Widespread Circulation of Echovirus Type 13 Demonstrated by Increased Seroprevalence in Toyama, Japan, between 2000 and 2003

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To confirm the magnitude of an echovirus type 13 (E13) outbreak in 2002 and to evaluate whether genetic and antigenic changes in E13 influenced the occurrence of the outbreak, we measured titers of neutralizing (NT) antibody against the Toyama, 2002-240-SF, and prototype Del Carmen E13 strains among inhabitants of Toyama before and after 2002. The rate of positivity for NT antibodies against both 2002-240-SF and Del Carmen in 2003 made a remarkable upturn in children 0 to 14 years old, compared to that in 2000. Titers of NT antibody against strain 2002-240-SF of inhabitants were slightly higher than those against Del Carmen, whereas anti-E13 rabbit serum raised against either strain Del Carmen or 2002-240-SF showed almost the same titer of NT antibody against both strains. These data indicate that the antigenic properties of the strains may be slightly different. Differences in amino acids between strains 2002-240-SF and Del Carmen in the VP4, VP2, VP3, and VP1 regions may affect both antigenic and receptor binding properties, even though they do not seem to be significant enough to escape widespread immunity. One of the factors of the outbreak was thought to be the increase in susceptibility in the young generation.

Echovirus type 13 (E13), belonging to the family Picornaviridae, human enteroviruses, includes more than 60 serotypes (24). The enterovirus genome is a single-stranded, polyadenylated, positive-sense RNA of about 7,400 bases. The single long open reading frame, which is flanked by 5′ and 3′ nontranslated regions (NTRs), encodes a polyprotein of about 2,200 amino acids that is processed during and after translation by viral proteases to yield the mature viral polypeptides. The P1 region encodes the capsid proteins VP4, VP2, VP3, and VP1. P2 encodes a protease (2Apro), 2B, and 2C. The 3B VPg precursor (3AB), the major viral protease (3Cpro), and the RNA-dependent RNA polymerase (3Dpol) are encoded in the P3 region (22).

Humans are the only known reservoir of human enteroviruses, and the main transmission route is fecal-oral (24). These infections may be unapparent or related to various disorders. Echoviruses are members of Human enterovirus species B of the genus Enterovirus and are associated with illnesses, including rashes, aseptic meningitis, encephalitis, and myositis, mainly during summer in temperate climates (24).

E13, mostly related to aseptic meningitis, was prevalent in Spain (2), Germany (6), and France (1) in 2000 and in the United States and Australia in 2001 (20). While E13 had not been isolated from 1981 to 2000 in Japan, it was detected in children with illnesses such as aseptic meningitis, gastroenteritis, pharyngitis, and viral exanthemata in Fukushima, Osaka, etc. in 2001 (10, 12). After that, the E13 outbreak spread throughout Japan in summer 2002 (8, 14, 19, 33). We have previously reported that partial VP1 nucleotide sequences (703 bases) of isolates from patients with aseptic meningitis and three from river water samples in Toyama in 2002 showed more than 98.7% identity and belonged to the same genetic cluster as those that circulated worldwide in 2000 to 2002. This evidence suggested that transmission of E13 had also occurred in Toyama (8). However, the magnitude of the prevalence and distribution of E13 infection remains unknown. Here we report a seroepidemiological study of E13 that found a significant increase in seroprevalence in Toyama Prefecture between 2000 and 2003. Moreover, to evaluate the possibility that genetic or antigenic changes in regions other than VP1 influenced the occurrence of the outbreak, we determined the complete sequences of four E13 isolates derived from two patients with aseptic meningitis and two river water samples and compared the titers of NT antibody against the isolates obtained in 2002 and prototype strain Del Carmen isolated in 1953 (22).

