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# Serologic and Genotypic Analysis of a Series of Herpes Simplex Virus Type 1 Isolates From Two Patients With Genital Herpes

**Kenichi Umene,[1]* Takashi Kawana,[2] and Yasuyuki Fukumaki[3]**

[1]*Faculty of Human Environmental Science, Department of Nutrition & Health Science, Fukuoka Woman's University, Fukuoka, Japan*
[2]*Department of Obstetrics and Gynecology, Mizonokuchi Hospital, Teikyo University, Kawasaki, Japan*
[3]*Division of Human Molecular Genetics, Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan*

Herpes simplex virus type 1 (HSV-1) has been reported increasingly as a cause of genital herpes, although HSV-1 is usually associated with oro-labial herpes. In the present study, serum specimens and materials for viral isolation were obtained serially from two patients with recrudescent HSV-1 genital infections to study serology and molecular epidemiology. Recurrent episodes, during which HSV-1 was isolated, were followed by an increase in the level of anti-HSV-1 antibody, suggesting a booster effect from re-exposure to viral antigens and the possible usefulness of the variation in the level of anti-HSV-1 antibody to diagnose recurrence. While genotypes of HSV-1 isolates obtained from one patient were different from those from the other patient, genotypes of sequential HSV-1 isolates obtained from the same patient were the same, implying that the recrudescent genital lesions of the two patients could be attributed to endogenous recurrence of a latent virus. Sera from one patient neutralized HSV-1 isolates obtained from the other patient as well as HSV-1 isolates obtained from the same patient. An HSV-1 isolate obtained during a later episode in one patient was neutralized by sera taken before/during the later episode of the same patient, as effectively as an HSV-1 isolate obtained during an earlier episode in the same patient; thus, in these two cases, HSV-1 was assumed to have multiplied during recurrence despite the presence of an anti-HSV-1 antibody that could neutralize experimentally HSV-1. ***J. Med. Virol. 81:1605–1612, 2009.*** © 2009 Wiley-Liss, Inc.

**KEY WORDS:** recurrence; antibody; RFLP; hypervariable region; molecular epidemiology

## INTRODUCTION

Herpes simplex virus (HSV) is a ubiquitous human pathogen that is classified into two serotypes, HSV-1 and HSV-2: HSV-1 is the usual cause of oro-labial herpes, while HSV-2 is usually acquired as a genital infection. Typical HSV infection proceeds through three stages of primary infection, latency, and recurrence; hence, HSV has the ability to reactivate periodically, resulting in a productive infectious virus. Clinical and sub-clinical reactivation of HSV with resultant viral shedding is related with the transmission of HSV; thus, anti-viral therapy is expected to reduce the frequency and degree of viral shedding and to lower the transmission rate [Sacks et al., 2004]. Genital herpes, a disease marked by recurrent ulcerative lesions, is one of the most prevalent sexually transmitted diseases [Geretti, 2006; Gupta et al., 2007]. HSV-2 is the most common cause, but recent reports suggest that an increasing percentage of genital herpes is caused by HSV-1 [Kawana et al., 1982; Sucato et al., 1998; Haddow et al., 2006]. HSV-1 genital infection is less likely to recur than that caused by HSV-2 [Reeves et al., 1981; Lafferty et al., 1987].

The two HSV strains are differentiated usually by analyzing DNA when they are unrelated epidemiologically; hence, transmission of a strain can be traced [Buchman et al., 1978, 1979; Chaney et al.,

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*Correspondence to: Kenichi Umene, Faculty of Human Environmental Science, Department of Nutrition & Health Science, Fukuoka Woman's University, Fukuoka 813-8529, Japan. E-mail: umene@fwu.ac.jp

