specific mutations, because other *Neisseria* spp. did not have this mutation. Interestingly, a PenA nonmosaic strain, NG-120, tended to be more sensitive to aztreonam, a monobactam, unlike other PenA mosaic strains. It remains possible that these strains have other features, but it was difficult to explain such phenomena in this study.

In PenA mosaic strains with reduced susceptibility to cefixime and ceftriaxone isolated in 2006, the PenA amino sequences of NG-109, -110, -118, -121, and -122 were quite similar to the PenA of *N. perflava/sicca* and *N. cinerea* as well as *N. flavescens* and *N. meningitidis* (Fig. 2). In other *Neisseria* spp., it was reported that one of the donors conferring *penA* to *N. meningitidis* was identified as a naturally penicillin-resistant species, *N. flavescens*.[37] These gene transformations between commensally resistant *Neisseria* spp. and the original susceptible gonococci might have been caused by widespread commercial oral sex, and have enhanced the spread of reduced susceptibility to cephems.[17] An *N. gonorrhoeae* PenA with mosaic structures that confers reduced susceptibility to cefixime might have been constructed by various partial PenA from other *Neisseria* spp. (Fig. 3A,B). In this modeling study of mosaic PenA in NG-109, it is suggested that the PenA 3D conformation of the mosaic strain exchanged with other *Neisseria* spp. reduced the affinity to PenA of cephem antibiotics, including cefixime or ceftriaxone. Moreover, each domain in PenA of NG-109 had other alterations of amino acid residues which were not found in other *Neisseria* spp., suggesting that these alterations were induced to maintain the activity of PenA, since they were not found in other *Neisseria* spp.

In conclusion, we consider that the cause of reduced susceptibility to cephems is a conformational alteration of the β-lactam-binding pocket in PenA. A homology modeling simulation of PenA suggests that PenA mosaic sequences and the mutation of Ala-501 to Val near the β-lactam binding site Ser-310 of the PenA nonmosaic strain caused reduced susceptibility to penicillins or other cephem antibiotics. *N. gonorrhoeae* PenA with mosaic structures might have emerged by the transduction of regions from PenA of other *Neisseria* spp.

The reduction of susceptibility to antimicrobial agents has obviously progressed. Continual surveillance and monitoring of susceptibility to antimicrobial agents and genetic analysis are needed not only to reveal the detailed mechanism of resistance in *N. gonorrhoeae*, but also to select new therapeutic agents.

