Surveillance of rotavirus strains reveals evidence of emerging G12 and unusual human-animal reassortant strains in Manipur, North-eastern India

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Background: The rotaviruses are the major cause of severe gastroenteritis and account for an estimated 100,000 deaths and 400,000 hospitalizations in children under five years of age in India. To date, 23 G and 31 P genotypes have been reported in Group A Rotaviruses on the basis of differences of the outer capsid VP7 and VP4 encoding genes. North-Eastern states of India are geographically distinct with temperate weather compared to rest of the India. Few outbreaks were reported during the year 1979 to 1988 in Manipur with evidence of zoonotic transmission but systematic surveillance study was not done till date.

Methods: The samples were collected from children (<5 years) hospitalized with acute diarrhea at the Regional Institute of Medical Sciences (RIMS), Manipur between December 2005 to March 2008. Rotavirus positivity was screened by ELISA or PAGE. RT-PCR was used to determine specific genotypes. Sequencing and blast analysis was also performed to analyze evolutionary and genetic diversity of the circulating strains.

Results: The globally common genotypes G1P[8] and G2P[4] constituted 58% of the total positive strains, while 3% and 8% strains were of the emerging genotypes, G9P[6] and G12P[6]. G12 strains have been reported from Manipur for the first time. The G12 strains clustered with lineage III strains and had >98% identity with Bangladeshi, Thailand and USA strains. Few unusual G-P combinations like G4P[6], G10P[6] and G9P[19], along with some G-P non-typables were also found. G4 and G10 strains clustered with the porcine and bovine strains, indicating zoonotic transmission.

Conclusion: High frequency of rotavirus infection (A 50%) and predominance of the common strains G1P[8] and G2P[4] among children with acute diarrhea emphasizes the need for implementation of currently available vaccines to reduce the burden of rotavirus induced diarrhea in high risk regions of developing countries.

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The nucleocapsid protein of measles virus and other morbillivirus blocks host interferon signaling pathway

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Background: Interferon (IFN)-β/α and IFN-λ are potent antiviral cytokines that are induced in direct response to viral infection and have important roles in the inhibition of viral replication and regulation of host immunity. Against these functions, viruses have developed many mechanisms to escape the antiviral action of IFN. Measles virus (MV) belongs to the genus Morbillivirus of the family Paramyxoviridae, which contains canine distemper virus and rinderpest virus, and has negative-sense RNA genome and consists of six tandemly linked genes that encode eight proteins. A number of paramyxoviruses contribute to viral evasion of the host IFN response by various mechanisms involving one or more their nonstructural accessory proteins. In the case of MV, it has been reported that the accessory V and C proteins inhibit host IFN signaling pathways. However, the mechanisms are unclear in detail. In this study, we confirmed the effect of not only the accessory proteins of MV-HL strain but also other proteins on the IFN signaling pathways.

Methods: HEK-293T cells were transfected with IFN-β/α or IFN-λ inducible reporter plasmid together with expression plasmid encoding various viral proteins and then treated with each IFN. To clarify the mechanism of the inhibition, we further analyzed details using western blotting and indirect immunofluorescence.

Results: We found that the MV nucleocapsid (N) protein (MV-N) inhibits both IFN-β/α and IFN-λ signal transduction like the accessory proteins. In the results of western blot analyses, MV-N neither prevents the phosphorylation of STAT and Jak nor induces STAT degradation. On the other hand, MV-J inhibits the nuclear import of activated STAT. We confirmed that the N protein of the other morbilliviruses also disturbs the IFN signaling.

Conclusion: The N protein is the most abundant of the viral proteins and enwraps the genomic RNA into helical filamentous structures referred to as nucleocapsids. In this study we revealed a novel function of the N protein of morbillivirus that acts as an IFN-antagonist. The presented here give insight into understanding how the viral proteins of morbilliviruses perform to inhibit IFN signaling.

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Analysis of phosphorylation residues on Nipah virus nucleocapsid protein and role of the phosphorylation

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Background: Nipah virus (NiV) is a recently emerged zoonotic virus that causes encephalitis and respiratory illness in humans and livestock, with a high mortality rate (40-70%) in humans. NiV is a negative single-stranded RNA virus that belongs to the family Paramyxoviridae and is composed of six structural proteins. The viral nucleocapsid (N) protein encapsidates the genomic RNA and forms a nucleocapsid. This serves as a template for viral replication and transcription, which is catalyzed by an RNA-dependent RNA polymerase that is composed of viral phosphoprotein (P) and large (L) proteins. NiV is related closely to Measles virus (MV), which has a well-characterized N protein and also belongs to the family Paramyxoviridae. It has been reported that MV-N undergoes phosphorylation modification. Recently, we identified the phosphorylation sites of MV-N, and the phosphorylation was required for efficient viral transcription. However, phosphorylation of NiV-N has not been reported. In this study, we investigated that if NiV-N also undergoes phosphorylation.