High Prevalence of Varicella-Zoster Virus Reactivation in Herpes Simplex Virus–Seronegative Patients with Acute Peripheral Facial Palsy

Yasushi Furuta,1 Fumio Ohtani,1 Hiroki Kawabata,2 Satoshi Fukuda,1 and Tomas Bergström1

Varicella-zoster virus (VZV) and herpes simplex virus (HSV) are considered to be the major causes of acute peripheral facial palsy (APFP). One hundred and forty-two patients with APFP were analyzed by serological assays and polymerase chain reaction analysis. Ramsay Hunt syndrome was diagnosed in 21 patients. Of the remaining 121 patients clinically diagnosed with Bell’s palsy, VZV reactivation without zoster (zoster sine herpetic disease) was detected in 35 patients (29%). The prevalence of antibodies to HSV among patients with Bell’s palsy was significantly higher than the prevalence among those with VZV reactivation (Ramsay Hunt syndrome or zoster sine herpetic disease). In contrast, a high incidence (88%) of VZV reactivation among HSV-seronegative patients with APFP was observed. Our data indicate that VZV is one of the major etiologic agents of clinically diagnosed Bell’s palsy and that VZV reactivation causes APFP in most patients who lack antibodies to HSV.

Many infectious causes of acute peripheral facial palsy (APFP) have been identified, such as otitis media, Lyme disease, and some viral infections. Reactivation of varicella-zoster virus (VZV) is a known cause of APFP. Ramsay Hunt syndrome is characterized by zoster around the ear, facial palsy, and eighth cranial nerve symptoms (hearing loss and/or vertigo). In some cases of Ramsay Hunt syndrome, zoster rash appears several days after the onset of facial palsy; idiopathic peripheral facial palsy (Bell’s palsy) is initially diagnosed in these patients. Furthermore, VZV causes APFP without skin lesions; these cases have been termed zoster sine herpetic disease (ZSH), and diagnosis is made by serological assays and/or PCR analysis. We previously showed that PCR analysis is useful for the early diagnosis of ZSH and found that some cases of ZSH did not generate an antibody response to VZV [1].

Herpes simplex virus (HSV) is also suspected of causing APFP [2, 3]. Serological studies have shown that the prevalence of antibodies to HSV among patients with Bell’s palsy is higher than that among healthy control subjects, which suggests that reactivation of HSV may be involved in the pathogenesis of Bell’s palsy [4–6]. In these previous studies, however, patients with ZSH were not differentiated from those with Bell’s palsy.

In addition, HSV type–specific serological assays were not performed for patients with APFP. In the present study, we distinguished ZSH from Bell’s palsy by means of PCR analysis and serological assays and then compared the prevalence of HSV type–specific antibodies among patients with VZV reactivation (Ramsay Hunt syndrome or ZSH) and with the prevalence among patients without VZV reactivation (Bell’s palsy). We also determined the causes of APFP in HSV-seronegative patients.

Patients and Methods

Patients. The study included 142 patients who from September 1995 through March 1999 visited 3 clinics (Hokkaido University Hospital, Sapporo, Tenshi Hospital, Sapporo, and Shinmishitsu Muroran General Hospital, Muroran, Japan) within 10 days after the onset of APFP. Patients aged <4 years were excluded from the study. Paired serum samples were obtained from 138 patients at their initial visit and again 2–3 weeks later (convalescent phase). Serum samples from 4 patients with Ramsay Hunt syndrome were analyzed once at the initial visit. Saliva samples were collected from 139 patients. These samples were taken 4–10 times at every visit. Ramsay Hunt syndrome was diagnosed if, in addition to APFP, there were typical zoster lesions around the ear or in the oral epithelium. ZSH was diagnosed if VZV DNA was detected in saliva samples by PCR analysis or when serological assays indicated recent VZV infection. Bell’s palsy was diagnosed in all other patients.

