved in virion assembly using another genotype and cell culture for replication [59].

Great efforts have been made towards understanding the HEV life cycle in recent years by developing cellular systems and infectious HEV clones [60]. Polarized cell models have been developed to closely mimic in vivo infection with HEV, which are highly permissive to infection, making them a good tool for molecular studies of the HEV cycle. For example, human hepatoma-derived HepaRG and porcine hepatocyte-like PICM-19 cell lines have been shown to support HEV replication, and are useful for studying virus–host interactions and species barrier crossing, especially since HEV infection is a zoonosis in developed countries [61]. Capelli et al. [62][63] showed that different HEV genotypes release more than 90% of the virus from the apical membrane after infecting polarized human hepatocellular carcinoma HepG2/C3A cells, suggesting the main route of release for infectious virions [62][63]. In recent years, the key steps of HEV's natural infectious cycle in vivo have been confirmed by employing polarized human stem-cell-derived, hepatocyte-like cells (HLCs). Infection of these cells with HEV results in the secretion of two different progeny particle types, including quasi-enveloped particles from the basolateral membrane and naked highly infectious virions from the apical membrane [64]. These findings provide novel insights into the HEV infectious cycle. The release of HEV particles basolaterally could spread the infection in the host and lead to extrahepatic manifestations [65].

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