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1983; Sakaoka et al., 1995; Umene, 1998a,b]. Differences in DNA detected between HSV strains using restriction endonuclease (RE) are divided into two types: restriction fragment length polymorphism (RFLP) and "common-type variation" [Umene et al., 1984; Umene, 1998a,b]. RFLP, which is due mostly to the gain or loss of an RE cleavage site, is stable and serves as a physical marker of the HSV genome in genetic and epidemiological studies [Buchman et al., 1978; Chaney et al., 1983; Sakaoka et al., 1994; Umene and Kawana, 2000]. The other type variation ("common-type variation") is located in fragments containing tandemly repeated sequences and is also called a hypervariable region [Umene and Yoshida, 1989; Maertzdorf et al., 1999]. Reiterated sequences in "common-type variation" have a tendency to be more variable than other sequences, and this property of reiteration makes way for a beneficial marker when attempting to differentiate HSV-1 strains [Umene and Yoshida, 1989; Umene, 1998a,b; Maertzdorf et al., 1999]. The use of a "common-type variation" as a marker should be avoided if the copy number of reiterations changes so rapidly that it would not be feasible to trace the strain back to the source. The"common-type variation," reiteration VII within the protein-coding regions of genes US10 and US11, proved sufficiently stable to differentiate HSV-1 strains [Umene and Yoshida, 1989; Maertzdorf et al., 1999; Remeijer et al., 2001, 2002; Umene and Kawana, 2003].

HSV can cause recrudescent lesions and the responsible viruses are postulated to derive from two sources: (i) a virus that remains in the body following primary infection (endogenous recurrence), in which case the genomic profiles of HSV isolates would be the same; (ii) re-infection with exogenous virus (exogenous re-infection), in which case the genomic profiles of HSV isolates would be different [Buchman et al., 1979; Sakaoka et al., 1995; Umene et al., 2007].

Primary infections with HSV are followed by the production of antibodies to the viral antigen: IgM antibodies are produced transiently, while IgG antibodies persist. HSV infections recur in spite of host immune responses to the virus [Whitley and Miller, 2001; Koelle and Corey, 2003; Ramachandran and Kinchington, 2007]. Although the possible role of antibodies against viral antigens in the development of recurrent lesions was explored, differences of opinion remain regarding the relationship between the level of anti-HSV antibody and recurrent HSV infection. The present report describes the serologic status of two patients with recrudescent HSV-1 genital infections and genotypes of a series of HSV-1 isolates obtained from each patient.

## METHODS

### Serologic Studies

Samples were assayed using two enzyme-linked immunosorbent assay (ELISA) kits, the Herpes Simplex IgG detection kit and Herpes Simplex IgM detection kit (Denka Seiken, Tokyo, Japan), which detect IgG and IgM antibodies to HSV, respectively, according to the manufacturer's instructions [Kawana et al., 1995; Hashido et al., 1997; Kumaki et al., 2001]. Antibody index values were calculated by dividing the optical density values for test specimens by the average of the optical density values for the standard pooled human serum containing low-titer IgG and IgM-type antibody to HSV, respectively. Two other ELISA kits, HerpeSelect-1 ELISA and HerpeSelect-2 ELISA (Focus Technologies, Inc., Cypress, CA), which detect IgG antibodies to glycoproteins G of HSV-1 and HSV-2, respectively, were used to distinguish serologically between HSV-1 and HSV-2 [Geretti, 2006]. Index values were calculated by dividing specimen optical density values by the mean of the cut-off calibrator absorbance values. Neutralizing antibodies of a patient were assayed using HSV-1 isolates obtained from the same and the other patient (Tables I and II) [Kawana et al., 1982].

### HSV-1 Isolation and Extraction of HSV-1 DNA

Specimens for herpes simplex viral culture were obtained by swabbing with cotton applicators, and separate swabs were used to sample the cervix, vulva, and anal areas of patients (Tables I and II) [Kawana et al., 1982]. Specimens were inoculated onto cultures of Vero cells, which were examined daily for a cytopathic effect. Working stocks of HSV-1 isolates were made on Vero cells in Eagle's MEM supplemented with 2% fetal bovine serum at a low multiplicity of infection [Umene et al., 1984]. A Vero cell monolayer infected with HSV-1 stock was collected by low-speed centrifugation and viral DNA was extracted by the method of Hirt [Umene and Kawana, 2000].

### Polymerase Chain Reaction (PCR) and Sequencing

PCR to amplify the region encompassing the reiteration VII region was carried out using a pair of primers: 5′-GTGGGTTGGGCTTCCGGTGG-3′ (nucleotide number 12,032–12,051) and 5′-CCAGAGACCCCAGGGTACCG-3′ (12,288–12,307), as described [Umene et al., 2007] [the nucleotide numbering system was a short unique region of HSV-1 strain 17, McGeoch et al., 1985]. The nucleotide sequences of HSV-1 isolates C81–C88, corresponding to the short unique region between 12,073 and 12,276 of strain 17, were submitted to DDBJ/EMBL/GenBank. The accession numbers are AB426482 to AB426489.

