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Isolation of Echovirus Type 13 in Osaka City during 2001-2002

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Echoviruses, components of human enteroviruses, are etiological agents for aseptic meningitis, nonspecific rashes, and encephalitis (1). They consist of 28 serotypes numbered from 1 to 33, with the omissions of 8, 10, 22, 23, and 28.

Between November 2001 and September 2002, we obtained 40 isolates of Echovirus type 13 (E13) from 36 cases. Forty isolates were from 30 patients with aseptic meningitis, 6 with gastroenteritis, 2 with rashes, 1 with acute encephalitis, and 1 with fever. Clinical specimens were 4 throat swabs, 25 cerebrospinal fluid samples, and 11 stool specimens. They were isolated most frequently (37.5%) in July (Fig. 1). Among E13-positive patients, 40.0% were <3 years old, 55.0% were between 4 to 12, and 2.5% were between 13 to 15 years. One of the patient's age was unknown. Most of the patients in Osaka City were under the age of 15, as previously reported in the United States (U.S.) (2) and Fukushima Prefecture, Japan (3).

Original E13 isolation was from a 1-year-old boy with a rash in Osaka City. A clinical specimen (OC/01397) from the throat swab was inoculated to Vero and RD-18S cells. Only RD-18S cells showed cytopathic effects (CPE). By using the primer pair EVP2 and OL68-1, the VP4 region of the enterovirus genome was amplified (4,5). However, microneutralization tests using pooled antisera (Denka Seiken, Tokyo, Japan), against enteroviruses including EP95, or an in-house monospecific antibody for coxsackievirus A type 10 (anti-CA10/G-10), CA16, and enterovirus 71 (anti-EV71/BrCr and anti-EV71/C7; National Institute of Infectious Diseases, Tokyo) were all negative. We then amplified the region from VP1 to 2C of this enterovirus using the primers EUG3C and EUC2 (6). The amplified fragment contained the 3′ terminal region of VP1, which is important for serotyping by means of phylogenetic analysis. The amplified PCR fragment was purified and 351 nucleotides (nt) at the 3′ end of VP1 were sequenced using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA). A BLAST2 search (http://blast.genome.ad.jp/) of the sequence showed high homology (81%) of the target sequence with E13 (GenBank No: AY302539). To confirm this finding, we performed microneutralization tests using a specific antiserum against E13 (Denka Seiken). The antiserum specifically inhibited CPE, indicating that the serotype of this enterovirus was E13.

The other 39 strains were identified using the same method. We compared the 3′ end sequence of the VP1 region of OC/01397 (DDBJ/EMBL/GenBank accession No.: AB178768), OC/02175 (AB178769), OC/02223 (AB178770), OC/02247 (AB178771), and OC/02348 (AB178772) with isolates previously obtained in Japan and other countries. OC/01397 was the initial isolate from the Osaka outbreak, whereas OC/02175, OC/02223, and OC/02247 were obtained in July 2002. OC/02348 was the final isolate during that period. Phylogenetic analyses of the 3′ end of the VP1 region showed that the E13 isolates from Osaka City were genetically close to those commonly detected elsewhere in the world during 2000-2002 (amino acid identity, 97 to 100%; nucleotide identity, 95 to 100%; Fig. 2, cluster A). E13 isolates obtained before 2000 belonged to a different cluster (Fig. 2, cluster B). Amino acid identity between the E13 isolates in clusters A and B was 90 to 98% and nucleotide identity was 73 to 82%. A previous report partially supports these findings (7).

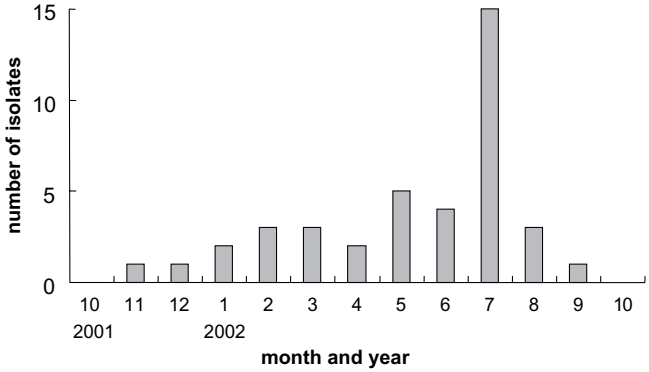

Fig. 1. Monthly isolation of E13 in Osaka City, between November 2001 and September 2002.

