

Abstract

Enterovirus (EV) is a RNA virus. Mutation was often found in enterovirus isolates due to the lack of RNA proof reading. The EVs that can not be typed by FA test had increased since 1999. The NT test is laborious and time consuming. Besides, the NT antibodies are expensive and are running out worldwide. To address the typing of EVs in recent years and to study the molecular epidemiology of EVs in the last two decades, the sequences of the VP4 region were analyzed. The monoclonal antibody(mAb) against EV71, CA16 and E30 were prepared. Eighty four strains of NPEV from 1999 to 2004 were analysed by sequence analysis. The results revealed that the major identified serotype of NPEV in each year were different. CA4 was the major type in 2004 (from CDC data) CA16 was the major type in 2003,2000,1999. E6 was the major type in 2002. E30 was the major type in 2001. The molecular epidemiological study of EV71 isolated in 2004 suggested the reemergence of genotype C instead of the genotype B that predominated in 1999-2003. It is suggested to keep alert on this reemerged EV71 genotype.

For the preparation of monoclonal antibodies (mAbs) one each strains of EV71 from the genotype B and genotype C and one each strains of CA16 and E30 were prepared for the immunization of BALB/c mouse. Both CA16 and E30 strains did not react with the commercialized monoclonal antibody. Nine, three and twelve clones of mAb of EV71, CA16 and E30 were found, respectively. The nine mAb of EV71 react very well with EV71 VP1 antigens by FA and WB or immunodot. The isotype of immunoglobulin is IgG₁. Six of the nine mAbs showed with the following viruses : no cross reaction CA16, EV70, CA9, CA10, CB3~CB6, E6, E7, E9, E30. A NT titer of 1 : 2 was found in clone E611G2G. The analysis of the location of antigen determinant will be further studied using the VP1 expression systems constructed in our lab i.e. *E coil* (pET32a) and pcDNA3.1. The characterization of the mAbs of CA16 and E30 are in progress.

Keywords : Enterovirus (EV) ; monoclonal antibody(mAb) ; immunoglobulin