Oropharyngotonsillitis Associated With Nonprimary Epstein-Barr Virus Infection

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Objective: To identify distinct clinical features of pharyngotonsillitis or oropharyngitis associated with Epstein-Barr virus (EBV) infection from herpes simplex virus infection.

Design: Clinical studies by case exploration.

Setting: Institutional practice at a university hospital.

Patients: Thirty-three patients with pharyngotonsillitis and 4 patients with oropharyngitis of nonbacterial infection underwent biopsy of pharyngotonsilar lesions.

Main Outcome Measure: The specimens were examined by histopathology, immunohistochemistry, in situ hybridization, and polymerase chain reaction. In addition to serological testing and routine laboratory data, photographic oropharyngeal findings were collected for clinical evaluation.

Results: In situ hybridization to detect EBV-encoded small nuclear RNA-1 and -2 disclosed 8 cases of pharyngotonsillitis and 4 cases of oropharyngitis associated with EBV infection. Immunohistochemical analysis identified 5 cases of pharyngotonsillitis associated with herpes simplex virus infection. Serological examination showed that, among 12 cases positive by in situ hybridization, 3 cases were primary infection with infectious mononucleosis and 9 were nonprimary infection. The staining pattern of in situ hybridization was different, ie, a linear pattern in cases of nonprimary infection and a scattered pattern in cases of primary infection. The clinical manifestations of EBV pharyngotonsillitis were distinct from those of herpes simplex virus pharyngotonsillitis and were characteristic irrespective of infectious status, while those of EBV oropharyngitis were more variable.

Conclusions: Epstein-Barr virus–associated pharyngotonsillitis was demonstrated in patients with nonprimary infection unaccompanied by infectious mononucleosis. Epstein-Barr virus should be considered a potential causative agent of oropharyngotonsillitis even in absence of infectious mononucleosis, especially in a young adult.


Both viruses and bacteria are important pathogens in the etiology of pharyngitis and acute tonsillitis.1,2 According to the guidelines for acute pharyngitis of the Infectious Diseases Society of America, viruses are the most common nonbacterial pathogens causing acute pharyngitis.3 Viral pharyngotonsillitis has been known to be caused by herpes simplex virus (HSV), Epstein-Barr virus (EBV), cytomegalovirus, and human herpesvirus 6 infection.4-6 Herpes simplex virus is the virus most frequently associated with acute pharyngitis, while exudative tonsillitis was found to be caused by EBV in 19% of the cases, with EBV being the second most common viral agent in the children studied.1,7 Whereas the diagnosis and treatment of bacterial pharyngotonsillitis have been established, it has been impossible to define the pathogenesis of viral pharyngotonsillitis because of the difficulty in identifying the causative agents in an outpatient clinic. Actually, some patients with viral pharyngotonsillitis show severe or prolonged symptoms, and their lesions do not respond well to treatment with antimicrobial agents.

Identification of the clinical characteristics of lesions with viral causes should improve their early diagnosis and treatment. We therefore investigated the oropharyngeal findings of pharyngotonsillitis and oropharyngitis caused by EBV and HSV infection, by serological, histological, and immunohistochemical studies and in situ hybridization (ISH) of biopsy specimens in combination with the polymerase chain reaction.
PATIENTS AND METHODS

PATIENTS

Among patients with acute pharyngotonsilitis or oropharyngitis of nonbacterial origin seen at the Ear, Nose, and Throat Clinic, Daimi Hospital, Tokyo Women’s Medical University, Tokyo, Japan, punch biopsy specimens were collected from 37 cases composed of 32 cases of acute pharyngotonsilitis and 5 cases of oropharyngitis.

There were 20 females and 17 males, and their ages ranged from 13 to 53 years (mean, 23.6 years). The patients with acute pharyngotonsilitis complained of sore throat, high fever (temperature, >38°C), and dysphagia. Physical examination showed exudative tonsillitis accompanied by exudation and/or erosion of the oropharyngeal mucosa and cervical lymphadenopathy. Most of the patients needed to be admitted for treatment. In contrast, the patients with oropharyngitis had less severe symptoms than those with acute pharyngotonsilitis. Their symptoms continued for 2 weeks despite antibiotic treatment. In addition, 2 of these cases recurred, 10 months and 4 years after treatment. Twenty-eight of 37 patients had not responded to previous treatment with antibiotics for 7 days. Because of the severe and prolonged symptoms, all 37 patients underwent biopsy after informed consent was obtained. The tonsillar and oropharyngeal findings of the patients were checked in detail and recorded in photographs.