Typically, E13 is not a common enterovirus serotype, but this virus became prevalent in Europe (8,9) during 2000 and in the U.S. (2) during 2001. Outbreaks of E13 have been reported in Fukushima, Yamagata Prefecture, and Kobe City, Japan between 2001 and 2003 (3,10,11). In contrast, only 65 E13 isolates were detected between 1970 and 2000 in the U.S. (2), and E13 was last identified in Japan in 1980. Thus, genetically close strains of E13 seemed to suddenly arise and spread globally after the year 2000.

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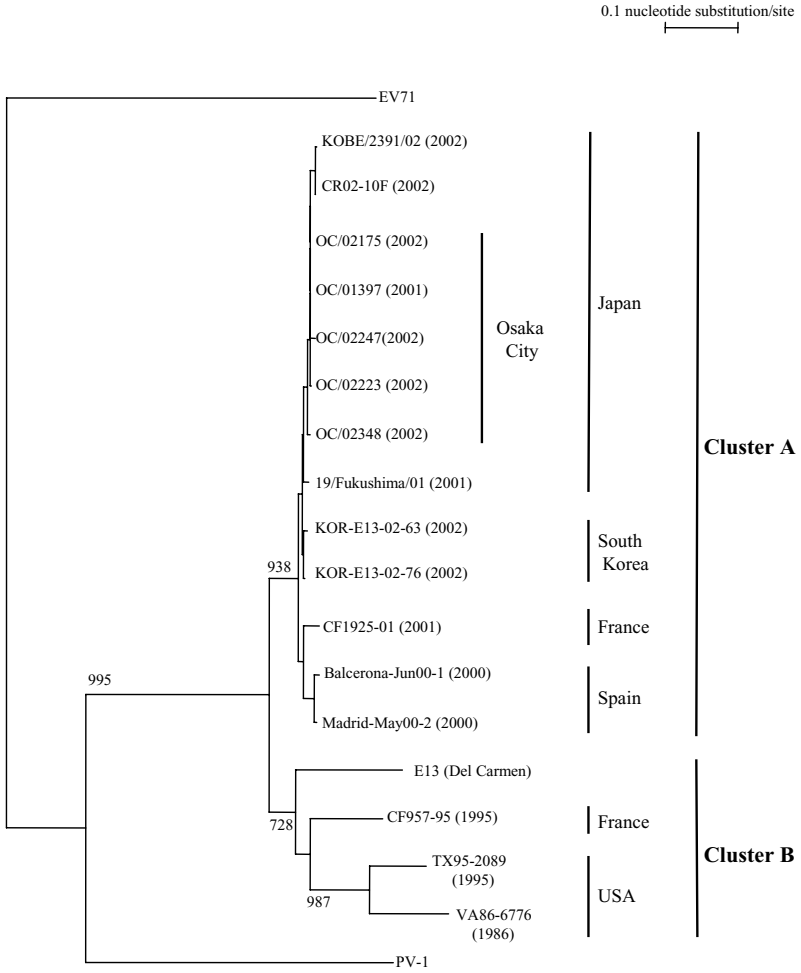

Fig. 2. E13 isolates in Osaka City were genetically close to other isolates obtained after 2000 in Japan and in other countries. Phylogenetic analysis of the 3′ end of VP1 gene containing 351 nt was done using Clustal X (version 1.83) by the Kimura two-parameter method. The phylogenetic tree was reconstructed by the neighbor-joining method with 1000 pseudoreplicate datasets of bootstrap analysis. Numbers at nodes denote bootstrap value and marker shows measurement as relative phylogenetic distance. Del Carmen strain represents the E13 prototype. GenBank accession numbers are shown below. KOBE/2391/02 (AB112423), 19/Fukushima/01 (AB086858), CR02-10F (AB092985), KOR-E13-02-63 (AY268579), KOR-E13-02-76 (AY268580), VA86-6776 (AF152299), Balcerona-Jun00-1 (AY227313), Madrid-May00-2 (AY227343), TX95-2089 (AF081635), CF957-95 (AJ537605), CF1925-01 (AJ537609), E13 (Del Carmen) (AF081327), PV-1 (NC_002058), EV71 (U22521).

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