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Neutralization Assays for Echovirus 18 Isolates in 2006

Shinichi Takao*, Kiyoko Wakatsuki[1], Hiromu Yoshida[2], Hiroyuki Shimizu[2] and Takaji Wakita[2]

Hiroshima Prefecture Institute of Health and Environment, Hiroshima 734-0007;
[1]Fukuoka City Institute for Hygiene and the Environment, Fukuoka 810-0065; and
[2]Department of Virology II, National Institute of Infectious Diseases, Tokyo 162-8640, Japan

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According to the National Epidemiological Surveillance of Infectious Diseases (NESID), epidemics caused by echovirus 18 (E18) have occurred mainly in the western area of Japan ever since the E18 isolation in Kitakyusyu City in 2006 (1). In general, enterovirus infection causes mild clinical symptoms such as rashes, etc., in infants, and severity such as aseptic meningitis in older children. Early in 2006, E18 was isolated from infants with rashes and upper respiratory inflammation; in the subsequent summer season of 2006, the number of patients with aseptic meningitis increased.

The 2006 epidemics by E18 in Hiroshima Prefecture and Fukuoka City followed a series of outbreaks by E18 in the same area during 2001 - 2004. The domestic EP95 enterovirus antiserum pool is routinely available for the identification of enteroviruses; however, it was difficult to identify the 2006 isolates by neutralization assay using EP95. Therefore, nucleotide sequencing analysis with isolates was performed in Fukuoka and Hiroshima to study whether the antigenic drift occurring on the VP1 region of the viral genome provided a major antigenic site, or not.

For the isolation of E18, a throat swab, cerebrospinal fluid, or stool sample was used for patients with rashes, upper respiratory inflammation, or aseptic meningitis, respectively, and isolates were the more sensitive to RD-18S cells.

Of the clinical isolates in 2006, most isolates were not well-typed using EP95. The result of neutralization assay (NT) using EP95 should be carefully interpreted owing to the breakthrough phenomena, and then confirmed using type-specific E18 antisera (20 - 50 units) made of a prototype Metcalf strain supplied by the Enterovirus Reference Center at the Department of Virology II, National Institute of Infectious Diseases, Tokyo.

Though it was reported that the commercial type-specific E18 antiserum might have cross-reactivity to E6, E30, and other serotypes (2), the NT assay using it should be carefully performed. E6 can be grown well in both HEp-2 and RD-18S cells with remarkable cytopathic effect (CPE). On the other hand, the CPE in RD-18S cells caused by E18 proceeds more slowly than that by E6, and the growth of E18 in HEp-2 cells is less susceptible (2). Thus, the unique CPE pattern by E18

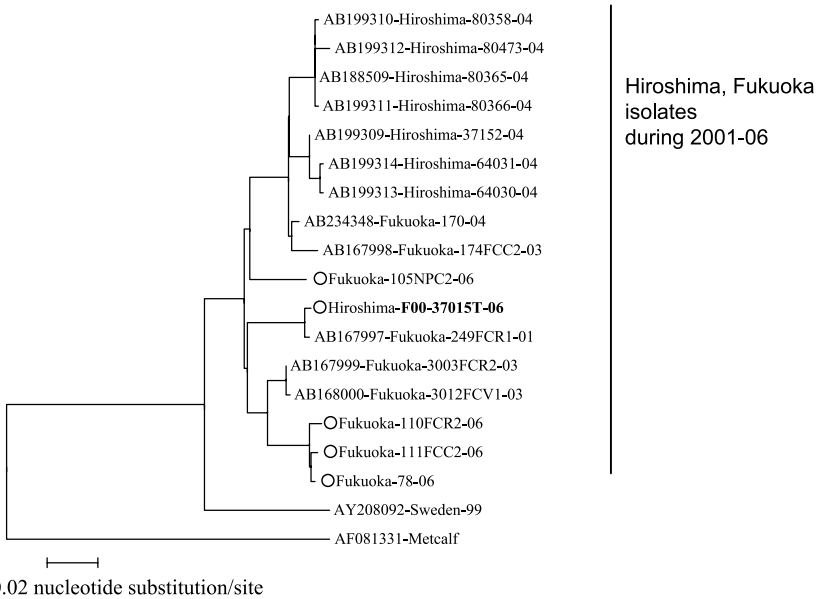

Fig. 1. Phylogenic relationship among isolates from 2001 to 2006. Phylogenetic tree for E18 using the VP1 partial region (694 bp), generated by neighbor-joining method with 19 isolates. Circles indicate isolates in 2006. They were compared with, Prototype Metcalf (AF081331) and Sweden strain (AY208092).

*Corresponding author: Mailing address: Hiroshima Prefecture Institute of Health and Environment, Minami-machi 1-6-29, Minami-ku, Hiroshima 734-0007, Japan. Fax: +81-82-252-8642, E-mail: s-takao84335@pref.hiroshima.jp

found in RD-18S dutin virus isolation may be helpful in differentiating it from other enteroviruses before the NT assay.

To determine the genetic relationship among isolates, we compared isolates in 2006 with those isolated during 2001-2004. After viral RNA purification and RT-PCR to amplify the partial VP1 region of the viral capsid genome using a pan-entero primer set (3,4), the 2006 sequencing analysis was performed for 4 and 1 isolates, respectively, in Fukuoka City and Hiroshima Prefecture, and results were compared with those of 12 other isolates deposited previously in the same area.

Though the serotype of the F00-37015T isolate in Hiroshima Prefecture, as a representative isolate, was difficult to determine by NT assay, it had a high identity of amino acid sequence with previous isolates at the VP1 region (Fig. 1). The nucleotide divergence among isolates was at most 5%, and 1-2 amino acid substitutions on the VP1 region. Therefore, a few amino acid substitutions might affect antigenicity.

The findings of our virus investigation for E18 in 2006 may be useful for responding to and preventing future epidemics.

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