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Using human embryonic fibroblast (HEF) and HEp-2 cell cultures, adenoviruses were isolated from 989 (3.7%) out of 26,793 pediatric patients with ART in Yamagata, Japan from January, 1986 to December, 1991. All isolates were identified as types 1 (Ad1)-6 and no other serotypes were identified. Epidemiologic feature was different depending on the subgenus group. Ad1, 2, 5 and 6 (group C) were endemic and the infections occurred frequently in the summer season. Ad3 (group B) was epidemic in the autumn to winter season, although the virus was isolated every month in non-epidemic season. No seasonal distribution of Ad4 (group E) could be determined because the number of patients was limited. Neutralizing antibody positive ratio for group C were more than 40% at 1-2 years of age and almost 100% by 10 years of age but those for Ad3 (group B) were 40% by 10 years of age. The neutralizing antibodies for Ad4 (group E) or Ad7 (group B) became negative by 10 years of age. With group C infections, most cases were infants and young children less than 2 years of age, but Ad3 infections were older children with the peak at 4 and 5 years of age.

Approximately 5% of acute respiratory infections (ARI) among infants and children are due to adenoviruses (Ad). Recently, the 47 different serotypes of human adenoviruses have been classified into subgenera A through F on the basis of their DNA properties (Wadell 1984). Common serotypes isolated from pediatric patients with ARI are types 1, 2, 5 and 6 (group C), types 3 and 7 (group B), and type 4 (group E). The most common serotypes involved in ARI are type
In Japan, however, no longitudinal investigation of epidemiologic and clinical features of adenovirus infections has never been conducted on the basis of virus isolation from infants and children with ARI. Starting in 1986, employing a microplate method with five kinds of cell cultures, HEF (human embryonic fibroblast), HEp-2, Vero, MDCK and HMV-II, developed by Numazaki et al. (1987) and Moriuchi et al. (1990), we have therefore continuously performed such a study utilizing the large number of pediatric patients with ARI in Yamagata, Japan, to clarify the yearly changes in respiratory viruses. We document here our findings about epidemiologic feature of all adenovirus infections in this population covering 6 years.

**MATERIALS AND METHODS**

**Study population**

During the 6 year period from January, 1986 to December, 1991, throat swab specimens were collected from pediatric patients (<15 years of age), with a temperature of >37.5°C or symptoms and signs of a respiratory illness, seen in Yamagata City Hospital Saiseikan, Yamagata, Japan. The Yamagata City Hospital functions for both primary care and as a referral center for a population of approximately 250,000, and cares for about 130 pediatric outpatients per day and 1,600 pediatric inpatients per year.

**Virus isolation and identification**

Virologic diagnosis was based on virus isolation studies (Numazaki et al. 1987; Moriuchi et al. 1990). Briefly, throat swabs were suspended in 3 ml of Eagle's minimum essential medium with 0.5% gelatin, 0.2% bovine serum albumin, and antibiotics. The collected specimens were kept for 1 to 4 days in Yamagata City Hospital at 4°C and then transported to the Virus Research Center, Sendai National Hospital. After being clarified by centrifugation at low speed, the supernatant (0.1 ml) was inoculated into two microplates, HEF-HEp-2-Vero-MDCK (Numazaki et al. 1987) and HMV-II (Moriuchi et al. 1990), to isolate not only the adenoviruses but also other respiratory viruses. The inoculated plates were incubated at 33°C in a CO₂ incubator for 10 days. Adenovirus-like CPE (cytopathic effect) agents cultivated in HEF or HEp-2 were identified by neutralization test using the antisera prepared in this laboratory (types 1, 2 and 3) or supplied from the National Institute of Health, Tokyo, Japan (types 4, 5 and 6).

**Antibody**

One hundred and twelve serum samples obtained from, children less than 10 years of age with acute phase ARI in 1993 were tested for the presence of neutralizing antibody against adenovirus types 1 to 7. The sera to be tested were diluted 1:4 beforehand.
RESULTS

Adenovirus serotypes isolated from children with ARI

Adenoviruses were recovered from 989 (3.69%) out of 26,793 patients tested in Yamagata from January, 1986 to December, 1991, by using HEF and HEp-2 cell cultures. All of these isolates could be identified as either one of the types 1 (Ad1) to 6 and no other serotypes were found.

Out of 989 isolates, 832 (84.1%) were recovered from the cultures of HEF only and HEF & HEp-2, and 542 (54.8%) in HEp-2 only and HEF & HEp-2 (Table 1). According to the sensitivity to the types of cell culture used for the isolation, viruses could be divided into three groups: Ad3, group B, (more cultivatable in HEF than in HEp-2); Ad1, 2, 5 and 6, group C, (almost equally cultivatable in both cultures but a little more in HEp-2 than in HEF); Ad4, group E, (cultivable in HEp-2 but not in HEF). As a result, by using both HEF and HEp-2 cell cultures, all of the groups B, C and E viruses could be efficiently isolated.

