Abstract

Dengue fever and Japanese encephalitis (JE) are the two most important reportable diseases belonging to Flavivirus family in Taiwan. Due to the increased intensity of dengue fever in many part of the world, World Health Organization (WHO) had listed the development of rapid and accurate diagnostic assays for early diagnosis of dengue fever as the first priority in the studies of dengue prevention and control. Our lab has been devoted to develop a system that is able to differentially diagnose all flavivirus (dengue virus, JE virus, yellow fever virus, and West Nile virus) potentially cause outbreaks in Taiwan. A comprehensive system based on virus isolation, RT-PCR, various ELISA and sequence analysis had been developed to detect viral nucleic acid and NS1 antigen in the acute-phase serum samples and virus-specific antibodies in the convalescent-phase serum samples. Although we have setup various forms of ELISA using native antigens (Envelop and NS1 antigens) secreted in the culture supernatants of virus infected cells, little is known about the immune responses elicited to the NS proteins that are not secreted outside the infected cells. We have so far succeeded in the cloning and expression of DEN-2 capsid and NS3 recombinant proteins in E. Coli expressing system. Initial studies were performed to analyze the anti-NS3 IgM and IgG antibody response using Ni-NTA agarose purified recombinant proteins. The results showed that the convalescent and post-convalescent phase serum samples from confirmed dengue patients had significant higher titers in ELISA compared to normal control sera. The specificity of NS1-specific IgM and IgG ELISA are 80-93%, respectively. The sensitivity, however, is still very low and is not suitable for clinical diagnosis at its present format. In the future, we will try to improve the sensitivity of this NS3-specific ELISA assay by increasing the purity and native antigenicity of recombinant proteins. The availability of dengue and JE NS3 and NS5 recombinant proteins will eventually used for the serodiagnosis and seroepidemiological studies. This will contribute significantly for the decision-making and development of effective control strategy.

Keywords：flavivirus；dengue fever；Japanese encephalitis；ELISA；non-structural protein