## CASE REPORTS

### Case 1

A 40-year-old woman (patient 1), without a previous history of genital herpes infection, presented with an uncomfortable vulvar ulcer with palpable inguinal lymph nodes (1st day in Table I). Serologic tests and attempts at viral isolation were carried out. When she

TABLE I. Patient 1

| | Antibody to HSV | | | | | | HSV-1 isolation | | |
|---|---|---|---|---|---|---|---|---|---|
| | ELISA (index value) | | | | Neutralization | | | | |
| Days | IgG[a] | IgM[b] | Anti-HSV-1[c] | Anti-HSV-2[d] | HSV-1[e] | Titers | Derivation of materials | Viral culture[f] | Isolate no. |
| 1 | 0.4 | 0.33 | 0.06 | 0.02 | C81 | ≤4 | Vulva | + | C81 |
| | | | | | C84 | ≤4 | Cervix | + | C82 |
| | | | | | (C85) | ≤4 | | | |
| | | | | | (C88) | ≤4 | | | |
| 3 | | | | | | | Vulva | + | |
| | | | | | | | Cervix | + | |
| 6 | | | | | | | Vulva | + | |
| 8 | | | | | | | Vulva | + | |
| | | | | | | | Cervix | − | |
| 13 | | | | | | | Vulva | − | |
| | | | | | | | Cervix | − | |
| 31 | 21.9 | 9.78 | 1.61 | 0.07 | C81 | 45 | Vulva | − | |
| | | | | | C84 | 32 | Cervix | − | |
| | | | | | (C85) | 32 | | | |
| | | | | | (C88) | 32 | | | |
| 80 | 11.3 | 4.39 | 1.02 | 0.05 | C81 | 23 | Vulva | − | |
| | | | | | C84 | 32 | Cervix | − | |
| 157 | 8.0 | 4.21 | 0.54 | 0.03 | C81 | 11 | Vulva | − | |
| | | | | | C84 | 11 | Cervix | − | |
| | | | | | (C85) | 16 | | | |
| | | | | | (C88) | 11 | | | |
| 227 | 28.6 | 3.19 | 1.03 | 0.03 | C81 | 64 | Vulva | + | C83 |
| | | | | | C84 | 45 | Cervix | − | |
| | | | | | (C85) | 90 | | | |
| | | | | | (C88) | 90 | | | |
| 230 | | | | | | | Vulva | − | |
| | | | | | | | Cervix | − | |
| 237 | 82.2 | 2.64 | 2.32 | 0.03 | C81 | >128 | Vulva | − | |
| | | | | | C84 | >128 | Cervix | − | |
| 290 | 29.1 | 2.84 | 1.51 | 0.04 | C81 | 90 | Vulva | − | |
| | | | | | C84 | 128 | Cervix | − | |
| 414 | 18.8 | 2.62 | 2 | 0.03 | C81 | 23 | Vulva | + | C84 |
| | | | | | C84 | 23 | Cervix | − | |
| | | | | | (C85) | 23 | | | |
| | | | | | (C88) | 45 | | | |
| 419 | 63.1 | 2.46 | 4.11 | 0.05 | C81 | 45 | Vulva | − | |
| | | | | | C84 | 45 | Cervix | − | |
| | | | | | (C85) | 45 | | | |
| | | | | | (C88) | 80 | | | |

[a]Herpes Simplex IgG detection kit (Denka Seiken) [Kumaki et al., 2001].
[b]Herpes Simplex IgM detection kit (Denka Seiken) [Kumaki et al., 2001].
[c]HerpeSelect-1 ELISA (Focus Technologies, Inc.) [Geretti, 2006].
[d]HerpeSelect-2 ELISA (Focus Technologies, Inc.) [Geretti, 2006].
[e]HSV-1 isolates obtained from patient 2 are indicated in parentheses.
[f]A positive culture result (+) or a negative culture result (−) are indicated for each viral culture attempt.

