Thesis for doctoral degree (Ph.D.)

PATHOLOGIC POTENTIAL OF VARIANT VIRUSES AND RETROSPECTIVE STUDY OF CLINICAL DIAGNOSIS ON FLAVIVIRUS ENCEPHALITIS IN VIETNAM

Le Xuan Luat

At
Institute of Tropical Medicine (NEKKEN)
&
Graduate School of Biomedical Science,
Nagasaki University, Japan

2013
Arthropod-borne flavivuses cause a variety of clinical symptoms such as meningoencephalitis, hemorrhagic fever, and arthritis. Infection caused by encephalitic flaviviruses may vary from mild to a severe form. This could be attributed to the degree of virulence of each virus strain. In this dissertation, I tried to find any molecular determinant(s) of virus virulence of flaviviruses.

Tick-borne encephalitis virus (TBEV) is a zoonotic agent that causes acute central nervous system (CNS) disease in humans. It is widely believed that immune response in addition to CNS infection contribute to mouse mortality following TBEV infection. However, it is not well elucidated whether virus variants have any difference on pathogenesis of the virus infection. Therefore, in this study, we investigated the biological and pathologic potentials of the variant clones in the TBEV Oshima strain. We isolated eight variant clones from the stock virus of the Oshima 5-10. These variants exhibited different plaque morphologies in BHK cells and pathogenic potentials in mice. Full sequences of viral genomes revealed that each of the variant clones except one had specific combination of nucleotide and amino acid changes at certain positions different from the parent strain. We also showed that an amino acid substitution of Glu122→Gly in the E protein could have likely affect virus infection and replication in vivo, and the attenuated pathogenicity in mice. These data confirm the presence of virus variants or quasispecies from the parent strain. Further elucidation of the effect of each variant clones for immune response such as T cell response is important priority to enable the development of effective vaccine and treatment strategies for TBE.

In developing countries in South East Asia including Vietnam, Japanese encephalitis (JE) is the most important encephalitic flavivirus infection. While, Echovirus 30 (E30) has
been reported to be the equally common causative agents of acute meningitis among patients in the same region. And thus differential diagnosis is essential in clinical field. In view of this, an outbreak in Vietnam in 2001–2002 gained our interest because the initial clinical diagnosis of infected patients was due to JEV infection. There are few clinical insights regarding E30 cases, and there are no reports comparing E30 and JEV acute meningitis/encephalitis cases based on clinical symptoms and case histories. We therefore aimed to identify reliable clinical methods to differentiate E30 and JEV acute meningitis/encephalitis.

A retrospective, cross-sectional study was conducted to compare E30 and JEV acute meningitis/encephalitis cases. We collected and analyzed the clinical records of 43 E30 confirmed cases (E30 group) and 60 JEV confirmed cases (JEV group). Clinical data were compared between the E30 and the JEV groups. Differences of clinical parameters were analyzed by certain statistical tests. Fever, headache, and vomiting were the most common symptoms in both the E30 and the JEV groups. Combined symptoms of headache and vomiting and the triad of symptoms of fever, headache, and vomiting were observed in more patients in the E30 group (E30 vs. JEV: 19% vs. 0%, p< 0.001; 74% vs. 27%, p< 0.001, respectively). On the other hand, strong neurological symptoms such as seizure (5% vs. 73%, p<0.001) and altered consciousness (12% vs. 97%, p<0.001) were manifested primarily in the JEV group. CSF leukocytosis was observed predominantly in the E30 group (80 vs. 18 cells/μL, p=0.003), whereas decreasing CSF sugar level was observed predominantly in the JEV group (58.7 vs. 46.9 mg/dL, p< 0.001). Fever, headache, vomiting, absence of neurological symptoms (seizure, altered consciousness), and presence of CSF leukocytosis are important parameters to consider in differentiating E30 from JEV cases during early infection. Then, proper measures can be adopted immediately to prevent the spread of the disease in the affected areas.