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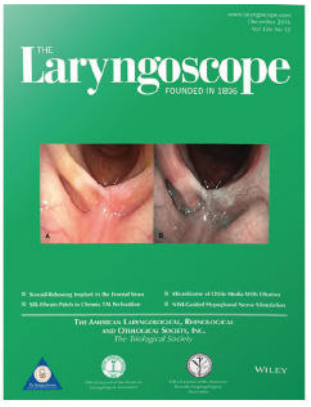

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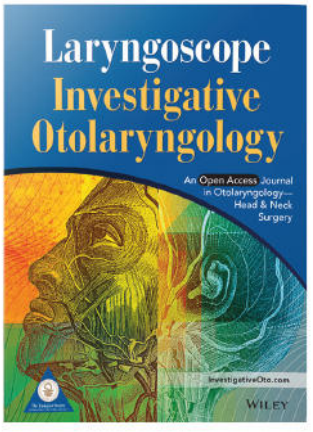

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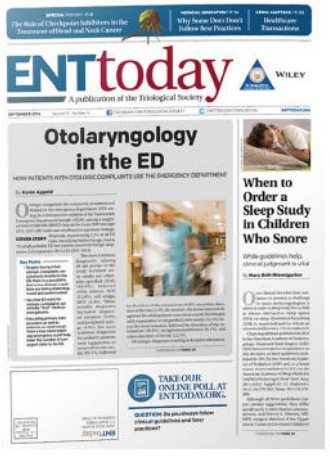

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Western Blot Analysis for Diagnosis of Lyme Disease in Acute Facial Palsy

Yasushi Furuta, MD; Hiroki Kawabata, PhD; Fumio Ohtani, MD; Haruo Watanabe, MD

Background: **Lyme borreliosis has been implicated in the pathogenesis of acute peripheral facial palsy (APFP). Few studies, however, have used Western blot analyses to confirm the serological diagnosis.** ***Purpose:*** **To analyze the prevalence of anti-*Borrelia* antibodies in patients with APFP compared with healthy control subjects living in Hokkaido Island, Japan.** ***Patients and Methods:*** **In total, 113 patients with APFP were analyzed. They included 32 patients with varicella zoster virus (VZV) reactivation (Ramsay Hunt syndrome and zoster sine herpete) and 81 patients with Bell's palsy. Fifty-eight healthy control subjects were also included. IgM and IgG antibodies to *Borrelia garinii* and *afzelii* were tested by Western blot, and diagnoses were made according to the Centers for Disease Control and Prevention criteria.** ***Results:*** **Five of 81 (6.2%) patients with Bell's palsy, 1 of 32 (3.1%) patients with VZV reactivation, and 1 of 58 control subjects (1.7%) were judged to have both IgM and IgG antibodies to *Borrelia*. This difference was not significant ($P > .05$, χ^2 test). Patients with Bell's palsy who had herpes simplex virus type 1 (HSV-1) reactivation at the onset of palsy had a higher IgM-immunoreactivity to *Borrelia afzelii*.** ***Conclusions:*** **Although it is one of the endemic areas of Lyme disease in Japan, the prevalence of APFP caused by Lyme borreliosis is low in Hokkaido Island. In addition, cross-reactivity to *B. afzelii* in IgM blots is often observed in patients with HSV-1 reactivation, suggesting that careful interpretation of *Borrelia* IgM immunoblot data are needed for accurate serological diagnosis.** ***Key Words:*** **Acute peripheral facial palsy, Bell's palsy, Lyme disease, anti-*Borrelia* antibody, CDC criteria.**

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INTRODUCTION

Several causes of acute peripheral facial palsy (APFP) have been identified, such as otitis media, temporal bone fracture, and some viral infections. In most cases, however, the cause remains obscure, and often a diagnosis of idiopathic peripheral facial palsy (Bell's palsy) is made. Reactivation of varicella zoster virus (VZV) is a known cause of Ramsay Hunt syndrome. Our previous study has shown that VZV reactivation without zoster (zoster sine herpete) is one of the major causes of clinically diagnosed Bell's palsy.[1] Herpes simplex virus type 1 (HSV-1) is also suspected of causing APFP.[2,3] Although reactivation of these herpes viruses is the major cause of clinically diagnosed Bell's palsy, other pathogenic factors may be involved in the development of Bell's palsy.