SEROLOGICAL AND LABORATORY EXAMINATIONS

Each patient’s serum was submitted for serological studies within 3 days of the initial examination and checked again 12 or 21 days later. The second check could not be performed in 10 patients. Antibody titers were determined by the fluorescent antibody test for anti-EBV antibodies to nuclear antigens (EBNA); IgG, IgM, and IgA antibodies to viral capsid antigens (VCA); and IgG and IgA antibodies to diffuse and restricted early antigens (EA-DR), and by the complement fixation test for anti-HSV antibody and/or neutralization test for anti-HSV-1 and -2 antibodies. Tonsillar or pharyngeal swabs were collected and used for bacterial culture. White blood cell counts with differential counts and blood chemistry studies, particularly for liver enzymes, were routinely investigated.

PATHOLOGICAL EXAMINATIONS

Punch biopsy specimens, 2 to 3 mm in diameter, were collected from the margin of the lesions of tonsil or oropharyngeal mucosa within 5 days of our initial examination. All of the tissues were fixed in 10% buffered or nonbuffered formalin. Of them, 18 tissue samples were divided in 2 parts immediately after biopsy. One part was fixed in 10% buffered formalin and the other was stored at −80°C until use. The formalin-fixed tissue was embedded in paraffin and consecutively sectioned for histopathological examinations by hematoxylin-eosin staining, immunohistochemistry, ISH, and PCR analysis.

Immunohistochemistry to detect HSV antigens was performed on the sections as described previously. Briefly, deparaffinized sections were treated with 0.3% hydrogen peroxide in methanol and incubated overnight at 4°C with rabbit polyclonal antibody against HSV or type-specific monoclonal antibodies to HSV-1 or HSV-2. Then biotinylated anti-rabbit or mouse IgG (Vector Laboratory, Burlingame, Calif) and avidin-biotin peroxidase complex (Vector Laboratory) were applied sequentially for 45 minutes each. The peroxidase activity was developed in 0.05-mol/L.

RESULTS

HISTOPATHOLOGY, IMMUNOHistoCHEMISTRY, AND ISH ON BIOPSY SpecIMENS

Histopathological examination in combination with EBER-ISH or immunohistochemical analysis for HSV antigen demonstrated EBV infection in the biopsy tissues of 8 patients with pharyngotonsilitis and 4 patients with oropharyngitis (Table 1), and HSV infection was identified in 5 of the patients with pharyngotonsilitis (Table 2). Inclusion-bearing cells were not observed in any specimens except 5 from patients with HSV-associated pharyngotonsilitis. No cases of double infection with HSV and EBV were detected in this study. Malignant lymphoma was found in the biopsy specimen from an EBV-infected patient with oropharyngitis (case 9). Biopsy specimens of the other 20 cases, composed of 19 cases of pharyngotonsilitis and 1 case of oropharyngitis, showed only nonspecific findings. Most of them showed necrosis, vesicles, or lymphocyte infiltration in the squamous epithelium and parenchyma of the tonsils (Figure 1, A and C), although all of the biopsy specimens were too small to observe structural changes in the tonsils.

Positive signals were found by EBER-ISH in the nuclei of lymphocytic cells located in tonsillar parenchyma and epithelium in 12 specimens, 8 from patients with pharyngotonsilitis (cases 1-8) and 4 from patients with oropharyngitis (cases 9-12). Two different patterns of distribution of EBER-positive cells were observed in the biopsy specimens. The first pattern consisted of positive cells distributed in the epithelium and parenchyma (Figure 1, B) and was designated the “scattered pattern.” The second showed positive cells extending from the subepithelial parenchyma of the tonsil into the lower two thirds of the epithelium (Figure 1, D) and was called the “linear pattern.” The EBER-ISH method did not detect any positive cells in the 24 tonsil specimens used as negative controls.

