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CLINICAL REPORT

Detection of Herpes Virus Genomes in Skin Lesions from Patients with Behçet's Disease and Other Related Inflammatory Diseases

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Although the aetiology of Behçet's disease is still poorly understood, viral infection has long been postulated as one of the factors. To investigate the relationship between herpes viruses and Behçet's disease, we used polymerase chain reaction to detect herpes simplex virus 1 (HSV-1) and 2 (HSV-2), and human herpes virus 6 (HHV-6) and 7 (HHV-7) DNA in samples of lesional tissues from patients with Behçet's disease and other related inflammatory disorders. Four cases were positive for HSV-1; 1 of 11 Behçet's disease cases, 2 of 3 Sweet's disease cases and 1 of 3 erythema nodosum cases. Two cases were positive for both HSV-1 and HSV-2; one Behçet's disease and one erythema nodosum. All cases were negative for HHV-6 and HHV-7. These findings indicate that there might be some relationship between Behçet's disease and the presence of HSV-1 and/or HSV-2 DNA and that HHV-6 and HHV-7 do not seem to be involved in the pathogenesis of Behçet's disease. However, HSV-1 and HSV-2 DNAs were also detected in non-Behçet's disease lesions, suggesting that HSV-1/2 is not correlated to the direct pathogenesis of Behçet's disease. ***Key words: Behcet's disease; herpes virus DNA; polymerase chain reaction.***

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Behcet's disease (BD) is a multi-system inflammatory disease characterized by oral and genital ulcerations and various cutaneous, arthritic, ocular, vascular and neurological manifestations (1, 2). There have been many studies about the relationship between microbial infections and the occurrence of BD. Although attention has focused on streptococci (3, 4), viral infection is thought to be another aetiologic factor (5–9). A number of different viruses have been suspected of being implicated in BD, and particular attention has been paid to evaluating the role of herpes viruses such as herpes simplex virus (HSV) (10–12), varicella zoster virus (13–15), human cytomegalovirus (15, 16) and Epstein-Barr virus (17). In view of their characteristics (18), human herpes viruses 6 (HHV-6) and 7 (HHV-7) are two agents that potentially could play an aetiological role in cutaneous lesions in BD. HHV-6 and HHV-7 cause exanthema subitum and may invade the central nervous system (CNS) or be involved in CNS manifestations (19). The aim of this study was to screen BD patients for the presence of HSV-1, HSV-2, HHV-6 and HHV-7 genomes in their skin lesions, and to compare the results with those from other related inflammatory disorders including erythema nodosum (EN) and Sweet's disease. A variety of methods can be employed to demonstrate a viral aetiology of BD. The most direct methods include visualization of the virus particles in the tissue and in situ hybridization or polymerase chain reaction (PCR) to detect viral genomes in the tissue. We used PCR to detect herpes virus DNA in skin samples from BD patients, including those with oral aphtha, genital ulcer, folliculitis, EN-like symptoms, and compared the results with those obtained from other related inflammatory diseases.

PATIENTS AND METHODS

Patients

We examined 11 patients (3 men and 8 women) who were diagnosed with BD based on the international diagnostic criteria (20) and 7 patients (1 man and 6 women) with other related inflammatory disorders, including 3 with Sweet's disease, 3 with EN, and one with phlegmone. The three types of BD are defined as follows according to the revised Japanese criteria (21): 1) complete type: a patient who has four major symptoms ($n=2$); 2) incomplete type: a patient who has three major symptoms or who has another major symptom other than ocular symptoms or two minor features ($n=8$); 3) suspected type: a patient who has two major symptoms ($n=1$). The patients with other related inflammatory diseases had no history of recurrent herpetic infection or of BD. The mean age in the BD group was 35 years (range 23–50) and 38 years (range 23–70) in the other group. The mean duration of disease was 5.5 years (1 month to 24 years) in the BD group and 4.8 weeks (1 week to 2.5 months) in the other related inflammatory disease group. The clinical features and complications for each patient during the course of their disease and at the time of the biopsy are given in Table I.