Lyme borreliosis is caused by infection with a spirochete, *Borrelia burgdorferi*, which is transmitted from rodents to humans by ticks. The disease shows a multisystem disorder involving the skin (erythema chronicum migrans), joints (arthritis), the central and peripheral nervous systems (meningoencephalitis and cranial neuritis), and the heart (cardiac arrhythmias). Unilateral or bilateral peripheral facial palsy is the most common symptom of neurologic Lyme disease (neuroborreliosis), especially in childhood.[4] In some patients, APFP has been the only manifestation of the disease.[5] Several endemic areas of Lyme disease have been identified in the world, and the number of patients has steadily increased in North America and Northern Europe. In Japan, Hokkaido Island and Nagano Prefecture are the two major endemic areas.[6]

The diagnosis of Lyme disease is generally made from clinical symptoms in combination with laboratory tests, such as culture isolations, antibody assays using serum samples or cerebrospinal fluid (CSF), and polymerase chain reaction (PCR). In patients whose only manifestation of the disease is APFP, analysis of anti-*Borrelia* antibodies in sera or CSF is the only reliable method of diagnosis. Previous investigations, by using enzyme-linked immunosorbent assay (ELISA) of serum samples, reported that the prevalence of anti-*Borrelia* antibodies in Japanese patients with Bell's palsy was lower than that in United States and Europe (0% vs 16%–22%).[7–10] Recently, the Centers for Disease Control and Prevention (CDC) criteria for the serological diagnosis of Lyme disease have been advocated in the United States to improve the specificity of serological diagnosis.[11] They recommend the use of a two-test protocol, consisting of ELISA for screenings

From the Department of Otolaryngology (Y.F., F.O.), Hokkaido University School of Medicine, Sapporo, Japan, and the Department of Bacteriology (H.K., H.W.), National Institute of Infectious Diseases, Tokyo, Japan.

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Send Correspondence to Yasushi Furuta, MD, Department of Otolaryngology, Hokkaido University School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan. E-mail: yfuruta@med.hokudai ac.jp

followed by Western blot for confirmation. Only one study out of the United States involved an epidemiological survey of patients with APFP using the CDC diagnostic criteria.[12] In addition, no study to date has evaluated the prevalence of anti-*Borrelia* antibodies in patients with APFP with respect to their virological backgrounds.

In the present study, we analyzed anti-*Borrelia* antibodies in patients with Bell's palsy, VZV reactivation (Ramsay Hunt syndrome and zoster sine herpete), and healthy control subjects by using Western blot, and sought to determine the prevalence of seropositive patients with APFP who live in Hokkaido Island, Japan.

PATIENTS AND METHODS

Patients and Control Subjects

In total, 113 patients over 15 years of age with unilateral APFP were analyzed in this study. These patients visited our clinic within 7 days of onset. After careful clinical examination and after the virological tests described previously were performed,[1] Ramsay Hunt syndrome was diagnosed in 12 patients and zoster sine herpete was diagnosed in 20 patients. Therefore, these 32 patients had APFP associated with VZV reactivation. The remaining 81 patients were diagnosed with Bell's palsy. By PCR tests of saliva samples,[3] HSV-1 reactivation was detected in 23 of the 81 patients within 5 days of the onset of palsy. The remaining 58 patients did not have VZV or HSV-1 reactivation at the onset of the disease. Sera obtained during the acute phase of the disease (less than 3 weeks after onset) were analyzed by Western blot.

Fifty-eight healthy control subjects were included in this study. The control sera were collected between September and November 1999. The age of the control group was matched to that of the Bell's palsy group. Informed consent was obtained from the patients and control subjects.