Immunohistochemical analysis detected HSV antigens in specimens from 5 cases of acute pharyngotonsilitis. Histopathologically, intranuclear inclusion bod-
Tris buffer (pH 7.6) supplemented with diaminobenzidine and hydrogen peroxide. Nuclei were counterstained with 2% methyl green.

In situ hybridization to detect EBV-encoded small nuclear RNA-1 and -2 (EBER) was carried out with digoxigenin-labeled RNA probe. The EBER sequence (GenBank accession number, J02078) encompassing EBER 1 and EBER 2 (708 base pairs [bpl]) was amplified by PCR, and the purified PCR product was cloned into pGEM-T vector (Promega, Madison, Wis). Antisense or sense RNA probe was labeled with digoxigenin by using an in vitro RNA transcription and labeling kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s instructions. Deparaffinized sections were first treated with 10-µg/mL proteinase K (Boehringer Mannheim) and then hybridized with 10 ng of antisense or sense RNA probe overnight at 55°C in hybridization buffer consisting of 50% formamide, 0.05-mol/L HEPES, pH 7.0, 5× Denhardt’s (0.02% each of polyvinylpyrrolidone, Ficoll, and bovine serum albumin fraction V), and 3× standard saline citrate. After hybridization, the sections were washed in 0.2× standard saline citrate at 50°C twice for 15 minutes each. They were blocked with Block ace (Snow Brand Milk Products, Tokyo, Japan), and antidigoxigenin antibody conjugated with alkaline phosphatase (Boehringer Mannheim) was applied sequentially for signal detection. Nitroblue tetrazolium and bromochloroindolyl phosphate in 0.05-mol/L Tris hydrochloride, pH 9.5, were used for signal visualization. For positive controls, the sections taken from a patient with malignant lymphoma with serological increases in anti-EBV antibody titers were used. In addition, 24 formalin-fixed tonsillar sections retrieved during tonsillec- tomy from EBV carrier patients positive for anti-EBNA antibody and IgG antibody to VCA, but negative for IgM antibody to VCA, were used for negative controls.

PCR ANALYSIS FOR EBV INFECTION

The PCR for EBV DNA successfully amplified specific products identified by hybridization in 8 of the 12 EBER-positive specimens. The other 4 EBER-positive specimens (cases 4, 5, 10, and 11 in Table 1) were negative by β-globin PCR, suggesting inappropriate DNA samples for PCR that were found to have been fixed in nonbuffered formalin. No specific products were detected by PCR in the 24 tonsil specimens used as negative controls. To confirm the sensitivity of PCR, 10-fold dilutions of the DNA extracted from B95-8 cell lines were amplified. Viral DNA could be detected only in the B95-8 cell line in greater than 50-fg DNA samples by gel analysis with ethidium bromide staining.

Total DNA was extracted from 37 formalin-fixed and paraffin-embedded sections by using a DNA extraction kit (Takara Dexpad; Takara Biomedicals, Kyoto, Japan) according to the manufacturer’s instructions, and DNA was also extracted from 18 unfixed frozen tissues by a standard procedure, as described previously. The PCR method for detection of EBV DNA was used, with modifications to amplify the 125-bp DNA sequences in BamHI-W fragments of the EBV genome. Amplification with Taq DNA polymerase (Boehringer Mannheim) was performed on 20 ng to 300 ng of extracted DNA. Thirty-five cycles of denaturation (1 minute at 94°C), annealing (1 minute at 66°C), and extension (2 minutes at 72°C) were carried out. The PCR products were separated by 2% agarose gel electrophoresis and visualized under UV light with ethidium bromide staining. To confirm the specificity of the products, they were transferred to a nylon membrane and subjected to Southern blot hybridization with an internal probe labeled with fluorescein-11-deoxy uracil triphosphate by using an ECL 3′-end labeling system (Amersham, Buckinghamshire, England). The signal was detected by enhanced chemilumi- nescence (Amersham) according to the protocol provided by the manufacturer. Total DNA specimens extracted from an EBV-infected cell line, B95-8, intermingled with 5% to 10% EBV-producing cells was used as a positive control, and specimens from the 24 formalin-fixed and paraffin-embedded sections of tonsillar tissue specimens from EBV carrier patients were used as negative controls. To check the integrity of the DNA extracted, all specimens were subjected to β-globin PCR amplifying a 162-bp fragment.