visited the hospital (1st day), serology for antibodies to HSV was negative and viral culture was positive for HSV-1 isolates (C81 and C82), which were obtained from materials taken on the 1st day from the vulva and cervix (Table I). On the 31st day, the anti-HSV antibody values of both IgG and IgM classes were positive, and the anti-HSV-1 antibody value was positive but anti-HSV-2 was not; hence, the episode on the 1st day was assumed to be the primary HSV-1 infection [Kalimo et al., 1977; Hashido et al., 1997]; thereafter, the levels of antibodies decreased gradually (on the 80th and 157th days). On the 227th day, she complained of recrudescent genital lesions, and HSV-1 isolate C83 was obtained (Table I): the level of anti-HSV-1 antibody increased on the 237th day (10 days later). On the 414th day, she visited the hospital because of recrudescent genital lesions, and HSV-1 isolate C84 was obtained (Table I): the level of anti-HSV-1 antibody increased on the 419th day (5 days later).

### Case 2

An 18-year-old woman (patient 2), with a previous history of genital herpes infection, presented with multiple vulvar vesicles without palpable inguinal lymph nodes (1st day in Table II), and HSV-1 isolates (C85 and C86) were obtained. On the 1st day, IgG and anti-HSV-1 antibody values were positive, albeit low,

TABLE II. Patient 2

| | Antibody to HSV | | | | | | HSV-1 isolation | | |
|---|---|---|---|---|---|---|---|---|---|
| | ELISA (index value) | | | | Neutralization | | | | |
| Days | IgG[a] | IgM[b] | Anti-HSV-1[c] | Anti-HSV-2[d] | HSV-1[e] | Titers | Derivation of materials | Viral culture[f] | Isolate no. |
| 1 | 1.9 | 0.31 | 1.06 | 0.04 | (C81) | ≤4 | Vulva | + | C85 |
| | | | | | (C84) | 4 | Cervix | + | C86 |
| | | | | | C85 | 4 | | | |
| | | | | | C88 | ≤4 | | | |
| 6 | | | | | | | Vulva | − | |
| | | | | | | | Cervix | − | |
| 13 | 85.0 | 0.43 | 6.08 | 0.08 | (C81) | 45 | | | |
| | | | | | (C84) | 45 | | | |
| | | | | | C85 | 64 | | | |
| | | | | | C88 | 45 | | | |
| 20 | | | | | | | Vulva | − | |
| | | | | | | | Cervix | − | |
| 169 | 10.0 | 0.32 | 1.75 | 0.04 | (C81) | 23 | Cervix | + | C87 |
| | | | | | (C84) | 23 | Anal areas | + | C88 |
| | | | | | C85 | 11 | | | |
| | | | | | C88 | 11 | | | |
| 178 | 105.0 | 0.35 | 6.75 | 0.04 | (C81) | >128 | Cervix | − | |
| | | | | | (C84) | 128 | Anal areas | − | |
| | | | | | C85 | >128 | | | |
| | | | | | C88 | >128 | | | |
| 272 | 17.8 | 0.29 | 2.26 | 0.05 | | | Vulva | − | |
| | | | | | | | Cervix | − | |
| 286 | | | | | | | Vulva | − | |
| | | | | | | | Cervix | − | |
| 370 | 10.3 | 0.48 | 1.93 | 0.11 | | | Vulva | − | |
| | | | | | | | Cervix | − | |
| | | | | | | | Anal areas | − | |
| 391 | | | | | | | Vulva | − | |
| | | | | | | | Cervix | − | |

[a]Herpes Simplex IgG detection kit (Denka Seiken) [Kumaki et al., 2001].
[b]Herpes Simplex IgM detection kit (Denka Seiken) [Kumaki et al., 2001].
[c]HerpeSelect-1 ELISA (Focus Technologies, Inc.) [Geretti, 2006].
[d]HerpeSelect-2 ELISA (Focus Technologies, Inc.) [Geretti, 2006].
[e]HSV-1 isolates obtained from patient 1 are indicated in parentheses.
[f]A positive culture result (+) or a negative culture result (−) are indicated for each viral culture attempt.

and no significant increase in the level of IgM was seen on the 13th day (12 days later), although a marked increase in IgG and anti-HSV-1 antibody was shown in comparison with that on the 1st day; hence, the episode on the 1st day was thought to be recrudescent HSV-1 infection. The levels of IgG and anti-HSV-1 antibody on the 169th day were lower than on the 13th day. On the 169th day, she visited the hospital with recrudescent genital lesions, and HSV-1 isolates (C87 and C88) were obtained (Table II): the level of anti-HSV-1 antibody increased on the 178th day (9 days later).