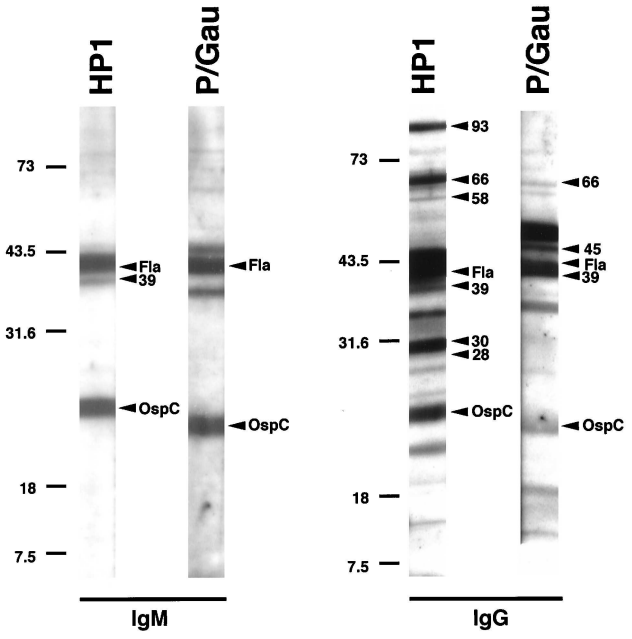

Fig. 1. Representative Western blot pattern of a patient (no. 128) who was determined to be positive both for IgM and IgG against *Borrelia garinii* strain HP1 (HP1) and *Borrelia afzelii* strain P/Gau (P/Gau). Molecular weight markers (73-7.5) are shown on the left in kilodaltons. The bands indicated by arrowheads were determined to be positive.

Bacterial Strains

For the antigens, we used *Borrelia garinii* strain HP1, which was isolated from *Ixodes persulcatus* in Hokkaido, and *Borrelia afzelii* strain P/Gau, which was isolated from human CSF in Germany. Although *Borrelia burgdorferi sensu stricto* is also associated with Lyme disease, it has not been detected in Japan.[6] Therefore, we excluded *Borrelia burgdorferi sensu stricto* as an antigen. These isolates were cultured in BSK II medium at 37°C and were harvested by centrifugation at 4°C. After washing three times with phosphate-buffered saline containing 5 mmol/L $MgCl_2$, the pellet was resuspended in sample buffer (1% sodium dodecyl sulfate, 1% 2-mercaptoethanol, 20 mmol/L Tris, pH 6.8, 10% glycerol), then frozen at -80°C until use.

Western Blot

Antibodies against *Borrelia* proteins were detected by Western blot analysis. Immunoblotting was carried out according to a method described previously.[13] Peroxidase-labeled antihuman IgM and IgG antibodies (ICN Pharmaceuticals Inc.–Cappel Products, Costa Mesa, CA) were used as the second antibodies. According to the CDC criteria, the presence of any two of the three IgM bands (the outer surface protein C [OspC], 39 kD, and 41 kD [Fla]) and the presence of any five of the 10 IgG bands (18, OspC, 28, 30, 39, Fla, 45, 58, 66, and 93 kD) were considered positive. Monoclonal antibodies G7 (provided by Dr. T. Masuzawa), 958, and 960 (Chemicon International Inc., Temecula, CA) were used to identify OspC, 55–100 kD antigens and 72 kD antigen, respectively.

Statistical Analysis

Statistical analysis was performed using StatView Version 4.5 software (Abacus Concepts, Inc., Berkeley, CA). The χ^2 test was used to calculate the significance of the correlations among the variables. A P value of less than .05 was considered significant.

RESULTS

Typical Western blot patterns are shown in Figure 1. In total, 5 of 81 (6.2%) patients with Bell's palsy were determined to have both IgM and IgG antibodies to *B. garinii* or *afzelii* by Western blot, suggesting that they had a recent *Borrelia* infection (Table I). Two of the five patients had immunoreactivity to both *B. garinii* and *afzelii*; two were positive to *B. garinii* only, and one was positive to *B. afzelii* only. When patients with Bell's palsy were divided into two groups, with or without HSV-1 reactivation, no difference in prevalence of immunoreactivity to *Borrelia* was observed between the two groups. In addition, we found one patient (3.1%) with VZV reactivation who had both IgM and IgG immunoreactivity to *B. garinii* and one healthy control subject (1.7%) who had positive immunoreactivity to *B. afzelii*. The difference in the prevalence of both IgM and IgG seropositivity among the Bell's palsy, VZV reactivation, and control groups was not significant ($P > .05$, χ^2 test).

