Human adenovirus causes a variety of diseases including upper respiratory tract infection, acute conjunctivitis, cystitis and gastroenteritis. Acute febrile exudative tonsillitis is caused by various agents, including group A β-hemolytic streptococci and viruses. Group A β-streptococcal tonsillitis is most common in children 6 years of age or older; by contrast, viral tonsillitis is most common in children younger than 3 years of age (1). Adenovirus is the most frequent agent among viral tonsillitis. Adenovirus-associated exudative tonsillitis induces high grade and prolonged fever, sore throat and poor appetite; hospitalization is often required for such patients. Strong inflammatory responses such as leukocytosis with neutrophilia and high C-reactive protein (CRP) values are usually observed. These laboratory findings are different from those of common viral infections, making it difficult to distinguish adenovirus respiratory infection from bacterial infection. However, the mechanisms of these findings induced by adenovirus infection have not been well elucidated. Real-time polymerase chain reaction (PCR) methods (2) are thought to be useful in the diagnosis and monitoring of Epstein-Barr virus (EBV) (3) and cytomegalovirus (CMV) infections (4). We have reported SYBR Green real-time PCR assay for the quantitative detection of adenovirus DNA (5). Measurement of adenovirus load in clinical samples from adenovirus infections may provide important information for analyzing the pathogenesis of various adenovirus infections. In this study, we evaluated the adenovirus DNA load in patients with adenovirus-associated exudative tonsillitis and analyzed the correlation between the viral load and clinical findings.

Thirty patients at Nakano Children’s Hospital, Osaka, Japan, between June 2003 and October 2004 were clinically diagnosed as having acute adenovirus-associated tonsillitis based on their high fever (>38.0°C) and exudative tonsillitis, and were admitted for fluid transfusion because of poor fluid intake. These patients did not have chronic tonsillitis as an underlying illness. Etiologic diagnosis was confirmed using an immunochromatographic rapid diagnostic kit (Adenoclone; TFB, Tokyo, Japan) with throat swabs. Patients included 20 boys and 10 girls aged from 8 months to 8 years (mean age, 4 years and 11 months). Forty-one throat swabs from 30 patients and 30 whole blood samples from 23 patients were collected. Throat swabs were collected by swabbing the tonsils of all patients. Throat swabs from 10 patients and blood from 6 patients were collected in the acute phase (within 4 days of the onset of fever) and recovery phase (between 5 and 10 days after the onset of fever), respectively. Throat swabs were collected and suspended in 1 ml of normal saline, and blood was collected in EDTA-coated tubes. Plasma was collected after centrifugation at 5,000 rpm for 5 min. Informed consent was obtained from the parents or guardians of all patients.

DNA was extracted from 200 µl of throat swabs, whole blood and plasma using a High Pure Viral Nucleic Acid Kit (Roche Applied Science, Mannheim, Germany), according to the manufacturer’s instructions. DNA was eluted in 50 µl of distilled water and stored at -20°C until use. Adenovirus DNA was tested by quantitative SYBR Green real-time PCR assay (5). Briefly, 1 µl of template DNA was added to a final volume of 25 µl containing 1 × SYBR Green PCR Master Mix (Applied Biosystems Japan, Tokyo, Japan) and 160 nM of the primers Hex3 and Hex4. The sequence of primer pair was as follows, Hexon 3: 5’-GACATGACTTTC GAGGTCAATCCCCATGA-3’, Hexon 4: 5’-CCCGCTGAG AAGGGTGTCGGCCAGTA-3’ (6). PCR amplification was performed using an ABI PRISM 7900 HT. Amplification was performed with 40 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min and elongation at 72°C for 1.5 min. During thermal cycling, emission from each sample was recorded and SDS software processed the raw fluorescence data to produce threshold cycle (Ct) values for each sample. The SDS software then computed a standard curve from the Ct values of the diluted standards and extrapolated absolute quantities for the unknown samples based on their Ct values. The minimum detectable adenovirus DNA level in this assay is 10⁶ copy/mL using adenovirus type 2 quantification standard plasmid DNA as previously reported (5).