MATERIALS AND METHODS

Viruses. Five E13 strains, 2002-240-SF, 2002-241-FC, 2002-243-SF, 2002-245-NP, and 2002-257-NP, were isolated from clinical specimens (cerebrospinal fluid, feces, or nasopharyngeal swabs) taken from five patients with aseptic meningitis in June and July 2002 (8). Eleven E13 strains, P1(1)-1, S3(1)-1, S7(1)-2, S7(1)-3, S7(1)-4, S7(1)-5, S7(2)-6, S7(2)-6, O3(1)-1, O7(1)-1, and O11(2)-1, were isolated from environmental specimens (water from the Itachi, Sembo, and Oyabe rivers) in May to December 2002 (8). The prototype E13 strain, Del Carmen (GenBank accession No. AY302539), which was isolated in the Philippines in
TABLE 1. Age distribution of sera used for neutralizing test against E13

| Age (yr) | 2000 | 2003 | 2008 |
|----------|------|------|------|
| 0–1      | 20   | 8    | 15   |
| 2–3      | 18   | 8    | 14   |
| 4–6      | 23   | 12   | 13   |
| 7–9      | 9    | 14   | 8    |
| 10–14    | 20   | 14   | 23   |
| 15–19    | 19   | 25   | 24   |
| 20–29    | 28   | 14   | 23   |
| 30–39    | 27   | 25   | 22   |
| 40–49    | 15   | 23   | 21   |
| 50–59    | 25   | 26   | 22   |
| ≥60      | 25   | 28   | 22   |
| Total    | 229  | 197  | 207  |

1953 (22), was obtained from the National Institute of Infectious Diseases (Tokyo, Japan).

Measurement of neutralizing (NT) antibody titers. Human serum specimens were collected from residents of Toyama Prefecture after informed consent was received from either the individual or a guardian between June and September 2000, 2003, and 2008 for the national epidemiological surveillance of vaccine-preventable diseases led by the Ministry of Health, Labor and Welfare, Japan. Serological study in this investigation was approved by the Committee for Ethical Review of the Toyama Institute of Health. Two hundred twenty-nine sera from 2000, 197 sera from 2003, and 207 sera from 2008 were used for this study. The age distribution is shown in Table 1.

For the seroepidemiological study, we measured titers of antibody against E13 strains 2002-240-SF and Del Carmen. The titers of these viruses were adjusted to 100 50% tissue culture infective doses (TCID50)/50 l of a cell suspension with 0.1% bovine serum albumin (BSA) of 1:4 to 1:1,024 prepared in duplicate in 96-well microtiter plates. Then, 50 μl containing 100 TCID50 of E13 virus was added to each well. After incubation at 37°C for 3 h, 100 μl of a cell suspension containing approximately 1 × 105 to 2 × 105 RD-18S cells in MEM supplemented with 10% fetal bovine serum (FBS), 100 μl of the cell suspension were inoculated to each well. The cytopathic effect was then scored after 7 days of incubation at 37°C in a 5% CO2 atmosphere. The NT titer of each specimen was calculated by the Karber method. Titers of ≥4 were regarded as seropositive. Titers of ≥1,024 and <4 were regarded as 1,024 and 2, respectively, for the calculation of geometric mean titers.

Immunization of rabbits. E13 isolate 2002-240-SF, which was originally isolated in RD-18S cells and was passaged three times in RD-18S cells, was used as an immunogen to elicit antisera in rabbits. All animal procedures were approved by the Committee for Animal Care and Use of Toyama University. The virus in the culture fluid was concentrated and purified by precipitation with 8% polyethylene glycol 6000 (Wako Pure Chemical Industries) and then centrifuged on 30% sucrose gradient at 40,000 rpm for 3 h. A stock of E13 strain 2002-240-SF was prepared (4.0 × 107 TCID50/ml) and inactivated by UV light (367 mJ/cm2) before use. Sera were collected from two 15-week-old female Japanese white rabbits (3 to 5 kg each) before immunization. The two rabbits were inoculated subcutaneously with 2 ml of inactivated 2002-240-SF stock in the RIBI adjuvant system (Corixa Corporation) at an interval of 2 weeks. Two weeks after the third immunization, serum samples were collected and stored at −40°C after heat inactivation (50°C for 30 min). The NT antibody titers of these sera were measured by the same method as described above for the seroepidemiological study. We used 16 E13 isolates of Toyama strains and strain Del Carmen adjusted to 100 TCID50/50 μl as the challenge viruses for the measurement of NT antibody titers.