References

1. Tapsall J. Antimicrobial resistance in *Neisseria gonor-rhoeae*. World Health Organization 2001; Report WHO/CDS/CSR/DRS/2001.3.
2. Belland RJ, Morrison SG, Ison C, Huang WM. *Neisseria gonorrhoeae* acquires mutations in analogous regions of *gyrA* and *parC* in fluoroquinolone-resistant isolates. Mol Microbiol 1994;14:371–80.
3. Deguchi T, Yasuda M, Nakano M, Ozeki S, Ezaki T, Saito I, et al. Quinolone-resistant *Neisseria gonorrhoeae*: correlation of alterations in the GyrA subunit of DNA gyrase and the ParC subunit of topoisomerase IV with antimicrobial susceptibility profiles. Antimicrob Agents Chemother 1996;40:1020–3.
4. Su X, Lind I. Molecular basis of high-level ciprofloxacin resistance in *Neisseria gonorrhoeae* strains isolated in Denmark from 1995 to 1998. Antimicrob Agents Chemother 2001;45:117–23.
5. Tanaka M, Nakayama H, Haraoka M, Saika T, Kobayashi I, Naito S. Susceptibilities of *Neisseria gonorrhoeae* isolates containing amino acid substitutions in GyrA, with or without substitutions in ParC, to newer fluoroquinolones and other antibiotics. Antimicrob Agents Chemother 2000;44:192–5.
6. Tanaka M, Sakuma S, Takahashi K, Nagahuzi T, Saika T, Kobayashi I, et al. Analysis of quinolone resistance mechanisms in *Neisseria gonorrhoeae* isolates in vitro. Sex Transm Inf 1998;74:59–62.
7. Centers of Disease Control for Prevention. Sexually transmitted diseases treatment guidelines, 2006. MMWR 2006;55(No. RR-11):1–94.
8. Kumamoto Y, Tsukamoto T, Nishiya I, Akaza H, Noguchi M, Kamidono S, et al. Sexually transmitted disease surveillance in Japan (rate per 100000/year by disease, age and gender: 1998). Jpn J Sex Transm 1999;10:40–60.
9. Akasaka S, Muratani T, Yamada Y, Inatomi H, Takahashi K, Matsumoto T. Emergence of cephem- and aztreonam-high-resistant *Neisseria gonorrhoeae* that does not produce beta-lactamase. J Infect Chemother 2001;7:49–50.
10. Muratani T, Akasaka S, Kobayashi T, Yamada Y, Inatomi H, Takahashi K, et al. Outbreak of cefozopran (penicillin, oral cephems, and aztreonam)-resistant *Neisseria gonorrhoeae* in Japan. Antimicrob Agents Chemother 2001;45:3603–6.
11. Japanese Society for Sexually Transmitted Disease. Guidelines for diagnosis and treatment of sexually transmitted disease 2006. Jpn J Sex Transm Dis 2006;17(1 Suppl):35–9.
12. Dougherty TJ, Koller AE, Tomasz A. Penicillin-binding proteins of penicillin-susceptible and intrinsically resistant *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 1980;18:730–7.
13. Brannigan JA, Tirodimos IA, Zhang QY, Dowson CG, Spratt BG. Insertion of an extra amino acid is the main cause of the low affinity of penicillin-binding protein 2 in penicillin-resistant strains of *Neisseria gonorrhoeae*. Mol Microbiol 1990;4:913–9.
14. Dowson CG, Jephcott AE, Gough KR, Spratt BG. Penicillin-binding protein 2 genes of non-beta-lactamase-producing, penicillin-resistant strains of *Neisseria gonorrhoeae*. Mol Microbiol 1989;3:35–41.
15. Smith JM, Dowson CG, Spratt BG. Localized sex in bacteria. Nature 1991;349:29–31.
16. Spratt BG. Hybrid penicillin-binding proteins in penicillin-resistant strains of *Neisseria gonorrhoeae*. Nature 1988;332:173–6.
17. Ameyama S, Onodera S, Takahata M, Minami S, Maki N, Endo K, et al. Mosaic-like structure of penicillin-binding protein 2 Gene (*penA*) in clinical isolates of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime. Antimicrob Agents Chemother 2002;46:3744–9.
18. Faruki H, Sparling PF. Genetics of resistance in a non-beta-lactamase-producing gonococcus with relatively high-level penicillin resistance. Antimicrob Agents Chemother 1986;30:856–60.
19. Gill MJ, Simjee S, Al-Hattawi K, Robertson BD, Easmon CSF, Ison CA. Gonococcal resistance to beta-lactams and tetracycline involves mutation in loop 3 of the porin encoded at the *penB* locus. Antimicrob Agents Chemother 1998;42:2799–803.
20. Ropp PA, Hu M, Olesky M, Nicholas RA. Mutations in *ponA*, the gene encoding penicillin-binding protein 1, and a novel locus, *penC*, are required for high-level chromosomally mediated penicillin resistance in *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 2002;46:769–77.
21. Zhao S, Tobiason DM, Hu M, Seifert HS, Nicholas RA. The *penC* mutation conferring antibiotic resistance in *Neisseria gonorrhoeae* arises from a mutation in the PilQ secretin that interferes with multimer stability. Mol Microbiol 2005;57:1238–51.
22. Tanaka M, Nakayama H, Huruya K, Konomi I, Irie S, Kanayama A, et al. Analysis of mutations within multiple genes associated with resistance in a clinical isolate of *Neisseria gonorrhoeae* with reduced ceftriaxone susceptibility that shows a multidrug-resistant phenotype. Int J Antimicrob Agents 2006;27:20–6.