SEROLOGICAL AND LABORATORY FINDINGS

Significant serological alterations of anti-EBV antibody titers in paired serum samples were found in 5 of the 12 cases of EBV-associated pharyngotonsillitis (cases 1, 2, 3, 9, and 10 in Table 1), consisting of 3 cases of acute pharyngotonsillitis and 2 cases of oropharyngitis. Three cases of pharyngotonsillitis with EBV infection appeared to represent a primary infection because they were positive for IgM, had increased IgG anti-VCA antibody levels, and were negative for anti-EBNA antibody. According to the diagnostic criteria of Evans, these cases corresponded to IM with laboratory findings such as increased liver enzyme levels (aspartate and alanine aminotransferase) and lymphocyte counts, with the presence of atypical lymphocytes. Another 2 cases of oropharyngitis were considered to represent reactivated EBV infection (nonprimary infection) because they were positive for both antibodies to diffuse and restricted early antigen and anti-EBNA antibody. One of them had elevated liver enzyme levels and mononucleosis (the total proportion of monocytes and lymphocytes was greater than 50%) and was diagnosed as malignant lymphoma on the basis of additional histopathological examinations (case 9). In the other 7 EBER-ISH–positive cases,
comprised of 5 cases of acute pharyngotonsillitis and 2 cases of oropharyngitis, the serological changes suggested previous infection (nonprimary) with EBV accompanied by normal liver enzyme levels and white blood cell differential count.

All of the individuals who proved immunohistochemically positive for HSV antigen had pharyngotonsillitis. Two of them (cases 14 and 17 in Table 2) showed a substantial increase in complement fixation antibody titer to HSV, and none was positive on the neutralization test for HSV. Serologically, all cases of HSV pharyngotonsillitis were considered to represent primary infection because of their low antibody titers. The neutralization test could not distinguish between HSV-1 and HSV-2 serotype.

Bacterial cultures of tonsillar or pharyngeal swabs detected no pathogenic bacteria including antibiotic-resistant strains: 11 cases had normal flora, 2 cases had.

### Table 1. Summary of EBV-Associated Pharyngotonsillitis

<table>
<thead>
<tr>
<th>Patient No./Sex/ Age, y</th>
<th>Clinical Diagnosis</th>
<th>Biopsy Findings</th>
<th>Serological Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RNA In Situ Hybridization†</td>
<td>PCR for EBV</td>
</tr>
<tr>
<td>1/F/24 Acute pharyngotonsillitis</td>
<td>S + 640/320</td>
<td>10/20</td>
<td>.../10</td>
</tr>
<tr>
<td>2/F/13 Acute pharyngotonsillitis</td>
<td>S + 640/160</td>
<td>40/10</td>
<td>&lt;10/10</td>
</tr>
<tr>
<td>3/F/26 Acute pharyngotonsillitis</td>
<td>S + 160/80</td>
<td>20/10</td>
<td>.../20</td>
</tr>
<tr>
<td>4/F/22 Acute oropharyngitis</td>
<td>L − 40/...</td>
<td>&lt;10/...</td>
<td>.../...</td>
</tr>
<tr>
<td>5/F/31 Acute oropharyngitis</td>
<td>L − 10/...</td>
<td>&lt;10/...</td>
<td>&lt;10/...</td>
</tr>
<tr>
<td>6/F/23 Acute oropharyngitis</td>
<td>L + 160/...</td>
<td>&lt;10/...</td>
<td>.../...</td>
</tr>
<tr>
<td>7/F/19 Acute oropharyngitis</td>
<td>L + 640/320</td>
<td>&lt;10/10</td>
<td>&lt;10/10</td>
</tr>
<tr>
<td>8/M/13 Acute oropharyngitis</td>
<td>L + 160/160</td>
<td>&lt;10/10</td>
<td>&lt;10/10</td>
</tr>
<tr>
<td>9/F/22 Recurrent oropharyngitis</td>
<td>L + 5120/2560</td>
<td>20/...</td>
<td>.../40</td>
</tr>
<tr>
<td>10/F/27 Refractory gingivitis</td>
<td>L − 640/160</td>
<td>&lt;10/10</td>
<td>&lt;10/10</td>
</tr>
<tr>
<td>11/F/18 Recurrent oropharyngitis</td>
<td>L − 160/80</td>
<td>&lt;10/10</td>
<td>&lt;10/10</td>
</tr>
<tr>
<td>12/F/25 Acute oropharyngitis</td>
<td>S + 640/320</td>
<td>&lt;10/10</td>
<td>&lt;10/1...</td>
</tr>
</tbody>
</table>