RT-PCR and nucleotide sequencing. Viral RNA was extracted from 140 μl of the culture fluid of cells that appeared cytopathic using a QIAamp Viral RNA Mini kit (Qiagen). cDNA was synthesized for 1 h at 42°C by SuperScript III reverse transcriptase (RT) (Invitrogen) with a dT15-18, according to the manufacturer’s procedures. PCR was performed using Pure Taq Ready-To-Go PCR beads (GE Healthcare) to amplify the complete viral genome. PCR was carried out under the following conditions: inactivation at 94°C for 1 min and 35 cycles of an annealing at 42°C for 30 s, polymerization at 72°C for 1 min, and denaturation at 94°C for 30 s. After 35 cycles, an additional elongation step of 72°C for 5 min was used. For amplification of the complete viral genome, several primers were used (Table 2). The sequences at the 5’ or 3’ end of the RNA were amplified by the rapid amplification of 5’ cDNA ends (5’ RACE) system (Invitrogen) or the SMART RACE cDNA amplification kit (Clontech). To determine the sequences of these viruses, the PCR products were directly applied for sequence analysis using an ABI Prism BigDye Terminator v3.1 cycle sequencing kit and an ABI Prism 3100 DNA sequencer (Applied Biosystems). Alignment of the sequences was carried out with Clustal W and MEGA 3.1 software (13, 15, 16).

Three-dimensional structure. Variations in the capsid protein amino acid sequence between strains 2002-240-SF and Del Carmen were projected onto the three-dimensional structure of echovirus type 11 (Protein DataBank code 1h8t) (30) by using PyMOL 0.99 (http://pymol.sourceforge.net) and UCSF Chimera (26).
**Statistical analyses.** To evaluate the difference between individual titers of NT antibodies against strains 2002-240-SF and Del Carmen, the correlation coefficient (r) was calculated and the Wilcoxon signed rank test was conducted by using the logarithm of the NT antibody titer.

**Nucleotide sequence accession numbers.** The nucleotide sequences of the complete E13 genomes determined in this study were deposited in GenBank under accession no. AB501329 to AB501332.

**RESULTS**

**Rates of NT antibody positivity.** To confirm the magnitude of the seroprevalence of E13 in Toyama Prefecture, we measured the rates of seropositivity against E13 isolates (2002-240-SP) in the sera collected in Toyama in 2000, 2003, and 2008 (Fig. 1-A). We also used E13 reference strain Del Carmen as the challenge virus to evaluate the differences in the antigenic characteristics between strains 2002-240-SF and Del Carmen. In 2000, while the rates of antibody positivity against both E13 strains in the >40-year-old group were more than 50%, they were only 0 to 21.1% in those <40 years old (Fig. 1A). On the other hand, the rates of positivity in the 0- to 14-year-old age group remarkably rose in 2003, whereas those of persons >40 years old did not change. Especially, the results in the 2- to 3-year-old and 4- to 6-year-old age groups were markedly high, 75.0% (6/8) and 91.7% (11/12), respectively. In 2008, the remarkable high rates of positivity shifted to older age groups.

The above results have suggested that E13 seroprevalence significantly increased, especially in the young generation <14 years old between 2000 and 2003, with no seroprevalence thereafter.

**NT antibody titers.** The geometric mean titers of NT antibody against E13 strain 2002-240-SF in the sera of inhabitants collected in 2000, 2003, and 2008 are shown in Fig. 1B. The titers in the 0- to 1-year-old, 2- to 3-year-old, 4- to 6-year-old, and 7- to 9-year-old age groups in 2003 were 9.5, 215.3, 542.4, and 33.6, respectively, which were markedly higher than those in 2000, when the corresponding titers were 2.2, 2.3, 2.1, and 2.0. Most of the NT antibody titers of the positive sera in the 0- to 9-year-old age group in 2003 were more than 1,024, while the titers in the >40-year-old age group were 4 to 128 (data not shown). This also indicates that E13 infection occurred in children just before 2003. In 2008, the markedly high titers shifted to higher age groups. The 0- to 3-year-old age group had almost no antibody against E13.