Three patients with IgG seroreactivity only, which suggests a prior infection, were observed in the Bell's palsy group (Table I). In contrast, the prevalence of patients and control subjects with IgM reactivity only (9.4%–18.8%) was higher than that with IgG reactivity only and than that with both IgM and IgG reactivity. Specifically,

TABLE I.
Results of Western Blot Analysis.

Patients and Control Subjects	Positive for *Borrelia garinii*			Positive for *Borrelia afzelii*			Total IgM+IgG*
	IgM	IgG	IgM+IgG	IgM	IgG	IgM+IgG	
Bell's palsy (n = 81)	11 (13.6%)	3 (3.7)	4 (4.9)	15 (18.5)	0 (0)	3 (3.7)	5 (6.2)
With HSV-1 reactivation (n = 23)	6 (26.1)	0 (0)	1 (4.3)	9 (39.1)	0 (0)	1 (4.3)	2 (8.7)
No HSV-1 reactivation (n = 58)	5 (8.6)	3 (5.2)	3 (5.2)	6 (10.3)	0 (0)	2 (3.4)	3 (5.2)
VZV reactivation (n = 32)	3 (9.4)	0 (0)	1 (3.1)	6 (18.8)	0 (0)	0 (0)	1 (3.1)
Control subjects (n = 58)	9 (15.5)	0 (0)	0 (0)	9 (15.5)	0 (0)	1 (1.7)	1 (1.7)

*Positive for both IgM and IgG for either *Borrelia garinii* or *afzelii*.

patients with HSV-1 reactivation had a higher IgM immunoreactivity to *B. afzelii* (39.1%, $P = .0215$, χ^2 test) and *B. garinii* (26.1%, $P > .05$). None of these patients with IgM reactivity had erythema chronicum migrans, and they could not recall a history of a tick bite. To confirm the presence of IgG seroconversion in patients with IgM reactivity only, paired sera or sequentially drawn sera obtained 3 to 20 weeks after the onset of palsy were analyzed in 8 of 20 patients. IgG seroconversion was not observed in any of the patients.

The clinical characteristics of the six patients with APFP who were positive for both IgM and IgG antibodies are shown in Table II. All six patients did not have erythema chronicum migrans and could not recall a history of tick bite. Headache and general fatigue, which are common symptoms of Lyme borreliosis, were observed in three patients. One patient with HSV-1 reactivation had herpes labialis at the onset of palsy. The month of presentation among the six patients was distributed throughout the year. Five of the six patients had complete facial paralysis (grade VI or V by the House and Brackmann grading scale[14]). Prednisone was administered to five patients, excluding one patient who was breast-feeding (case no. 118). Although antibiotic treatment was not performed, all patients had completely recovered facial movement 1 to 3 months after the onset of palsy.

DISCUSSION

Hokkaido Island is one of the endemic areas of Lyme borreliosis in Japan,[6] and two cases of APFP with erythema chronicum migrans have been reported in this area.[15,16] In the present study, 5 of 81 (6.2%) patients with Bell's palsy had both IgM and IgG antibodies to *Borrelia* and 3 of the 5 patients exhibited headache or general fatigue, suggesting an association between facial palsy and Lyme borreliosis. The prevalence of anti-*Borrelia* antibodies, however, was not significantly higher than that in patients with VZV-related APFP and than that in healthy control subjects. Simultaneous bilateral facial palsy is a unique characteristic of APFP caused by Lyme borreliosis.[17] We have examined approximately 300 patients with APFP in the last 5 years without encountering such a case. In addition, because APFP is the most common symptom of neuroborreliosis in childhood,[4] we also evaluated six children with APFP, but none had anti-*Borrelia* antibodies as determined by Western blot (data not shown).