## RESULTS

### Analyses of DNA of Sequential HSV-1 Isolates

Four HSV-1 isolates of C81–C84 were obtained sequentially from patient 1, and the other four isolates of C85–C88 were from patient 2 (Tables I and II). Analyses of the DNA of HSV-1 isolates obtained sequentially from the same individual are useful to determine whether recrudescent lesions are attributable to endogenous recurrence or exogenous re-infection [Buchman et al., 1979; Sakaoka et al., 1995; Umene et al., 2007]. DNA of eight HSV-1 isolates C81–C88 was analyzed with respect to RFLP and reiteration VII.

A set of 20 RFLP markers, which are distributed widely on the HSV-1 genome and used to classify HSV-1 isolates into genotypes, was defined previously (Table III) [Umene and Kawana, 2000]. These RFLP markers can be identified by Southern hybridization analyses of the DNA of HSV-1 isolates digested with each RE of *Bam*HI, *Kpn*I, and *Sal*I [Umene et al., 1984; McGeoch et al., 1988]. Southern hybridization analyses of the 20 RFLP markers were performed, and RFLP profiles were the same between HSV-1 isolates obtained from the same patient. In previous studies, HSV-1 isolates were classified into a number of genotypes based on the state of the 20 RFLP markers, and genotypes were defined. The genotypes of four isolates, C81–C84, from patient 1 were the same as genotype F35 defined previously [Umene and Kawana, 2000; Umene et al., 2007]. Four isolates of C85–C88 separated from patient 2 did not belong to any genotype defined previously, and the genotype of C85–C88 was named F85 in the present study (Table III).

TABLE III. RFLPs Used for Differentiation of HSV-1 Isolates

| | RFLPs[a] | | HSV-1 isolates (genotype) | |
|---|---|---|---|---|
| Name | Definition | *Eco*RI probe | C81–C84 (F35[b]) | C85–C88 (F85[c]) |
| VR11 | Gain of the *Sal*I site between fragments I and C (and F) | J | + | + |
| VR25 | Loss of the *Sal*I site between fragments Z and H' | D | + | – |
| VR24 | Loss of the *Kpn*I site between fragments Z and E | D | – | – |
| VR23 | Gain of the *Kpn*I site on fragment E generating two fragments of 5.5 and 5.7 kbp | D | + | – |
| VR21 | Loss of the *Bam*HI site between fragments A' and A | D | – | – |
| VR22 | Gain of a *Bam*HI site on fragment A generating two fragments of 1.7 and 9.5 kbp | D | – | – |
| VR3 | Loss of the *Kpn*I site between fragments Ma and Mb | F | + | + |
| VR5 | Smaller *Sal*I N fragment of 4.8 kbp instead of 5.0 kbp | F | – | – |
| VR6 | Gain of the *Bam*HI site between fragments W and K' | O | + | + |
| VR7 | Loss of the *Bam*HI site between fragments D and H | A | – | + |
| VR61 | Smaller *Bam*HI O fragment of 3.7 kbp instead of 3.9 kbp | A | – | – |
| VR8 | Loss of the *Sal*I site between fragments K and C' | A | + | – |
| VR64 | Gain of a *Kpn*I site on fragment Aa generating two 5.0 kbp fragments | A | – | – |
| VR9 | Loss of the *Kpn*I site between fragments Aa and Ab | A | – | + |
| VR67 | Larger *Sal*I T fragment of 4.0 kbp instead of 3.7 kbp | A | – | – |
| VR73 | Gain of a *Sal*I site on fragment Q generating two fragments of 3.5 and 0.6 kbp | I | + | – |
| VR10 | Gain of the *Kpn*I site between fragments Ab and Y | I | + | + |
| VR72 | Loss of the *Kpn*I site between fragments T and O | I | + | – |
| VR93 | Gain of a *Kpn*I site on fragment F generating two fragments of 6.9 and 3.5 kbp | H | – | – |
| VR94 | Loss of the *Kpn*I site between fragments F and K | H | – | – |

[a]Twenty RFLPs were defined previously and are arranged in the order on the HSV-1 genome [McGeoch et al., 1988; Umene and Kawana, 2000].
[b]Four isolates of C81–C84 from patient 1 were classified into genotype F35 defined previously [Umene and Kawana, 2000].
[c]Four isolates of C85–C88 from patient 2 did not belong to any genotype defined previously, and the genotype of C85–C88 was named F85 in the present study.