There was a high correlation between the titers of NT antibody against 2002-240-SF and Del Carmen in each serum in 2000, 2003, and 2008 (r = 0.866, 0.939, and 0.967, respectively; P < 0.001) (Fig. 2). However, the titers of NT antibody against strain 2002-240-SF were slightly higher than those of NT antibody against strain Del Carmen, and their geometric means were significantly different (Wilcoxon signed rank test: test statistic [t] = 405, number of observations [n] = 58, P < 0.001 in 2000; t = 365.5, n = 77, P < 0.001 in 2003; t = 472, n = 55, P < 0.05 in 2008). On the other hand, anti-E13 strain Del Carmen or 2002-240-SF serum raised in rabbits (Denka Seiken, Japan) showed almost the same titer of NT antibody against 16 E13 isolates of Toyama strains and strain Del Carmen (Table 3).
Comparison of nucleotide sequences of E13 from river water samples and aseptic meningitis patients. We previously reported that the nucleotide sequences in the partial VP1 region (703 bases) of E13 isolates from river water samples were closely related to those from patients with aseptic meningitis in Toyama and other areas of the world in 2000 to 2002 (8). However, it was unclear whether genetic changes in regions other than VP1 influenced the occurrence of the outbreak in 2002. To clarify this point, we determined the complete sequences of four E13 isolates from two patients with aseptic meningitis (2002-240-SF, 2002-245-NP) and from two river water samples [I5(1)-1, S3(1)-1] and compared them with that of strain Del Carmen. The total number of nucleotides in the sequences of the four isolates, except the poly(A) tail, was 7,411. The percent identities of the complete nucleotide sequences of the isolates and strain Del Carmen are shown in Table 4. The percent identities between the nucleotide and amino acid sequences of the isolates and strain Del Carmen in each region were 75.8% to 86.0% and 90.0% to 100%, respectively (Table 4). The full sequences of the four isolates closely resembled each other, with less than 1.6% and 0.5% differences in the nucleotide and amino acid sequences, respectively. It thus appeared that the complete nucleotide sequences of the E13 isolates from river water and patients with aseptic meningitis were almost identical.

TABLE 3. Titers of NT antibody against E13 strains 2002-240-SF and Del Carmen in rabbit antisera

| E13 strain* | 2002-240-SF (rabbit 1) | 2002-240-SF (rabbit 2) | Del Carmen* |
|------------|------------------------|------------------------|-------------|
| S3(1)-1    | 512                    | 4,096                  | 512         |
| O3(1)-1    | 1,024                  | 2,048                  | 256         |
| I5(1)-1    | 1,024                  | 2,048                  | 512         |
| S7(1)-2    | 1,024                  | 2,048                  | 256         |
| S7(1)-3    | 1,024                  | 4,096                  | 512         |
| S7(1)-4    | 1,024                  | 4,096                  | 512         |
| S7(1)-5    | 1,024                  | 2,048                  | 512         |
| S7(2)-6    | 1,024                  | 4,096                  | 256         |
| O7(1)-1    | 512                    | 2,048                  | 512         |
| O11(2)-1   | 512                    | 2,048                  | 256         |
| S17(2)-6   | 512                    | 2,048                  | 256         |
| 2002-240-SF | 1,024                  | 2,048                  | 256         |
| 2002-241-NP | 1,024                  | 2,048                  | 256         |
| 2002-243-SF | 1,024                  | 2,048                  | 256         |
| 2002-245-NP | 512                    | 2,048                  | 256         |
| 2002-257-NP | 1,024                  | 2,048                  | 256         |
| Del Carmen  | 512                    | 2,048                  | 256         |

*E13 strains isolated from river water in Toyama in 2002: S3(1)-1, O3(1)-1, I5(1)-1, S7(1)-2, S7(1)-3, S7(1)-4, S7(1)-5, S7(2)-6, O7(1)-1, O11(2)-1, and S17(2)-6 (8). E13 strains isolated from patients with aseptic meningitis in Toyama in 2002: 2002-240-SF, 2002-241-NP, 2002-243-SF, 2002-245-NP, and 2002-257-NP. The titer of these challenge viruses was 100 TCID<sub>50</sub> per ml, respectively.