PCR analysis. DNA was extracted from 100-μL saliva samples by means of a DNA extraction kit (SepaGene, Sanko Junyaku Co., Tokyo). VZV DNA and HSV type 1 (HSV-1) DNA in the saliva samples were detected by use of a nested PCR method, as described elsewhere [1, 7]. For detection of HSV type 2 (HSV-2) DNA, 2 pairs of primers specific for the promoter region of the glycoprotein D gene of HSV-2 were used [8].

VZV and HSV type–common ELISA. Levels of antibody to
VZV and antibody to HSV were measured by use of ELISA kits (Enzygnost Anti-VZV/IgG and IgM and Enzygnost Anti-HSV/IgG and IgM, respectively; Behring-Werke, Marburg, Germany) and an automatic ELISA processor (Processor III, Behring-Werke). As recommended by the manufacturer, either significant changes (>2-fold) in IgG antibody levels or the presence of IgM antibody was considered to indicate recent infections with VZV and HSV.

**HSV type-specific ELISA.** For the determination of HSV type, HSV-1 glycoprotein G–specific antigen (kindly provided by SmithKline Beecham, London) and HSV-2 glycoprotein G–specific antigen [9, 10] were used. Microtiter plates (Nunc Immuno plates; Nunc, Rosshilde, Denmark) coated with the antigens were incubated with serum samples at a dilution of 1:100. For determination of IgG antibodies, alkaline phosphatase–conjugated rabbit antibodies to human IgG antibody (Dako A/S, Glostrup, Denmark) were used. In each plate, 4 negative control serum samples were included; serum samples were considered positive if their absorbance was 3 SDs greater than the mean absorbance of serum from negative control patients.

**HSV type-specific Western blotting.** Confluent monolayers of Hep-2 cells were infected with HSV-1 strain 90:395 or HSV-2 strain B4327 (both strains isolated in Göteborg, Sweden) at a multiplicity of infection of 25 pfu/cell. The HSV-1 and HSV-2 antigens were prepared as described elsewhere [11]. Antigens were separated by PAGE with use of a 7% polyacrylamide gel (7% NuPAGE Tris-acetate gels; NOVEX, San Diego, CA) and were transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA). The membranes were dried and cut into strips (3-mm width). The immunoblot procedure was performed as described elsewhere [11].

**Analysis of other infectious agents.** For 3 HSV-seronegative patients for whom PCR analysis and serological assays did not indicate reactivation of VZV, levels of antibody to Epstein-Barr virus (EBV) and antibody to cytomegalovirus (CMV) were analyzed (determined by Enzygnost Anti-EBV/IgG and IgM and Enzygnost Anti-CMV/IgG and IgM, respectively; Behring-Werke). Western blotting for antibodies to *Borrelia* was performed by use of *Borrelia garinii* strain HP1 and *Borrelia afzelii* strain P/Gau as the antigens [12].

### Results

**Clinical and virological diagnoses of APFP.** Of the 142 patients with APFP, 13 had typical zoster lesions around the ear or in oral epithelium in addition to APFP at their first visit; these patients had a diagnosis of Ramsay Hunt syndrome (figure 1; table 1). Bell’s palsy was diagnosed clinically in the other 129 patients at the first visit. Zoster lesions appeared after the first visit in 8 patients in whom Bell’s palsy was initially diagnosed. Therefore, Ramsay Hunt syndrome was diagnosed in a total of 21 patients. VZV reactivation was confirmed in 11 (58%) of 19 patients whose saliva samples were tested by PCR analysis and in 15 (88%) of 17 patients whose paired serum samples were tested by serological assays. VZV reactivation was not detected by virological tests in 3 patients who had clinically apparent zoster lesions.

For the remaining 121 patients with a clinical diagnosis of Bell’s palsy, PCR analysis detected VZV DNA in saliva samples from 20 patients on at least 1 occasion in the acute phase of palsy. Eleven of the 20 patients had either a significant increase in levels of IgG antibody to VZV or the presence of IgM antibody, while VZV DNA was detected in saliva samples from the remaining 9 patients with an absence of a detectable antibody response to VZV. In addition, serological assays indicated VZV reactivation in 15 patients, although VZV DNA was not detected. These 35 patients did not have zoster lesions, and ZSH was diagnosed. Thus ZSH occurred in 29% (35) of 121 patients with a clinical diagnosis of Bell’s palsy.