The proportions of different serotypes are also shown in Table 1. Of the 989 adenovirus isolates, 544 (55.0%) were Ad3, 181 (18.3%) Ad2, 170 (17.2%) Ad1, 66 (6.7%) Ad5, 24 (2.4%) Ad6, and only 4 (0.4%) Ad4. The isolation rate of adenoviruses from the ARI patients was 3.7 per cent in total: 2.0% of Ad3, 0.7% of Ad2, 0.6% of Ad1, 0.2% of Ad5, 0.1% of Ad6 and 0.02% of Ad4.

Seasonal distribution of adenovirus isolates

The monthly distribution of adenovirus serotypes in Yamagata from 1986 to 1991 is illustrated in Fig. 1. Ad1 and Ad2 were isolated almost every month throughout 6 study years but were more frequently isolated in the summer season during April to July and decreased in August and September (Fig. 2). Ad5 and Ad6 were isolated only when a large number of specimens were tested, because the

| Table 1. Quantative data for adenoviruses isolated from children with ARI using HEF and HEp-2 cell culture |
|---|---|---|---|---|---|---|---|
| | Ad3 | Ad1 | Ad2 | Ad5 | Ad6 | Ad4 | Total (%) |
| HEF only | 344 (63) | 15 (9) | 20 (10) | 3 (5) | 1 (4) | 0 (4) | 385 (38.9) |
| HEF & HEp-2 | 188 (58) | 99 (58) | 104 (59) | 39 (71) | 17 (0) | 4 (45.2) | 447 |
| HEp-2 only | 12 (33) | 56 (32) | 57 (36) | 24 (25) | 6 (100) | 4 (15.9) | 157 |
| Total | 554 (100) | 170 (100) | 181 (100) | 66 (100) | 24 (100) | 4 (100.0) | 989 (100.0) |

(1) (17.2) (18.3) (6.7) (2.4) (0.4) (100.0)
isolation rate from the patients was very low. However, the seasonal distribution of Ad5 and Ad6 was quite similar to that of Ad1 and Ad 2.

In contrast to Ad1, 2, 5 and 6 (group C), Ad3 (group B) demonstrated epidemics with three outbreaks, all in autumn or winter, during the 6 study years, although the virus was isolated in almost every month of non-epidemic period (Fig. 1). Isolation of Ad3 was decreasing in number in the summer season, the pattern showing a sharp contrast with those of Ad1 and 2 (Fig. 2).

No seasonal distribution of Ad4 could be determined because the isolation was limited to only 4 cases.

**Age distribution of adenovirus infections in ARI patients**

Ninety cases (9.1%) out of 989 patients with adenovirus infections were infants of less than one year of age (Table 2). All serotypes Ad1 to 6 were found among the isolates. The youngest case was 3 months of age, and most of the cases were more than 5 months of age.

The age distribution of ARI patients with adenovirus infections showed
different patterns depending on the subgenotype (Fig. 3). With group C infections, most cases were infants and young children less than 2 years of age. The incidence was rapidly decreased in the children over 2 years of age. For Ad3 (group B) infections, however, the patients were found more frequently in older children with the peak at 3 to 5 years of age.

**Age distribution of the adenovirus antibody among children in Yamagata**

The age distribution of the antibody to Ad1 to 7 is illustrated in Fig. 4. The distribution of the antibody to Ad1, 2, 5 and 6 (group C) was similar: approximately 40–60% at 1–2 years of age, 40–70% at 3–4 years of age, 60–80% at 5–6 years of age and 90–100% at 10 years of age. All children had the antibodies to group C viruses by 10 years of age. However, the distribution pattern of the antibody to Ad3 (group B) was very different: The positive rate was 10% at 1–2

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**TABLE 2. Number of infants infected with adenoviruses**

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<th>3</th>
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<th>5</th>
<th>6</th>
<th>7</th>
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<th>9</th>
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<td>31</td>
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<td>(34.5)</td>
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<td>18</td>
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<td>11</td>
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<td></td>
<td></td>
<td>90</td>
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<td>(100.0)</td>
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</table>

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Fig. 2. Numbers of patients with specific adenovirus infections by month for the 6 year period (1986-1991). △, Ad1; ○, Ad2; ■, Ad3; ▲, Ad5; ●, Ad6.
years of age, 20% at 3–4 years of age, 30% at 5–6 years of age and 40% at 10 years of age. No antibodies to Ad4 (group E) or 7 (group B) were demonstrable in the children less than 10 years of age.