Three hundred twenty units of anti-E13 Del Carmen serum (Denka Seiken, Japan).

TABLE 4. Percent identity of nucleotide and amino acid sequences among E13 isolates from Toyama and strain Del Carmen

| Region | Toyama isolates<sup>a</sup> | Toyama isolates and strain Del Carmen |
|--------|-----------------------------|--------------------------------------|
|        | Nucleotide | Amino acid | Nucleotide | Amino acid |
| 5’ NTR | 98.7–99.7 | 85.2–86.0 | 79.6–80.0 | 98.6 |
| VP4    | 97.1–99.5 | 79.0–79.6 | 96.9–97.3 |
| VP2    | 98.5–99.4 | 78.7–79.4 | 96.9–97.3 |
| VP3    | 98.3–99.6 | 78.8–78.9 | 96.9–97.3 |
| VP1    | 98.4–99.9 | 78.9–80.2 | 90.0–92.0 |
| 2A     | 98.0–99.8 | 79.6–77.1 | 97.0–98.0 |
| 2B     | 97.0–100  | 79.4–80.3 | 96.0–97.0 |
| 2C     | 98.3–99.4 | 80.7–81.6 | 95.5       |
| 3A     | 99.3–100  | 80.7–81.6 | 95.5       |
| 3B     | 93.9–98.5 | 78.3–78.5 | 97.0–97.4 |
| 3C     | 98.5–99.5 | 80.2–83.2 | 97.0–97.4 |
| 3D     | 98.4–99.8 | 78.3–78.5 | 97.0–97.4 |

Total 98.4–99.6 | 99.5–99.7 | 79.7–79.8 | 95.7–95.9

<sup>a</sup>Sequences of E13 isolates from Toyama [2002-240-SF, 2002-245-NP, I5(1)-1, and S3(1)-1] were compared with each other.
When the amino acid sequences of strains 2002-240-SF and Del Carmen were compared, differences of 1, 7, 6, and 25 amino acids were observed in the VP4, VP2, VP3, and VP1 regions, respectively. Although the neutralization antigenic sites (N-Ags) and the crystal structure of E13 have not been determined, the N-Ags of other enteroviruses have been reported and some of them are in the same position (4, 17, 18, 25, 27, 29). Therefore, the amino acid sequence differences between strains 2002-240-SF and Del Carmen were compared with the N-Ags of other enteroviruses on the three-dimen-
sional structure of echovirus type 11 (Protein Data Bank no. 1h8t) (30) (Fig. 3A and B). The differences in amino acid residues 157, 158, and 160 of VP2 and 59 and 64 of VP3 are located in the N-Ags of coxsackievirus A9 in the puff region and the knob structure, respectively (4). These two regions also correspond to N-Ag IIb and N-Ag IIIb of poliovirus types 1, 2, and 3, respectively (Fig. 3B) (9, 17, 18, 23, 25). In addition, many amino acid differences were observed between strains 2002-240-SF and Del Carmen in the VP1 region, of which the N and C termini were, respectively, common epitopes of enteroviruses (27, 29).

**DISCUSSION**

In this study, we demonstrated that many children were infected with E13 in Toyama between 2000 and 2003, while E13 was only detected in five patients with aseptic meningitis in Toyama (8). Therefore, there were many asymptomatic or mild cases, because E13 was reportedly associated with a wider range of symptoms than aseptic meningitis, such as respiratory infection (11), enterocolitis, and bronchitis in infants (14). Because of the facts that (i) there were no reports of isolation of E13 between 1981 and 2000 in Japan and (ii) the rates of positivity for NT antibody against E13 were extremely low in the 0- to 40-year-old age group in 2000, it is proposed that E13 must have spread extensively in a short time. Consistent with this observation, the nucleotide sequences of all of the E13 isolates obtained in Toyama in 2002 were quite similar, within $2.2 \times 10^{-2}$ substitution/site/year of evolutionary distance (8).

In 2008, the distribution of NT antibodies against E13 shifted to older age groups, while children 0 to 3 years old hardly had any antibody detected. This means that seroprevalence of E13 has not occurred since 2003 in Toyama. In fact, E13 has not been isolated in Toyama since 2003, and only a few cases have been sporadically reported in Japan (21). Because the numbers of infants without NT antibodies against E13 have been increasing, reemergence of E13 infection may occur in the future, although its timing cannot be predicted.