*EBV indicates Epstein-Barr virus; PCR, polymerase chain reaction; VCA, viral capsid antigen; EA-DR, early antigen, diffuse and restricted; EBNA, EBV nuclear antigen; ellipses, not done; plus sign, positive; minus sign, negative; IM, infectious mononucleosis; and ML, malignant lymphoma.

†Positive cell pattern by in situ hybridization (ISH) to detect EBV-encoded small nuclear RNA-1 and -2 ISH (EBER-ISH); S, scattered pattern; L, linear pattern.

‡Detection of type-specific HSV antigen by polyclonal and type-specific monoclonal antibodies.

§Titer at initial consultation/titer at second consultation.

¶All biopsy samples were from palatine tonsils except those in case 12, which were taken from gingival mucosa.

### Table 2. Summary of Primary HSV Tonsillitis

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age, y</th>
<th>Clinical Diagnosis</th>
<th>Inclusion Body†</th>
<th>Immunohistochemical Analysis‡</th>
<th>Serological Findings§</th>
</tr>
</thead>
<tbody>
<tr>
<td>13/F/19 Acute tonsillitis</td>
<td>+</td>
<td>HSV</td>
<td>32/16</td>
<td>4/4</td>
</tr>
<tr>
<td>14/M/25 Acute tonsillitis</td>
<td>+</td>
<td>HSV-1</td>
<td>&lt;4/16</td>
<td>&lt;4/4</td>
</tr>
<tr>
<td>15/F/20 Acute tonsillitis</td>
<td>−</td>
<td>HSV</td>
<td>&lt;4/16</td>
<td>&lt;4/4</td>
</tr>
<tr>
<td>16/M/25 Acute tonsillitis</td>
<td>+</td>
<td>HSV-1</td>
<td>&lt;4/16</td>
<td>&lt;4/4</td>
</tr>
<tr>
<td>17/M/30 Acute tonsillitis</td>
<td>+</td>
<td>HSV-1</td>
<td>&lt;4/16</td>
<td>&lt;4/4</td>
</tr>
</tbody>
</table>

*HSV indicates herpes simplex virus; CF, complement fixation test; NT, neutralization test; and ellipses, not done.

†Intranuclear inclusion body identified in the sections stained by hematoxylin-eosin. Plus sign indicates present; minus sign, absent.

‡Detection of type-specific HSV antigen by polyclonal and type-specific monoclonal antibodies.

§Titer at initial consultation/titer at second consultation.

¶HSV serotype was not determined. (see the “Serological and Laboratory Findings” subsection of the “Results” section.)

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small numbers of colonies of *Haemophilus parainfluenzae*, 1 case had *Staphylococcus aureus*, and 1 case had *Candida albicans*.

**CLINICAL FEATURES**

After examination of the histopathological findings in the biopsy specimens and the serological studies, tonsillar and oropharyngeal findings recorded in photographs were reevaluated to identify the distinctive clinical features of the tonsils and oropharyngeal mucosa (Table 3).

Clinically, the patients with acute pharyngotonsillitis caused by HSV infection showed reddening and swelling of the tonsils (Figure 3, A) accompanied by aphthae on the tonsil surface and/or oropharyngeal mucosa. Characteristically, these cases also had other HSV-associated

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**Figure 1.** Histopathological findings and results of in situ hybridization to detect Epstein-Barr virus–encoded small nuclear RNA-1 and -2 (EBER) of biopsy specimens. A and B, Case 2; C and D, case 11. A and C, Lymphocytic infiltration into squamous cell epithelium. B and D, Numerous EBER-positive cells distributed in the parenchyma of tonsil and subepithelial region in a scattered pattern (B) and in squamous cell epithelium in a linear pattern (D) (A and C, hematoxylin-eosin; B and D, antisense EBER probe; original magnification ×100).