**DISCUSSION**

Our epidemiologic survey of the adenovirus infections among infants and
Epidemiology of Adenovirus Infection in Yamagata, Japan

children with ARI in Yamagata, Japan has revealed clear differences between serotypes. On the basis of virus isolation using throat swab specimens, approximately 4% of pediatric patients with ARI were associated with adenoviruses, this figure being very similar to that previously reported from USA (Brandt et al. 1969).

The sensitivity of adenoviruses in terms of cell culture was quite different depending on the subgenus group. Group B (Ad3) was more cultivatable in HEF than in HEp-2, group C (Ad1, 2, 5 and 6) almost equally in both cultures but a little more in HEp-2, and group E (Ad4) in HEp-2 but not in HEF. Previously Brandt et al. (1969) reported that human embryonic kidney (HEK) was the most sensitive system for adenovirus isolation by comparing to HEp-2 and other cell cultures. However, in the present study, all of 4 Ad4 (group E) could be cultivated in HEp-2 but not in HEF and group C was more in HEp-2 than in HEF. In another virus isolation study from 4,787 pediatric patients with ARI in Sendai, all of 6 Ad4 were cultivated in HEp-2 but only one of those in HEF and all of group C viruses were more cultivatable in HEp-2 than in HEF (unpublished data). Accordingly, it is suggested that the sensitivity of adenoviruses in terms of cell culture are different depending on the subgenus group. Our microplate system of both HEF and HEp-2 has been shown to be efficient for isolation of adenoviruses from ARI.

Epidemiologically, Ad1, 2, 5 and 6 (group C) appeared endemic and more frequently in the summer season in Yamagata. In the age distribution of group C infections, patients were mostly in infants and young children less than 2 years of age. This pattern indicates that infants and young children are usually exposed to the group C viruses while they are at home. These epidemiologic status in Japan is similar to other countries in the world (Sterner 1962; Fox et al. 1969; Hall et al. 1971; Cooney et al. 1972).

In group C, the isolation rates from pediatric patients for Ad5 and 6 were remarkably lower than those for Ad1 and 2, although there was no difference in the antibody positive ratio for these viruses: 40-60% at 1-2 years of age and almost 100% by 10 years of age. Therefore, the isolation rate of group C viruses from pediatric patients may not indicate the total infection rate, but rather indicate the symptomatic infection rate, that is, the virulency may be weaker for Ad5 and 6 than that for Ad1 and 2.

Non-group C virus infections were found immediately after birth. The youngest infant was 3 months of age, but most cases were more than 5 months of age. It was therefore reconfirmed that maternal antibody to the viruses provides effective protection in early infancy.

Although three outbreaks of Ad3 (group B) were observed in Yamagata during the 6 study years, this virus was in fact continuously isolated from pediatric patients throughout the year. On the basis of DNA analysis, we have previously reported that the predominant genome type of Ad3 may be perpetuated
locally in the Yamagata area with minor genomic variation and the outbreaks of Ad3 are not necessarily due to the appearance of a new genome type (Mizuta et al. 1994).

The age distribution peak for Ad3 infection between 4 to 5 years of age in Yamagata suggests that Ad3 epidemics originate from close contact at kindergarten. The low level of Ad3 infection in summer presumably means that swimming pools are not important sources of infection.

Only 4 Ad4 (group E) and no Ad7 (group B) were isolated from 31,580 cases tested during the 6 study years. In the sera collected in 1993, the antibodies to Ad4 and 7 were negative until 10 years of age in Yamagata. Accordingly, our negative findings for isolation of Ad4 and 7 from pediatric patients with ARI are reasonable. In 1968, Numazaki et al. reported that the antibody positive ratio was approximately 80% for Ad4 and 50% for Ad7 in children of 6–10 years of age in Sendai, a city nearby Yamagata. However, in the sera collected from nursing school students (19–20 years of age) in Sendai in 1993, the antibodies to both Ad4 and Ad7 were also negative (unpublished data). It is, therefore, interesting to note that the outbreaks of Ad4 and Ad7 have been absent at least for the last 10 years in Yamagata and for 20 years in Sendai. According to the report from the National Institute of Health of Japan, only 7 conjunctivitis cases due to Ad7 were described in the whole country from 1986 to 1991 (The National Institute of Health 1992). It may be possible that Ad7 is going to disappear in Japan, but the reason is unclear.

References


7) Moriuchi, H., Oshima, T., Nishimura, H., Nakamura, K., Katsushima, N. & Numazaki,