The "common-type variation" of reiteration VII is a beneficial marker for the differentiation of HSV-1 isolates [Umene and Yoshida, 1989; Maertzdorf et al., 1999; Remeijer et al., 2001, 2002; Umene and Kawana, 2003; Roest et al., 2004]. DNA regions encompassing reiteration VII of C81–C88 were amplified by PCR, and nucleotide sequences of PCR-amplified DNA fragments were determined (Fig. 1). Nucleotide sequences of C81–C84 from patient 1 were the same, and those of C85–C88 from patient 2 were also the same. Nucleotide sequences of C81–C84 were different from those of C85–C88 (Fig. 1). The results obtained in this study concerning RFLP and reiteration VII of C81–C88 suggested that the sources of HSV-1 isolates obtained from the same patient were the same; hence, the recrudescent genital lesions of patients 1 and 2 were thought to be attributable to endogenous recurrence, not exogenous re-infection.

### Neutralizing Antibodies to HSV-1 Isolates

HSV-1 isolates were obtained successfully in the present study from patients from whom sera were drawn; thus, the neutralizing antibody in sera could be tested with HSV-1 isolate from the same patient (Tables I and II). Titers of neutralizing antibodies were examined using HSV-1 isolates, C81 and C84, which were obtained from patient 1 on the 1st and 414th days, respectively (Table I), and C85 and C88, obtained from patient 2 on the 1st and 169th days, respectively (Table II).

Neutralizing antibody values in patient 1 appeared to be negative on the 1st day; however, they were positive for HSV-1 isolates from patients 1 (C81, C84) and 2 (C85, C88) on the 31st day (30 days later) (Table I). C84 obtained on the 414th day (the later episode) was neutralized by sera taken between the 31st and 414th days, as well as C81 obtained on the 1st day (the earlier episode); hence, C84 was supposed to have multiplied despite the presence of an antibody that could neutralize experimentally C84.

The level of neutralizing antibodies in patient 2 was low on the 1st day; however, a marked increase was shown for HSV-1 isolates from patients 1 (C81, C84) and 2 (C85, C88) on the 13th day (12 days later) (Table II). C88 obtained on the 169th day (the later episode) was neutralized by serum taken on the 13th day (156 days before the separation of C88), as well as C85 obtained on the 1st day (the earlier episode), suggesting the multiplication of C88 despite the presence of an antibody that could neutralize experimentally C88.

## DISCUSSION

HSV reactivation occurs in the presence of anti-HSV serum antibody and the relationship between HSV recurrence and the level of anti-HSV antibody is controversial. First, a difference of opinion over the level