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Table I. *Sample number and clinical details of patients with Behçet's disease (BD) and other inflammatory disorders*

No./sex/age	Diagnosis	Location	Duration and symptoms (complications)	Treatment#	HSV-1	HSV-2
1/F/30y	BD	Lower leg	2y: EN-like symptom	Colchicine	–	–
2/M/29y*	BD	Scrotum	5y: Genital ulcer	NSAIDs	–	–
3/M/29y*	BD	Scrotum	5y: Genital ulcer		–	–
4/F/47y*	BD	Lower leg	1mo: EN-like symptom	Amoxycillin	Yes	Yes
5/F/47y*	BD	Oral mucosa	1mo: Oral aphtha		–	–
6/F/29y	BD	Arm	7y: EN-like symptom	None	–	–
7/M/49y	BD	Scrotum	4y: Genital ulcer	None	–	–
8/M/50y*	BD	Trunk	3y: Folliculitis	None	–	–
9/M/50y*	BD	Finger	3y: Folliculitis		–	–
10/M/50y*	BD	Finger	3y: Folliculitis		–	–
11/F/23y	BD	Lower leg	2.5y: EN-like symptom	NSAIDs MINO	–	–
12/F/28y	BD	Upper leg	1.5y: EN-like symptom	None	–	–
13/F/25y	BD	Lip	7y: Oral aphtha	NSAIDs	–	–
14/F/39y	BD	Lower leg	4y: EN-like symptom	NSAIDs	–	–
15/F/49y	BD	Arm	24y: EN-like symptom	NSAIDs MINO	–	–
16/F/44y	SD	Lower leg	2mo: (Myelodysplastic syndrome)	Prednisolone	Yes	–
17/M/56y	SD	Chest	3w:	None	–	–
18/F/41y	SD	Lower leg	2.5mo: (Hyperthyroidism)	None	Yes	–
19/F/48y	EN	Lower leg	2w:	None	–	–
20/F/23y	EN	Lower leg	1w:	None	–	–
21/F/70y	EN	Lower leg	1mo: (Drug-induced)	None	Yes	Yes
22/M/21y	CD	Arm	1mo: Erythema	None	–	–
23/M/26y	AD	Trunk	3mo: Prurigo	Topical steroid	–	–
24/F/23y	CD	Trunk	1w: Vesicle	None	–	–
25/M/77y	BP	Lower leg	1w: Bulla	None	–	–
26/M/88y	BP	Foot	3mo: Bulla	None	–	–
27/F/69y	BP	Arm	2w: Bulla	None	–	–
28/F/49y	BP	Trunk	2mo: Bulla	None	–	–
29/F/23y	Phlegmone	Lower leg	2w: Abscess	Amoxycillin	–	–
30/M/53y	KVE	Chest	3day: (Erythroderma)	Topical steroid Methotrexate	Yes	Yes

*Patients with several lesions.
#Medications when the skin biopsy was performed.
NSAIDs = non-steroidal anti-inflammatory drugs; MINO = minocycline hydrochloride; SD = Sweet's disease; EN = erythema nodosum; AD = atopic dermatitis; CD = contact dermatitis; BP = bullous pemphigoid; KVE = Kaposi varicelliform eruption.

Preparation of DNA samples

Samples of skin lesions were obtained from patients with BD, some of whom had intraoral ulcers ($n = 2$), genital ulcers ($n = 3$), folliculitis ($n = 3$) and EN-like eruptions ($n = 7$), and from patients with other inflammatory diseases, some of whom had EN ($n = 3$), Sweet's disease ($n = 3$), phlegmone ($n = 1$), bullous pemphigoid ($n = 4$) and contact dermatitis, including atopic dermatitis ($n = 3$) (Table I). The skin lesion used as a positive control was from a patient with Kaposi's varicelliform eruption. DNA was extracted from each skin sample as described previously (22). Every DNA sample was extracted at least twice, and distilled water was extracted alongside the specimen as an extraction control.