**Figure 2.** Histopathological and immunohistochemical findings of primary herpes simplex virus pharyngotonsillitis (case 17). A, Intranuclear inclusion bodies in squamous cell epithelium (hematoxylin-eosin, original magnification ×150). B, Immunohistochemistry using a mixture of anti–herpes simplex virus type 1 monoclonal antibodies showing nuclear and cytoplasmic staining (avidin-biotin peroxidase complex method counterstained with methyl green, original magnification ×150).
lesions of the oropharyngeal mucosa, such as herpes gingivostomatitis or herpes labialis (Figure 3, B), and atypical herpetic lesions such as aphthae or white-coated erosions surrounded by reddening in the hypopharyngeal and epiglottic mucosa. No petechiae were observed on the oropharyngeal mucosa. Two of the patients (cases 13 and 15) were found to have concomitant genital herpes.

In contrast, 8 patients with acute pharyngotonsillitis in which EBV was detected showed physical findings in common, irrespective of the presence of IM or mode of infection, ie, primary infection or reactivation, that were distinctly different from those of HSV-associated pharyngotonsillitis. Most of the cases presented with reddening and enlargement of tonsils covered with exudation, which was thicker than that of lacunar pharyngotonsillitis (Figure 3, C and E). In addition, there was a white coating on the pharyngeal and lingual tonsils, erosions or ulcers on the pharyngeal and epiglottic mucosa, and petechiae on the hard palate mucosa (Figure 3, D and F).

The clinical findings in EBV-associated oropharyngitis were much more variable than those of HSV- or EBV-associated pharyngotonsillitis. The lesions of the oropharyngeal mucosa consisted of painful erosions and/or ulcers, edematous swelling of the uvula, and gingivitis; erosions of the lip and tongue were also noted. Each case had multiple lesions, with combinations of a variety of findings (Figure 3, G and H).

Table 3. Clinical Findings on the Cases With Epstein-Barr Virus (EBV)– or Herpes Simplex Virus (HSV)–Associated Oropharyngotonsillitis

| Case (Period*) | Tonsil Exudation† | Oropharyngeal Lesion§ | Cervical Lymph Adenopathy¶ | Skin Lesion| Genital Lesion^ |
|----------------|------------------|----------------------|---------------------------|-----------------------------------|
|                | Tonsil Pharynx‡ | Epipharynx Hypopharynx | Pharynx Tongue Gingiva Lip Hypopharynx | Epipharynx Erosion ++ | Herpes ++ | Aphtha ++ | Epithelial hyperplasia | Vulvar edema |
| 1 (13 d)       | ++ – ++ – | ++ Edema of vulva – – – | ++ – – | ++ – – | – – – | – – – | – – – | – – – | – – – |
| 2 (13 d)       | +++ – +++ – | ++ Petechiae# – – – | +++ General exanthema – | General exanthema | – – | – – | – – | – – | – – |
| 3 (13 d)       | ++ + +++ – | ++ Petechiae# – – – | +++ General exanthema | General exanthema | – | – | – | – | – |
| 4 (10 d)       | – – – – | – – – – | – – – | – – | – – | ++ + | – – | – – | – – |
| 5 (5 d)        | +++ +++ – | – – – – | – – – | – | ++ | – | – | – | – |
| 6 (3 d)        | +++ + ++ + | – – – – | ++ | – | – | – | – | – | – |
| 7 (14 d)       | – – ++ – | – – | – – | – | ++ | – | – | – | – |
| 8 (4 d)        | ++ + ++ – | – | – | – | ++ | – | – | – | – |
| 9 (10 mo)      | – – – – | – – | – | – | Aphtha and ulcer | – | – | – | – |
| 10 (5 d)       | – – – – | – | Ulcer and erosion | – | – | – | – | – | – |
| 11 (4 y)       | – – – – | Ulcer | – | – | – | – | – | – | – |
| 12 (15 d)      | – – – – | – | Ulcer | – | – | – | – | – | – |
| 13 (14 d)      | + ++ – – | – | Aphtha | – | – | – | – | – | – |
| 14 (3 d)       | ++ – – +++ | – | Aphtha | – | – | – | – | – | – |
| 15 (5 d)       | – ++ – +++ | – | Aphtha | – | – | – | – | – | – |
| 16 (2 d)       | ++ ++ – +++ | – | Aphtha | – | Erosion | – | – | – | – |
| 17 (7 d)       | – – ++ – +++ | – | Aphtha | – | – | – | – | – | – |