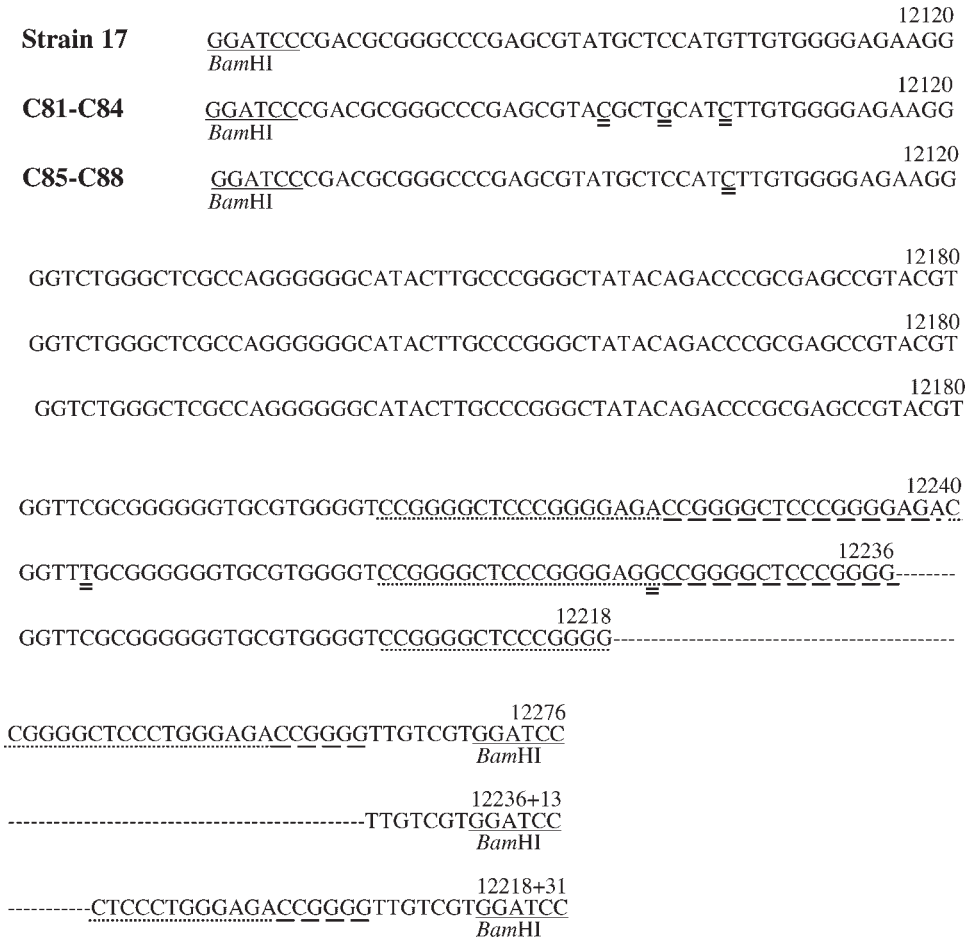


Fig. 1. Nucleotide sequences of a region encompassing reiteration VII of HSV-1. Nucleotide sequences of strain 17 (standard strain), isolates C81–C84 (F35 genotype), and isolates C85–C88 (F85 genotype). The nucleotide numbering system is the short unique region of strain 17 [McGeoch et al., 1985]. Nucleotide sequences recognized by *Bam*HI are single underlined. Nucleotides of HSV-1 isolates C81–C88 different from the corresponding nucleotides of strain 17 are double underlined. Each copy of 18-bp tandem repeats of reiteration VII is underlined by a dotted or broken line.

of anti-HSV antibody before/during recurrence was seen. Antibody titers were lower among those in whom herpes labialis was induced by experimental exposure to ultraviolet radiation compared to those who were exposed but did not develop lesions [Spruance et al., 1995], suggesting an association of a low antibody level with recurrence; however, individual susceptibility to recurrent herpetic facial infections did not correlate with changes in antibody levels in another study [Zweerink and Stanton, 1981]. Vaccination with recombinant HSV-2 glycoprotein had no significant influence on the subsequent frequency of genital herpes reactivation, although high levels of HSV-2-specific neutralizing antibodies were induced [Corey et al., 1999]. In the present study, the level of anti-HSV-1 antibody during a recurrent episode increased in patient 1 (157th to 227th days in Table I) and decreased in patient 2 (13th to 169th day in Table II) with inconsistent variation patterns.

Second, there was disagreement as to the level of anti-HSV antibody after recurrence. No increase in the titer of neutralizing antibodies following recurrence was detected in a study of individuals suffering from recurrent herpes labialis [Douglas and Couch, 1970], while multiple sera collected over 13 years from a sufferer of recurrent herpes labialis in another study revealed a gradual increase in neutralizing antibody titers [Ratner et al., 1980]. Titers of HSV-neutralizing antibody were revealed to be higher among patients with frequent herpes labialis than history-negative, HSV-seropositive control patients, consistent with a model in which antibody levels are driven by antigen load [Spruance et al., 1995]. In the present study, the level of serum anti-HSV-1 antibody increased after recurrence and HSV-1 isolation (Tables I and II), suggesting a boost of existing immune responses. The variation in the level of anti-HSV-1 antibody was considered potentially useful to diagnose recurrence.