Saliva samples were collected within 5 days after the onset of palsy from 75 of 86 patients with Bell’s palsy (no VZV reactivation); HSV-1 DNA was detected in samples from 23 patients. Three of the 75 patients did not have antibodies to HSV (table 2). Therefore, the incidence of HSV-1 reactivation among HSV-seropositive patients with Bell’s palsy during the acute phase of the disease was 32% (23/72). Of the 23 patients, 2 had typical herpes labialis at the onset of palsy. Another 2 patients had significant increases in levels of IgG antibody to VZV and antibody to HSV, respectively.

### Table 1. Virological diagnosis of varicella-zoster virus (VZV) reactivation in 142 patients with acute peripheral facial palsy.

<table>
<thead>
<tr>
<th>Virological diagnosis</th>
<th>No. of patients with diagnostic evidence of VZV reactivation/total no. tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramsay Hunt syndrome</td>
<td>21/19</td>
</tr>
<tr>
<td>Zoster sine herpete</td>
<td>35/20/34</td>
</tr>
<tr>
<td>Bell’s palsy</td>
<td>86/0/86</td>
</tr>
<tr>
<td>Total</td>
<td>142/31/139</td>
</tr>
</tbody>
</table>

**Discussion**

The results of this study suggest that VZV reactivation is a common event in patients with APFP. The incidence of VZV reactivation in the 142 patients with APFP was 41% (58% of the 19 patients whose saliva samples were tested by PCR analysis and 88% of the 17 patients whose paired serum samples were tested by serological assays). In addition, serological assays indicated VZV reactivation in 15 patients, although VZV DNA was not detected. These 35 patients did not have zoster lesions, and ZSH was diagnosed. Thus ZSH occurred in 29% (35) of 121 patients with a clinical diagnosis of Bell’s palsy.

Saliva samples were collected within 5 days after the onset of palsy from 75 of 86 patients with Bell’s palsy (no VZV reactivation); HSV-1 DNA was detected in samples from 23 patients. Three of the 75 patients did not have antibodies to HSV (table 2). Therefore, the incidence of HSV-1 reactivation among HSV-seropositive patients with Bell’s palsy during the acute phase of the disease was 32% (23/72). Of the 23 patients, 2 had typical herpes labialis at the onset of palsy. Another 2 patients had significant increases in levels of IgG antibody to VZV and antibody to HSV, respectively.
Prevalence of antibodies to herpes simplex virus (HSV) among patients with acute peripheral facial palsy.

<table>
<thead>
<tr>
<th>Virological diagnosis</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell’s palsy</td>
<td>83 (97)</td>
<td>3 (3)</td>
<td>86 (100)</td>
</tr>
<tr>
<td>VZV reactivation</td>
<td>33 (59)</td>
<td>23 (41)</td>
<td>56 (100)</td>
</tr>
<tr>
<td>Ramsay Hunt syndrome</td>
<td>12 (57)</td>
<td>9 (43)</td>
<td>21 (100)</td>
</tr>
<tr>
<td>ZSH</td>
<td>21 (60)</td>
<td>14 (40)</td>
<td>35 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>116 (82)</td>
<td>26 (18)</td>
<td>142 (100)</td>
</tr>
</tbody>
</table>

NOTE. VZV, varicella-zoster virus; ZSH, zoster sine herpete.

* P < .0001, Fisher’s exact test.

HSV. No HSV-2 DNA was detected. In total, 79 (56%) of 142 patients with APFP had either VZV or HSV-1 reactivation at the time of the onset of palsy.

Prevalence of antibodies to HSV among patients with APFP. Type-common ELISA showed that 116 (82%) of the 142 patients were HSV-seropositive (table 2). The prevalence of antibodies to HSV was compared between patients diagnosed with VZV reactivation (Ramsay Hunt syndrome or ZSH) and those diagnosed with Bell’s palsy. The difference in the prevalence of VZV reactivation was statistically significant (97% vs. 59%, respectively; *P* < .0001, Fisher’s exact test).

