STUDYING NEUROVIRULENT DETERMINANTS OF TBE VIRUS

Background

There are five known subtypes of TBEV - the European, Siberian, Far Eastern, Baikalian and Himalayan subtype. The genetic differences on the amino acid level within the subtypes is about 2% and 5-6%. The disease is generally milder when infected with the European subtype. Vaccines are available based on the Far Eastern and European subtypes which induce high neutralizing antibodies. The knowledge about viral determinants, which are responsible for the severity of disease, is limited. Adaption of TBEV to cell culture can result in amino acid (aa) changes in the envelope protein E (gE), resulting in stronger interaction with glycosaminoglycans, accompanied with attenuated neuroinvasiveness. No aa exchange has so far been described that leads to increased TBEV infection of the main target cell in the brain - the neurons. The authors tried to identify novel virulence determinants in TBEV infection.

Results

A Swedish TBEV strain - 93/783 - was identified that caused earlier death after i.p. infection with 104 focus forming units (FFU) in C57BL/6 mice, compared with other European subtype strains (Torö, Hypr, AS33, HM467/09 and Neudörfl), as well as with Siberian strains (M14/10 and Aina) and the Far Eastern subtype strain Sofjin, while Torö infected mice survived longer compared to Siberian strains.

To assess the neurovirulence of the two strains 93/783 and Torö, mice were intracranially infected with 102 FFU. Both strains revealed to be highly neurovirulent because all mice died within 8 days. 93/783 was the most neurovirulent strain that showed higher levels of viral replication in olfactory bulb, cerebellum, and brainstem of mice.

Two unique aa were found in gE of 93/783 – threonine 83 in the domain II and serine 463 in the stem anchor region, and a chimeric Torö virus was generated replacing the gE of Torö by that of 93/783. This chimeric strain was called Torö93E. The function of these aa in gE were analyzed. Strain 93/783 and the chimeric Torö strain showed much more efficient receptor binding and entry into neurons compared to Torö strain indicating that alanine 83 and serine 463 might be important virulence determinants by increasing neuronal infection.

Mice i.p. infected with 93/783 died after 7.5 days, while the survival time after infection with Torö was 11.5 days. After infection with Torö93E, the medium survival time was 10.4 days, indicating that the gE of 93/783 may contribute to the pathogenicity.

The authors hypothesized that TBE vaccine breakthroughs in Sweden may be caused by non-optimal neutralizing antibodies induced by the different vaccines (which have different aa sequences in their gE) used against naturally circulating strains in Sweden. Five aa substitution were found among the strains Neudörfl (antigen in FSMS Immun) and K23 (antigen in Encepur). K23 was found to have two unique aa substitutions in aa 52 and aa 136 which were not found in Torö, Neudörfl and 93/783, whereas Neudörfl possessed one unique aa substitution at position 167 compared to other strains.

Sera from 36 vaccinated individuals were analyzed for their neutralizing capacity: 11 from FSME Immun-vaccinees, 12 from Encepur vaccinees, and 13 from mixed FSME Immun/ Encepur vaccinees. Sera from individuals vaccinated with FSME Immun or from mixed FSME Immun/Encepur significantly neutralized Neudörfl better than Torö and 93/783 and the sera from FSME Immun and mixed FSME Immun/

Encepur neutralized Torö and 93/783 more efficiently compared to Encepur sera. Interestingly, sera from K23-based Encepur vaccinees showed overall lower neutralizing titers to all three strains tested with some not reaching to neutralizing titers. This indicates that aa residue 52 may be an important determinant for a neutralizing response.

Discussion

TBEV strain 93/783 was found to be more neurovirulent than Torö and this may be due to the gE of this strain which is involved in receptor binding and membrane fusion and could be of great importance for fast spread and infection in the brain. The gE of 93/783 mediated better binding and infection into neurons and contributed to the pathogenicity in mice.

Sera form individuals vaccinated with strain Neudörfl-based FSME Immun vaccine showed a higher neutralizing capacity to Swedish virus isolates compared to K23-based Encepur, for which the reason may be the unique aa substitution in the gE of K23. More studies are warranted to analyze the different antigenic behavior of K23-based Encepur and Neudörfl-based FSME Immun vaccines (and additional vaccines based on other strains) and to analyze if such differences in the gE antigens can be associated with vaccine breakthroughs.

Literature

Lindqvist et al.

The envelope protein of tick-borne encephalitis virus influence neuron entry, pathogenicity and vaccine protection

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