23. Obara Y, Yamai S, Nikkawa T, Shimoda Y, Miyamoto Y. Preservation and transportation of bacteria by a simple gelatin disk method. J Clin Microbiol 1981;14:61–6.
24. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. 7th ed. M7-A7 26 (2). Wayne: Clinical and Laboratory Standards Institute; 2007.
25. PRIME 1.5. Schrodinger, LLC, Portland.
26. Dessen A, Mouz N, Gordon E, Hopkins J, Dideberg O. Crystal structure of PBP2x from a highly penicillin-resistant *Streptococcus pneumoniae* clinical isolate: a mosaic framework containing 83 mutations. J Biol Chem 2001;276:45106–12.
27. Case DA, Cheatham III TE, Darden TA, Simmerling CL, Wang J, Duke RE, et al. AMBER 8. San Francisco: University of California; 2004.
28. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of simple potential functions for simulating liquid water. J Chem Phys 1983;79:926–35.
29. Goffin C, Ghuysen JM. Biochemistry and comparative genomics of SxxK superfamily acyltransferases offer a clue to the mycobacterial paradox: presence of penicillin-susceptible target proteins versus lack of efficiency of penicillin as therapeutic agent. Microbiol Mol Biol Rev 2002;66:702–38.
30. Hagman KE, Pan W, Spratt BG, Balthazar JT, Judd RC, Shafer WM. Resistance of *Neisseria gonorrhoeae* to antimicrobial hydrophobic agents is modulated by the *mtrRCDE* efflux system. Microbiology 1995;141(Pt 3):611–22.
31. Hagman KE, Shafer WM. Transcriptional control of the *mtr* efflux system of *Neisseria gonorrhoeae*. J Bacteriol 1995;177:4162–5.
32. Zarantonelli L, Borthagaray G, Lee EH, Shafer WM. Decreased azithromycin susceptibility of *Neisseria gonorrhoeae* due to *mtrR* mutations. Antimicrob Agents Chemother 1999;43:2468–72.
33. Saika T, Nishiyama T, Kanayama A, Kobayashi I, Nakayama H, Tanaka M, et al. Comparison of *Neisseria gonorrhoeae* isolates from the genital tract and pharynx of two gonorrhea patients. J Infect Chemother 2001;7:175–9.
34. Tanaka M, Nakayama H, Notomi T, Irie S, Tsunoda Y, Okadome A, et al. Antimicrobial resistance of *Neisseria* gonorrhoeae in Japan, 1993–2002: continuous increase of ciprofloxacin-resistant isolates. Int J Antimicrob Agents 2004;24(Suppl 1):S15–22.
35. Takahata S, Senju N, Osaki Y, Yoshida T, Ida T. Amino acid substitutions in mosaic penicillin-binding protein 2 associated with reduced susceptibility to cefixime in clinical isolates of *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 2006;50:3638–45.
36. Whiley DM, Limnios EA, Ray S, Sloots TP, Tapsall JW. Diversity of *penA* alterations and subtypes in *Neisseria gonorrhoeae* strains from Sydney, Australia, that are less susceptible to ceftriaxone. Antimicrob Agents Chemother 2007;51:3111–6.
37. Spratt BG, Zhang QY, Jones DM, Hutchison A, Brannigan JA, Dowson CG. Recruitment of a penicillin-binding protein gene from *Neisseria flavescens* during the emergence of penicillin resistance in *Neisseria meningitidis*. Proc Natl Acad Sci USA 1989;86:8988–92.

Journal of Medical Virology 81:1605–1612 (2009)

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

© 2009 WILEY-LISS, INC.

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.,

Grant sponsor: Ministry of Education, Science, Technology, Sports and Culture of Japan (partially supported).

*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

Accepted 26 May 2009
DOI 10.1002/jmv.21581
Published online in Wiley InterScience
(www.interscience.wiley.com)

WILEY InterScience DISCOVER SOMETHING GREAT

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′-CCAGAGACCCCAGGGTA-CCG-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

