Abstract

Hepatitis E virus is the sole member of the genus *Hepevirus* in the family of *Hepeviridae* and is a causative agent of acute hepatitis. Based on geographical distribution HEV can be grouped under four genotypes: genotype 1, 2, 3 and 4. Genotype 1 is restricted to humans whereas genotype 4 has been shown to infect pigs in addition to humans. The viral genomic region responsible for this phenomenon is not yet clear.

In this study we developed full genome cDNA clones of genotype 1 (T1FG) and genotype 4 (T4FG) viruses. We observed that though genotype 1 was positive for replicative intermediates in liver cells of human origin they were negative for the same in kidney cells of swine origin. On the other hand genotype 4 was positive for replicative intermediates in swine kidney and human liver cells. IFA positivity of both genotypes was very less though.

We further constructed chimeras by replacing regions of type 1 with corresponding regions of type 4. We also procured genotype 1 clone pSK-HEV2 from Dr. Emerson’s laboratory for comparison. We assumed that replacement of the viral sequence of type 1 virus, responsible for deciding the species specificity, would impart permissiveness to the type 1 virus which was initially unable to grow in swine cells. In view of this, keeping genotype 1 virus full genome clone pSK-HEV2 as the backbone we developed chimeras by replacing regulatory regions- 5'NCR, 3'NCR, putative sub-genomic promoter (junction region), ORF2/3 encoding region and complete ORF1 (encoding replicative enzymes).

S10-3 cells were used as model system for genotype 1 virus and PK-15 cells for genotype 4. All chimeras were tested for their replication competency in S10-3 cells and PK-15 cells. All parental and chimeric constructs showed positivity in S10-3 cells indicating that they could establish successful replication. T4FG was positive for replicative intermediate in both the cells. In addition, we found that chimera harbouring ORF1 along with its adjacent 5'NCR region of genotype 4 also gave positive results in both the cells. 5'NCR of the genotype 4 genome either alone, or in combination with other type 4 regions did not yield
positive results in PK-15 cells, indicating crucial role of ORF1 in crossing the species barrier.

All chimeras remained negative in IFA till 12 days post transfection in PK-15 cells. However, all chimeras were IFA positive in S10-3 cells on day 12, though with different intensity and different percent positivity.

It was observed that though 3’NCR from type 4 in chimera, T4 (3’NCR) resulted in enhancing the replication efficiency; it was further enhanced with addition of adjacent type 4 Jn+ORF2 region in the construct. Whereas, the construct with 5’NCR replacement (chimera, T4 [5’NCR]) exhibited drastic reduction in the replication efficiency than that of the pSK-HEV2.

We hypothesized that infection of PK-15 cells with chimeric virus (generated after transfection of S10-3 cells), would help us determine if the host restriction of genotype 1 virus is at the receptor level or it is dependent upon the host cell environment (host cell proteins). In addition we also used HepG2/C3A cells as these cells have been reported to be suitable for infectivity studies. HepG2/C3A cells infected with the lysates of S10-3 cells transfected with the chimeric construct T4 (Jn-ORF2-3’NCR) were positive for both nsRNA and IFA. Fluorescence could not be detected for other constructs. Samples for pSK-HEV2 and chimera T4 (Jn-ORF2-3’NCR) were positive for replicative intermediate. Most likely the virus particles were very less to establish a successful infection.

Further studies are needed to evaluate the role of ORF1 with context to host specificity.