* Period from onset to initial consultation in our clinic.
† Exudation pattern: diffuse (+++), moderate (++), slightly (+), not found (–).
‡ Oropharyngeal mucosa except palatine tonsil.
§ Except clinical change of palatine tonsil.
¶ Not found (–).
§ Severe (+++), moderate (++), slight (+), not palpable (–).
# Petechiae on hard palate.
Cervical lymphadenopathy was present in all cases in which the biopsy specimens were positive for EBER-RNA or HSV antigen. Generalized skin eruptions were observed in 2 of the 3 cases of EBV pharyngotonsillitis with primary infection. Although EBV is present in the saliva of many healthy individuals, the virus is an important causative agent in oropharyngeal diseases, such as oral hairy leukoplakia.

Figure 3. Oropharyngeal findings. A and B, Case 17; C and D, case 3; E and F, case 8; G and H, case 11. A and B, Tonsillar enlargement and reddening with small exudation and accompanied by herpes labialis. C and E, Tonsillar enlargement and reddening coated with thick exudation. D and F, Petechiae on hard palate. G and H, Various mucosal lesions, ulcer, aphthae, and erosion in the pharynx and oral mucosa.
and nasopharyngeal carcinoma.15-17 Only exudative tonsillitis complicating IM has been recognized as an oropharyngeal inflammatory disease associated with primary EBV infection.18 To our knowledge, EBV-associated pharyngotonsillitis and oropharyngitis in the absence of IM have never been reported with examination of biopsy specimens.

In this study, we identified 12 cases of EBV-associated pharyngotonsillitis and 5 cases of HSV-associated acute pharyngotonsillitis among 37 cases of nonbacterial pharyngotonsillitis and oropharyngitis examined by histopathological, immunohistochemical, and PCR analyses; ISH; and serological studies. The cases of EBV-associated oropharyngotonsillitis included 3 cases of acute pharyngotonsillitis with IM, and 5 cases of acute pharyngotonsillitis and 4 cases of oropharyngitis without IM. Based on the serological studies, 3 cases of the acute pharyngotonsillitis with IM were considered primary infection with EBV, and the other 9 cases were thought to represent nonprimary infection without IM. The use of EBER-ISH in biopsy specimens demonstrated different staining patterns in primary and nonprimary infection with EBV. This study also identified the distinct clinical features of oropharyngeal mucosal lesions in viral infections. The distribution of exudative lesions differed in acute pharyngotonsillitis caused by EBV and that caused by HSV infection, and lesions characteristic of EBV pharyngotonsillitis, EBV oropharyngitis, and HSV pharyngotonsillitis were identified.

**Tonsillar Exudations** were observed in most of the cases of pharyngotonsillitis, but not in any of the cases of oropharyngitis. Infections with group A β-hemolytic streptococci, measles virus, and adenovirus should be differentiated as causative agents of exudative tonsillitis.2,3 In our cases, group A β-hemolytic streptococci and measles virus infections were ruled out by the results of bacterial cultures and clinical features, respectively. Adenovirus infection usually occurred among children aged 3 to 6 years and was not detected by histopathological examination. The exudation of EBV pharyngotonsillitis and HSV pharyngotonsillitis was found exclusively in the epitharynx and hypopharynx, respectively.

Palatine petechiae were a characteristic lesion of EBV pharyngotonsillitis and were observed in 3 cases of primary and nonprimary EBV pharyngotonsillitis, but not in HSV pharyngotonsillitis. The petechiae resembled the clinical finding of Forchheimer sign, which is known to occur in rubella virus infection. They have also been recognized in tonsillitis associated with group A β-hemolytic streptococci and EBV pharyngotonsillitis with IM.19,20 The mucosal lesions of nonprimary EBV oropharyngitis varied compared with those of HSV and EBV pharyngotonsillitis, and consisted of nonspecific erosions, aphthae and/or ulcers with pain, and various combinations of them distributed widely throughout the oropharynx. The presence of herpes aphthae indicated HSV pharyngotonsillitis in cases of nonbacterial infection.