HSV type-specific antibodies and VZV reactivation in patients with APFP. Type-common ELISA showed that 26 (18%) of 142 patients were HSV-seronegative (table 3). The prevalence of antibodies to HSV was lower among HSV-seronegative patients, and virological evidence of VZV reactivation indicated ZSH in 14 of the 26 HSV-seronegative patients. Thus VZV reactivation was detected in 23 (88%) of the 26 HSV-seronegative patients who had APFP.

HSV type-specific ELISA was performed for the 116 HSV-seropositive patients with use of HSV-1 glycoprotein G–specific and HSV-2 glycoprotein G–specific antigens. In total, 109 of 116 serum samples reacted with HSV-1 glycoprotein G–specific antigen, whereas 26 of 116 serum samples were positive for HSV-2 glycoprotein G–specific antigen. HSV type-specific Western blotting was used to analyze serum samples negative for HSV-1 glycoprotein G–specific antigen or positive for HSV-2 glycoprotein G–specific antigen because, in our control study, Western blotting showed that serum samples that reacted with HSV-1 glycoprotein G–specific antigen but not with HSV-2 glycoprotein G–specific antigen were positive only for HSV-1. By use of Western blotting, 4 (3%) of 116 patients had only antibody to HSV-2, and 18 patients (16%) had antibodies to both HSV-1 and HSV-2. The remaining 94 patients (81%) had only antibody to HSV-1 (table 3). VZV reactivation was detected in 29 (31%) of 94 patients with only antibody to HSV-1 and in 2 (5%) of 4 patients with only antibody to HSV-2. Thus VZV reactivation was detected in 31 (32%) of 98 patients who had either antibody to HSV-1 or HSV-2, whereas only 2 (11%) of 18 patients with antibodies to both HSV-1 and HSV-2 had VZV reactivation. The difference, however, was not statistically significant (*P* = .09, Fisher’s exact test).

Age-related prevalence of antibodies to HSV and VZV reactivation. Because the prevalence of antibodies to HSV has been decreasing among the younger population in Japan, as well as in populations in other developed countries [13, 14], we compared the prevalence of antibodies to HSV with the incidence of VZV reactivation, as they relate to patient age (figure 2). The prevalence of antibodies to HSV was lower among patients with APFP who were aged <24 years (40%–44%) than among patients aged >55 years (almost 100%). Conversely, the incidence of APFP caused by VZV reactivation was higher (60%–75%) among patients aged <24 years than among older patients. It is interesting that among 25- to 34-year-old patients the incidence of VZV reactivation was lower (19%), and the prevalence of antibodies to HSV was higher (81%).

Analysis of other infectious agents in HSV-seronegative patients. In 3 of 26 HSV-seronegative patients with APFP, we were not able to detect VZV reactivation by either serological assays or PCR analysis. To investigate other infectious causes of APFP in these HSV-seronegative patients, we analyzed the patients’ serum samples for EBV, CMV, and *Borrelia*. All 3 patients lacked IgM antibodies to EBV and CMV, and no seroconversion to EBV or CMV was observed. Two patients had IgM and IgG antibodies to *B. garinii* and *B. afzelii*; these findings were confirmed by Western blotting (table 4). The results matched the Centers for Disease Control and Prevention’s criteria for the serological diagnosis of Lyme disease [15]. These 2 patients complained of headache but did not have the typical skin lesions (erythema migrans). The third patient (case 25) had only IgG antibody to *B. garinii*.