While echovirus types 11, 18, and 30 were frequently reported as causative agents of aseptic meningitis, E13 was rarely observed in Japan before 2001 (21), as well as in Europe and the United States from 1970 to 2000 (1, 2, 5, 6, 20). On the other hand, Bingjun et al. reported that E13 isolates in Yunnan, China, from 1997 to 2004 were assumed to be indigenous viruses due to their genetic divergence (3). Because the nucleotide sequences of E13 isolates in Toyama in 2002 were similar to those of the strains isolated in Yunnan in 2000 and those detected worldwide in 2000 to 2002 (3, 8), the seroprevalence of E13 in Toyama seemed to be derived from the epidemics in those areas. Considering that more than 50% of the inhabitants of Toyama Prefecture 40 years of age or older had NT antibody against E13 in 2000 and E13 was not isolated from 1981 to 2000, E13 is predicted to have been prevalent in the community at least in the 1960s. Consistently, other serological studies in Fukui, Yamagata, and Hiroshima Prefectures in 2000 to 2001 showed similar results (19, 31, 32). These data suggested that E13 was present in these areas of Japan until 40 years ago but thereafter disappeared or circulated among extremely few inhabitants.

Titers of NT antibody against strain 2002-240-SF among the inhabitants of Toyama were slightly higher than those against strain Del Carmen (Fig. 2), whereas anti-E13 rabbit serum raised against either strain Del Carmen or 2002-240-SF showed almost the same titer of NT antibody against E13 isolates from Toyama and strain Del Carmen (Table 3). Some of the amino acid sequence differences between strains 2002-240-SF and Del Carmen, e.g., D59A of VP3 in putative N-Ag IIb, may alter the electric charge and size of the viruses. Therefore, these amino acid sequence differences may affect the virus’s antigenic properties, even though the difference does not seem to be significant enough to escape widespread immunity.

VP1, VP2, and VP3 of enteroviruses are thought to create a deep cleft or “canyon” on the viral surface that is presumed to be the host receptor binding site (28). This canyon separates the major part of five VP1 subunits clustered around a pentamer axis from the surrounding VP2 and VP3 subunits (28). Most of the amino acid differences between strains 2002-240-SF and Del Carmen especially observed in VP1 are concentrated in the carboxy-terminal region that lines the south canyon wall. Because the carboxy termini of VP1 and VP3 intertwine with each other (28), T231S observed in the carboxy terminus of VP3, which partially lines the north canyon wall, might correlate with the change in VP1. Thus, amino acid alterations between the two strains may affect not only their antigenicity but also their receptor binding properties. The latter possibility might alter virulence as well as host specificity.

We have previously reported that outbreaks of coxsackievirus A16 and enterovirus 71 infections occurred at intervals of several years and seem to depend on an increase in a susceptible generation (7). In contrast, the outbreak of E13 infection occurred at an interval of at least 20 years. Since the antigenic characteristics of E13 have not changed significantly in more than 40 years, a receptor binding alteration which possibly affects virulence or host specificity might play some role in such a long interval. The virus might have continued infection in an extremely local area without an apparent outbreak to accumulate a genetic alteration that is required to cause an outbreak. Further analyses are needed to clarify the above hypothesis.

In conclusion, seroepidemiology revealed a significant increase in E13 seroprevalence in the young generation in Toyama between 2000 and 2003. One of the major factors in this outbreak was thought to be the increase in susceptibility in the young generation. A combination of surveillance of causative agents from patients and seroepidemiological study would contribute to our understanding of the comprehensive prevalence of E13, including asymptomatic infections.

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**REFERENCES**

1. Archimbaud, C., J. L. Bailly, M. Chambon, O. Tournilhac, P. Travade, and H. Peigne-Lafeuille. 2003. Molecular evidence of persistent echovirus 13 meningoencephalitis in a patient with relapsed lymphoma after an outbreak of meningitis in 2000. J. Clin. Microbiol. 41:4605–4610.