The distribution of oropharyngeal lesions suggests different patterns of spread of EBV or HSV into the oropharynx. The cases of HSV pharyngotonsillitis represented primary infection, and the lesions were observed on the lips, oral cavity, pharynx, and hypopharynx, suggesting that the viral infection was spread in the process of swallowing saliva. In contrast, the lesions of EBV-infected cases with primary or nonprimary infection may have been caused by direct oral infection or indirect infection mediated by EBV-infected lymphocytes, because the exudative lesions of the epipharynx were not explained by the passage of saliva alone.

Examinations of EBER-ISH and PCR in combination with histopathological analysis enabled us, for the first time, to identify 5 cases of EBV pharyngotonsillitis and 4 cases of EBV oropharyngitis without clinical evidence of IM. These cases were determined to represent nonprimary EBV infection on the basis of the serological analysis. The clinical manifestations in the tonsils and/or oropharynx, however, were distinct, irrespective of whether it was primary or nonprimary EBV pharyngotonsillitis. The laboratory findings, such as elevated liver enzyme levels; the mononucleosis; and general skin eruption were found exclusively in primary EBV pharyngotonsillitis, except the malignant lymphoma case. The histological findings in the biopsy tissues were indistinguishable, but EBER-ISH demonstrated 2 staining patterns in the distribution of signal-bearing cells. The EBER-positive cells in the specimens of primary EBV pharyngotonsillitis were distributed in the parenchyma of the tonsil and over the squamous epithelium (scattered pattern), while those in the specimens of nonprimary EBV tonsillitis and oropharyngitis were grouped in the basal cell layer of squamous epithelium (linear pattern). In terms of the presence or absence of general symptoms, serological alterations, and distinctive patterns of EBER-ISH, the pathogenesis of primary and nonprimary EBV infection seems to be different.

Primary infection has been thought to occur by transmission of cell-free virus and/or productively infected cells in saliva, followed by virus replication in the oropharyngeal epithelium and transmission into circulating B cells.18,20 Recirculation of virus-infected B cells but not epithelial cells causes IM and EBV primary infection.21 In contrast, nonprimary EBV infection has been thought to occur because continuous viral shedding occurs in individuals with the carrier state and physiological stimuli impacting on circulating B cells on the mucosal surface trigger initiation of the lytic cycle, resulting in EBV reactivation. These pathogenic mechanisms may in part explain the difference between the clinical and EBER-ISH findings in primary and nonprimary EBV infection of pharyngotonsillitis and oropharyngitis.18

In this study, we used EBER-ISH to detect EBV infection of biopsy tissues, because EBER is the most abundant EBV-encoded RNA expressed in latently infected cells.22 The EBER-ISH and immunohistochemical analyses have shown considerable numbers of latently infected B cells in the extrafollicular areas of the tonsils of patients in the acute phase of IM.23 Detection of EBV by ISH and in situ PCR in tonsil specimens from patients with chronic tonsillitis have demonstrated EBV-positive cells in the upper and lower epithelial cell layer.24 These results were consistent with our EBER-ISH findings in this...
study. The scattered and the linear patterns of EBER-ISH were detected in EBV pharyngotonsillitis with IM and in EBV pharyngotonsillitis and oropharyngitis without IM, respectively. All 24 tonsil specimens from individuals with EBV carrier state yielded negative results by EBER-ISH and PCR for EBV, indicating that EBER transcripts in the latent state were not detectable beyond the sensitivity of our EBER-ISH and PCR methods. Thus, positive signals with EBER-ISH and PCR suggest relatively active replication of EBV in oropharyngeal lesions.

The results of this study suggest that careful and detailed observation of oropharyngeal lesions can provide evidence of pharyngotonsillitis or oropharyngitis associated with EBV or HSV infection. Most of the patients were in the young age group. Complaints of high fever, sore throat, and dysphagia were common in EBV and HSV pharyngotonsillitis, but not severe in EBV oropharyngitis, and all of the latter cases were refractory to treatment. To correctly diagnose pharyngotonsillitis and oropharyngitis of viral origin, it is important to identify precisely the clinical features according to the characteristics of viral infection.

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