Occasionally, molecular fingerprinting of serial genital HSV isolates has yielded more than one HSV strain [Koelle and Corey, 2003]. Genomes of both HSV-1 and HSV-2 were detected widely in human spinal

ganglia [Obara et al., 1997], and both HSV-1 and HSV-2 isolates were obtained from an individual with genital herpes infections [Sakaoka et al., 1995; Sucato et al., 1998]. Studies of sequence diversity between HSV-1 and HSV-2 isolates revealed evidence of recombination, which requires the co-existence of two viral genomes; hence, co-infection by genetically distinct strains is suggested as an important aspect in HSV epidemiology [Bowden et al., 2004; Norberg et al., 2004, 2007]. The separation of HSV isolates with different genomic profiles from the same individual suffering from genital herpes has been reported; that is, (i) 2 of 8 cases of HSV-2 genital infections [Buchman et al., 1979], (ii) 1 of 63 cases of HSV-2 genital infections [Sakaoka et al., 1995], and (iii) 2 of 13 cases of HSV-1 genital infections [Roest et al., 2004] were demonstrated to be attributable to exogenous re-infection by analyzing RFLP or a hypervariable region ("common-type variation"). Since HSV-1 isolates from the same patient were not differentiated in either RFLP (Table III) or reiteration VII (a hypervariable region) (Fig. 1), recrudescent genital lesions in the patients analyzed in the present study were supposed to be ascribable to endogenous recurrence of a latent virus, not exogenous re-infection with other strains.

After natural, wild-type infections, viral pathogens are supposed ordinarily to elicit immune responses that lessen the severity and transmissibility of subsequent infection with the same viral type; however, prior HSV infection did not prevent subsequent HSV infection [Whitley and Miller, 2001; Koelle and Corey, 2003; Ramachandran and Kinchington, 2007]. It is assumed that natural viral infection might protect against subsequent infection with the same viral genotype (usually due to endogenous recurrence of a latent virus) more effectively than with a different genotype (generally attributable to exogenous re-infection with other strains). As HSV-1 isolates from the same patient analyzed in the present study had the same genotype (Table III, Fig. 1), decreased serologic reactivity resulting from a difference in genotype seemed unlikely. Genotypes of HSV-1 isolates obtained from patient 1 (F35) were different from those from patient 2 (F85) (Table III, Fig. 1). HSV-1 isolates obtained from patients 1 (C81, 84) and 2 (C85, C88) were neutralized similarly by sera drawn from each patient (Tables I and II); thus, in these two patients, sera from one patient appeared to be able to neutralize HSV-1 isolates from the other patient as well as from the same patient.

Serologic type conversion of an HSV-1 to an HSV-2 epitope was shown to result from single amino acid substitution on an HSV-1 molecule [Kimmel et al., 1990], and the lack of reactivity of several HSV-2 clinical isolates to anti-HSV-2 monoclonal antibodies was attributable to single frameshift mutations [Liljeqvist et al., 1999]; hence, it is possible that a single mutation produced in the genome of an HSV clone could affect the serologic reactivity of the HSV clone. Although HSV-1 isolates obtained from the same patient in the present study were the same in RFLP and reiteration VII, other variations produced in the genome of an HSV-1 clone might cause a difference in serologic reactivity. An HSV-1 clone with a variation, as a result of which the HSV-1 clone is neutralized less effectively by sera taken before/during a later episode in a patient, is supposed to multiply preferentially during the later episode in the same patient. In the present study, an HSV-1 isolate obtained during a later episode was shown to be neutralized by sera taken before/during this episode from the same patient, as effectively as an HSV-1 isolate obtained during an earlier episode in the same patient (Tables I and II); thus, the majority of HSV-1 clones present during a later episode was assumed not to have a variation that could render an HSV-1 clone more resistant to sera drawn before/during the later episode in the same patient.

HSV-1 is thought to have replicated during recurrence in these two patients despite the existence of an antibody that could neutralize experimentally HSV-1; hence, HSV-1 antibody seems to offer little, if any, protection against HSV-1 recurrence, suggesting the necessity of developing an immunologic strategy for HSV vaccination with consideration of HSV-encoded immune evasion functions directed at the humoral response [Whitley and Miller, 2001; Koelle and Corey, 2003; Ramachandran and Kinchington, 2007].

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