Polymerase chain reaction

Nested PCR was carried out for amplification of HSV-1, HSV-2, HHV-6 and HHV-7 DNA using two primer sets, as described previously in detail (23, 24). When the nested double PCR was performed, approximately 10 copies of each viral DNA were detectable, as described previously (24). The specificity of each PCR product was confirmed by solid phase hybridization with specific probes for each viral DNA (23, 24). The amplification condition was 3 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C, and a final extension for 5 min at 72°C. All DNA samples were confirmed to be amplifiable using PCR primers specific for a conserved region of the human β-globin gene. Every sample was tested at least three times for each virus. Negative controls, including distilled water, replaced the DNA sample and an extraction control were added to a PCR mix. After amplification, the PCR products were analysed by 2% agarose gel electrophoresis. Each band of HSV-1 and HSV-2 was analysed by DNA sequencing to confirm that the correct PCR products were obtained, as previously described (25).

RESULTS

The results are summarized in Table I. Three of the patients with BD had several lesions at the same time. One of 15 BD samples (no. 4) and 1 of 3 EN samples (no. 21) tested positive for both HSV-1 and HSV-2. The possible BD sample was from a patient with incomplete type of BD and EN-like skin symptom; his oral apthae sample (no. 5) was, however, negative for both HSV-1 and HSV-2. Two of 3 samples from Sweet's disease tested positive for only HSV-1 (no. 16 and 18). The positive control, a Kaposi's varicelliform eruption

sample (no. 30), was strongly positive for HSV-1 and weakly positive for HSV-2. The negative controls (bullous pemphigoid and contact dermatitis samples were all negative for HSV-1 and 2.

The PCR-positive patient with BD (no. 4) was shown by the neutralizing test method to have antibodies to HSC-1 and HSV-2 (data not shown).

No samples were positive for HHV-6 or HHV-7 on PCR, whereas hypersensitivity syndrome-positive controls were appropriately positive (data not shown).

DISCUSSION

A viral aetiology of BD has long been postulated. HSV-1 and HSV-2 RNA have been found in peripheral blood mononuclear cells from some patients with BD by in situ hybridization (10). The HSV-1 genome has been found by PCR in peripheral blood mononuclear cells and saliva from some patients with BD but not in biopsies of oral aphthae from BD patients (11, 12). To the best of our knowledge, the HSV-1 or HSV-2 genome has not been shown previously in the skin lesions of patients with BD. We first demonstrated by PCR the presence of HSV-1 and HSV-2 genomes in the skin sample of an EN-like symptom in a patient with BD (no. 4) but not in the patient's oral aphthae (no. 5). This suggests that there might be some connection between BD and the presence of HSV-1 and/or HSV-2 DNA; however, HSV may not be a direct cause of BD. It is most likely implicated through an effect on T-cell immunoregulation (28), and may be associated with the development of oral aphthae in BD.

Since an association has been found between intra-oral ulcers and HSV among immuno-compromised patients with hematologic malignancies (26), HSV-1 DNA might have been positive in the skin lesion of one patient with Sweet's syndrome based on an association with myelodysplastic syndrome (no. 16). However, some of the other patients with EN (no. 21) and Sweet's syndrome (no. 18) who had no hematological disorders were positive for the HSV-1 genome by PCR in their skin lesions. We also found the simultaneous presence of the HSV-2 genome in a patient with EN who had been taking an oral H_2-blocker (famotidine) against acute gastritis for 2 weeks before the lesion appeared (no. 21, Table I). It has been suggested previously that the spectrum of associated conditions in EN, including BD, is similar to that in Sweet's syndrome (27). Our findings also suggest a close relationship among these diseases with respect to the spectrum of reactive inflammatory diseases and also in the presence of HSV-1 and HSV-2 genomes.

In this study we found no cases positive for HHV-6 or HHV-7 DNA in specimens of skin lesions from patients with BD and other inflammatory disorders, which suggests that HHV-6 and HHV-7 are probably not involved in the pathogenesis of BD. However, since HHV-6 and HHV-7 are tropic to neurological tissues (18, 19), further studies are required using the skin lesions and/or cerebrospinal fluid from patients with BD associated with neurological manifestations.

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