The mean duration of fever in patients was 5.5 days. The mean values of CRP and leukocyte (WBC) counts at the acute phase were 4.8 mg/dl and 14,200/µl, respectively. Changes in the copy number of adenovirus in throat swabs and blood are shown in Fig. 1(A). Adenovirus DNA was detected in throat swabs from 30 patients (100%) in the acute phase. The copy number of adenovirus DNA ranged from 10⁴ to 10⁷ copy/mL. Adenovirus DNA was detected in 16 (88.9%) out of 18 samples in the recovery phase. Changes in the copy number
of adenovirus DNA in throat swabs in the acute and recovery phases are shown in Fig. 1(B). The copy number of adenovirus in throat swabs decreased in 9 patients in the recovery phase compared with the acute phase, but adenovirus DNA was still detected in 9 patients in the recovery phase. Adenovirus DNA was detected in blood from 23 patients (77%) in either the acute or recovery phases. The copy number of adenovirus DNA in blood ranged from $10^4$ to $10^7$/mL. The changes in the copy number of adenovirus DNA in blood in the acute phase and recovery phase are shown in Fig. 1(B). No adenovirus DNA was detected in plasma. The WBC count and CRP level did not correlate with the copy number of adenovirus DNA in either throat swabs or blood (data not shown). The copy number of adenovirus DNA in blood was positively correlated with the duration of fever ($r = 0.55; P < 0.005$) (Fig. 2).

We have reported that SYBR Green real-time PCR assay is a useful quantitative tool for analysis of adenovirus DNA and more sensitive than the IC kit (5). Strong inflammatory responses such as leukocytosis and a high CRP level are commonly observed in patients with adenovirus-associated exudative tonsillitis. The serum interleukin-6 (IL-6)-concentration is higher in patients with adenovirus infection compared with influenza virus and respiratory syncytial virus (RSV) infections. A good correlation between the CRP level and serum IL-6 concentration in adenovirus respiratory infection has been reported (7). These data suggest that adenovirus stimulates IL-6 production by vascular endothelial cells, fibroblasts or activated T lymphocytes. In this study, the CRP value and WBC number did not correlate with the copy number of adenovirus DNA, suggesting that the clinical severity is not dependent on the adenovirus load in the throat. However, the positive correlation between the duration of the fever and the adenovirus load in the blood suggests that high levels of adenovirus DNA in blood may play an important role in the persistence of fever.

There are a few reports that detect adenovirus DNA in blood from immunocompetent individuals. Aberle et al. reported that adenovirus DNA was detected in 41% of children with an acute respiratory adenovirus infection, and the majority (93%) of serum samples were collected within the first week after the onset of symptoms (8). Shike et al. reported that adenovirus DNA was detected in whole blood from only one out of 6 patients with culture-proven adenovirus infection (9). In our study, 42% of throat swabs and 46% of blood out of adenovirus DNA positive samples were from the recovery phase. Two cases showed an increase of the copy number of adenovirus DNA in the recovery phase. In one patient, the copy number of adenovirus DNA increased to $10^7$ copy/mL and the fever continued for 6 days, then rose again on day 9. This finding may be related to the increase of adenovirus DNA in the blood. These findings indicate that adenovirus replicates in the lymph tissue of the throat and pharynx, and then spreads into the bloodstream. Detection of adenovirus DNA from blood is not unusual phenomenon. For instance, varicella-zoster virus was isolated during the late incubation period and the acute phase (10). Varicella-zoster virus DNA declined gradually with time and was undetectable after 15 days from the onset (11). In this study, we evaluated neither serum antibody nor virus isolation. It is not clear whether detection of adenovirus DNA in blood indicates the presence of infectious adenovirus.

It has been reported that adenovirus is an important cause of morbidity and mortality among allogeneic bone marrow transplantation (BMT) recipients (12-15). Quantification of viremia with the use of real-time PCR is used in routine tests for adenovirus (16,17). It has been suggested that children who are viremic are at the greatest mortality risk from disseminated adenovirus infection (18-20). In immunocompromised individuals with disseminated adenovirus infection, viral load reflects disease activity and can be used to monitor the response to antiviral treatment. Walls et al. reported that there was no correlation between the quantity of virus in the blood and the severity of symptoms in BMT recipients (21). These data imply that the pathogenesis of adenovirus replication may be different in immunocompetent and immunocompromised individuals. Further study is needed to clarify the mechanisms by which adenovirus induces fever and inflammatory responses.
REFERENCES