Discussion

The present findings indicate that VZV is one of the major etiologic agents of clinically diagnosed Bell’s palsy. We applied the sensitive PCR method and serological assays to analyze paired serum samples and found that VZV reactivation with the absence of zoster (ZSH) occurred in 29% of patients clin-
of saliva samples, even in those obtained within 5 days after the onset of palsy (data not shown). Of the 23 HSV-seronegative patients who had VZV reactivation, 6 were diagnosed with Ramsay Hunt syndrome and 17 were clinically diagnosed with Bell’s palsy at the first visit. We were unable to detect VZV DNA by PCR analysis of saliva samples obtained at the first visit from 7 of the 17 patients. Our data suggest that the absence of antibodies to HSV is a reliable marker for the diagnosis of ZSH and Ramsay Hunt syndrome in patients who are clinically diagnosed with Bell’s palsy at the first visit; therefore a rapid immunoassay for the detection of antibodies to HSV might be useful to predict VZV reactivation before zoster lesions appear or before serological assays with use of paired serum samples indicate VZV reactivation in patients for whom PCR analysis is negative. This diagnostic approach may be most appropriate for young patients, who are less likely to have antibodies to HSV.

This study also confirmed that the prevalence of antibodies to HSV among patients with Bell’s palsy is significantly greater than that among patients with VZV reactivation, although HSV type–specific assays have not been performed yet for patients with APFP. Our study showed that the prevalence of antibody to HSV-2 only is very low among both groups of patients with VZV reactivation and Bell’s palsy. These results are consistent with a recent serological analysis in Japan [14]. We found only 2 patients with Bell’s palsy who had only antibody to HSV-2. Because HSV-2 reportedly causes facial palsy [20, 21], we investigated the shedding of HSV-2 into saliva in patients who had antibody to HSV-2; no HSV-2 DNA was detected. In contrast, we found 23 patients with Bell’s palsy who shed HSV-1 into saliva within 5 days after the onset of palsy. Although further studies are needed to analyze the mechanism by which HSV-1 reactivation causes APFP, these findings support the hypothesis that HSV-1 reactivation is involved in the pathogenesis of Bell’s palsy.

In 3 of 26 HSV-seropositive patients, we were not able to detect VZV reactivation. PCR analysis and serological assays were run in duplicate, and no degradation of DNA in the saliva samples was found by α-tubulin primers used as an internal control (data not shown). Because the cause of APFP in HSV-seronegative patients who have no VZV reactivation might be miscellaneous, we sought to determine other infectious causes of APFP in the 3 patients. It is interesting that 2 of the 3 patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (y), sex</th>
<th>Skin lesion</th>
<th>Associated symptom(s)</th>
<th>IgM antibody</th>
<th>IgG antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>27, M</td>
<td>None</td>
<td>None</td>
<td>Negative</td>
<td>Weakly positive&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>113</td>
<td>23, F</td>
<td>None</td>
<td>Headache, fatigue</td>
<td>Positive&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Positive&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>128</td>
<td>28, F</td>
<td>None</td>
<td>Headache, fatigue</td>
<td>Positive&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Positive&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Positive only for Borrelia garinii strain HP1.
<sup>b</sup> Positive for both B. garinii strain HP1 and Borrelia afzelii strain P/Gau.
had IgM and IgG antibodies to *Borrelia*, which suggests an association between APFP and Lyme disease. In some patients with Lyme borreliosis, APFP has been described as the only manifestation of the disease [22]. Because Hokkaido Prefecture, where all our patients live, is one of the areas in Japan where Lyme disease is highly endemic [23], we plan to analyze more patients with Bell’s palsy for antibodies to *Borrelia*. In the third HSV-seronegative patient (case 25), we must consider other causes of APFP. Further analysis of such cases may help us elucidate the etiology of Bell’s palsy.

In conclusion, our results provide strong evidence that VZV is 1 of the major etiologic agents of clinically diagnosed Bell’s palsy. Our data also indicate that the incidence of VZV reactivation among patients who lack antibodies to HSV is high. These findings should be considered especially for younger (aged <24 years) patients with APFP among whom the prevalence of antibodies to HSV has been decreasing. Conversely, our results demonstrate that the prevalence of antibodies to HSV among patients with Bell’s palsy is significantly higher than that among patients with VZV reactivation, supporting the hypothesis that HSV reactivation is involved in the pathogenesis of Bell’s palsy.

Acknowledgments

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References