Different genospecies of *B. burgdorferi* have been associated with different pathogenic potentials.[18] Although most Japanese isolates are *B. garinii*, Japanese and European *B. garinii* isolates are antigenically and genetically different.[19,20] The main clinical manifesta-

TABLE II.
The Clinical Features of Patients With Anti-Borrelia IgM and IgG Antibodies.

Case No.	Age (y), Gender	Other Symptom(s)	Month of Onset	Grade of Palsy*	Recovery of Palsy	Virological Tests	No. of Positive Bands by Western Blot			
							Borrelia garinii		*Borrelia afzelii*	
							IgM	IgG	IgM	IgG
113	23, F	Headache	February	IV	Complete 1 mo	None	3+	9+	2+	5+
118	29, F	None	March	V	Complete 1 mo	None	3+	5+	3+	–
128	28, F	Headache, general fatigue	June	V	Complete 2 mo	None	3+	8+	2+	5+
68	25, M	Herpes labialis	January	V	Complete 1 mo	HSV-1	2+	5+	–	–
199	56, F	General fatigue	December	V	Complete 3 mo	HSV-1	–	–	2+	5+
119	70, M	None	February	VI	Complete 2 mo	VZV	3+	5+	3+	–

*House and Brackmann grading scale.

tions of Japanese patients are reportedly mild, with nearly all patients showing erythema chronicum migrans only.[6] Taken together with these prior observations, our results indicate that the prevalence of Lyme borreliosis in patients with Bell's palsy is lower in Hokkaido Island than it is in the United States and Europe.

Several difficulties arise in the serological diagnosis of Lyme borreliosis in patients with APFP: 1) The criteria for a positive diagnosis have differed among several reports; 2) ELISAs have produced high false-positive rates; and 3) the presence of seropositive control subjects in the endemic area suggests that some incidental seropositivity may exist in the patient groups. Therefore, in cases of APFP that show facial palsy as the only clinical manifestation, the relationship between facial palsy and Lyme borreliosis remains speculative by serological testing alone. Interestingly, some studies in Europe reported that all patients with Lyme facial palsy had additional clinical manifestations not present in patients with Bell's palsy.[21,22] Furthermore, both serum and CSF analyses are advocated to verify the diagnosis of Lyme borreliosis in patients with Bell's palsy.[22]

IgM antibody appears early in Lyme disease and usually persists for a long time.[4] In contrast, IgG antibody is often negative in the acute phase of the disease. As such, IgM immunoblots are reportedly useful for the early diagnosis of *Borrelia* infection.[12] In the present study, a high prevalence of positive IgM immunoblots was observed not only in patients with Bell's palsy, but also in patients with VZV reactivation and in healthy control subjects. Furthermore, IgG seroconversion was not observed in any of the patients examined. In a study by Jain et al.,[12] one patient who had positive IgM immunoblots in the acute phase of APFP lacked IgG seroconversion. The presumably false-positive results by IgM immunoblots may be caused by the presence of antibodies that cross-react with the OspC protein of *B. burgdorferi*.[23] In addition, our data indicate that patients with HSV-1 reactivation had significantly higher IgM immunoreactivity to *B. afzelii*. Sera from patients with Epstein-Barr virus and cytomegalovirus infection have also shown false-positive results by IgM immunoblots as a result of cross-reactivity with OspC.[24] Furthermore, a case of VZV meningoencephalitis with facial palsy was reported in which the IgM immunoblot for *B. burgdorferi* was positive with the lack of a seroconversion demonstrated in the IgG blot.[25] These findings suggest that herpes viruses have homologous epitopes to *B. burgdorferi*. Because HSV-1 or VZV reactivation is the major cause of APFP, positive IgM immunoblots for *Borrelia* antibodies should be carefully evaluated.

In conclusion, this study suggests that, although it is one of the major endemic areas of Lyme disease in Japan, the prevalence of APFP caused by Lyme borreliosis is low in Hokkaido Island. In addition, cross-reactivity to *B. afzelii* in IgM immunoblots is observed in a high percentage of patients with HSV-1 reactivation, suggesting that careful interpretation of *Borrelia* IgM immunoblots is required for an accurate diagnosis, especially in patients with Bell's palsy.

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