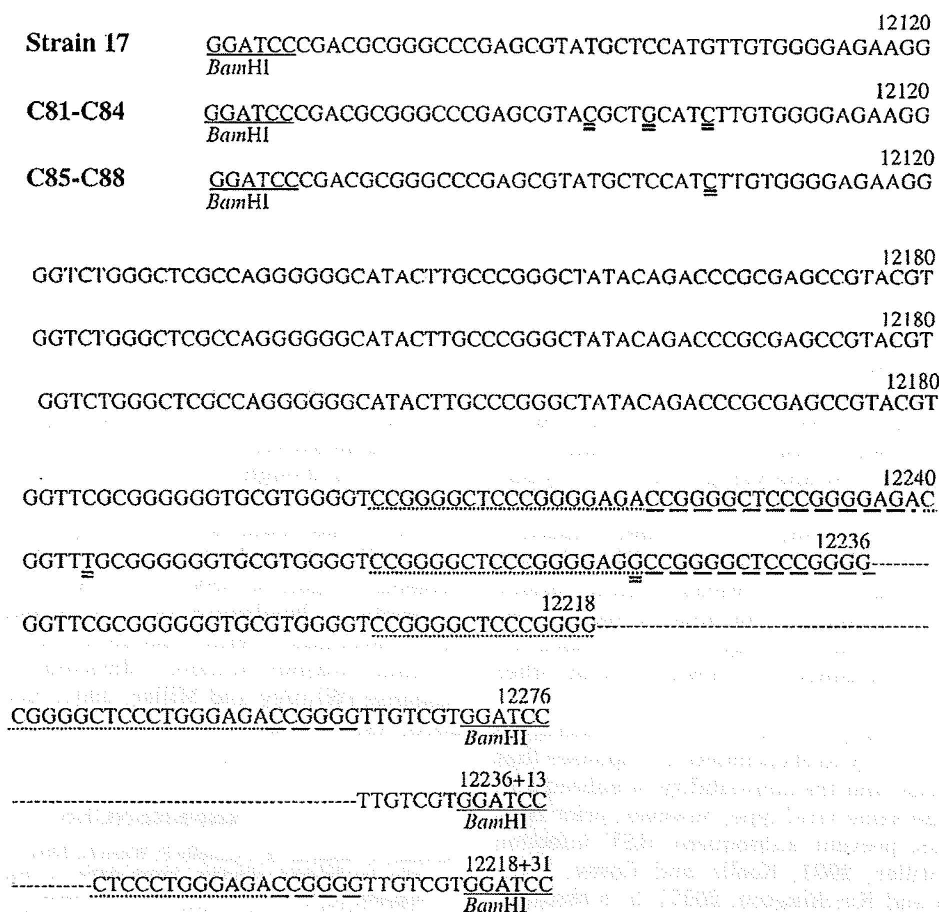

Fig. 1. Nucleotide sequences of a region encompassing reiteration VII of HSV-1. Nucleotide sequences of strain 17 (standard strain), isolates C81–C84 (F35 genotype), and isolates C85–C88 (F85 genotype). The nucleotide numbering system is the short unique region of strain 17 [McGeoch et al., 1985]. Nucleotide 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].

REFERENCES

Bowden R, Sakaoka H, Donnelly P, Ward R. 2004. High recombination rate in herpes simplex virus type 1 natural populations suggests significant co-infection. Infect Genet Evol 4:115–123.

Buchman TG, Roizman B, Adams G, Stover BH. 1978. Restriction endonuclease fingerprinting of herpes simplex virus DNA: A novel epidemiological tool applied to a nosocomial outbreak. J Infect Dis 138:488–498.

Buchman TG, Roizman B, Nahmias AJ. 1979. Demonstration of exogenous genital reinfection with herpes simplex virus type 2 by restriction endonuclease fingerprinting of viral DNA. J Infect Dis 140:295–304.

Chaney SMJ, Warren KG, Kettyls J, Zbitnue A, Subak-Sharpe JH. 1983. A comparative analysis of restriction enzyme digests of the DNA of herpes simplex virus isolated from genital and facial lesions. J Gen Virol 64:357–371.

Corey L, Langenberg AGM, Ashley R, Sekulovich RE, Izu AE, Douglas JMJ, Handsfield HH, Warren T, Marr L, Tyring S, DiCarlo R, Adimora AA, Leone P, Dekker CL, Burke RL, Leong WP, Straus SE. 1999. Recombinant glycoprotein vaccine for the prevention of genital HSV-2 infection. J Am Med Assoc 281:331–340.

Douglas RG, Jr., Couch RB. 1970. A prospective study of chronic herpes simplex virus infection and recurrent herpes labialis in humans. J Immunol 104:289–295.

Geretti AM. 2006. Genital herpes. Sex Transm Infect 82:iv31–iv34.

Gupta R, Warren T, Wald A. 2007. Genital herpes. Lancet 370:2127–2137.

Haddow LJ, Dave B, Mindel A, McPhie KA, Chung C, Marks C, Dwyer DE. 2006. Increase in rates of herpes simplex virus type 1 as a cause of anogenital herpes in western Sydney, Australia, between 1976 and 2003. Sex Transm Infect 82:255–259.

Hashido M, Inouye S, Kawana T. 1997. Differentiation of primary from nonprimary genital herpes infections by a herpes simplex virus-specific immunoglobulin G avidity assay. J Clin Microbiol 35:1766–1768.

Kalimo KOK, Marttila RJ, Granfors K, Viljanen MK. 1977. Solid-phase radioimmunoassay of human immunoglobulin M and immunoglobulin G antibodies against herpes simplex virus type 1 capsid, envelope, and excreted antigens. Infect Immun 15:883–889.

Kawana T, Kawagoe K, Takizawa K, Chen JT, Kawaguchi T, Sakamoto S. 1982. Clinical and virologic studies on female genital herpes. Obstet Gynecol 60:456–461.

Kawana T, Hashido M, Koizumi Y. 1995. Class-specific antibody response in acyclovir-treated and adenine arabinoside-treated patients with primary genital herpes simplex virus infection. Microbiol Immunol 39:795–799.

Kimmel KA, Dolter KE, Toth GM, Levine M, Glorioso JC. 1990. Serologic type conversion of a herpes simplex virus type 1 (HSV-1) to an HSV-2 epitope caused by a single amino acid substitution in glycoprotein C. J Virol 64:4033–4036.

Koelle DM, Corey L. 2003. Recent progress in herpes simplex virus immunobiology and vaccine research. Clin Microbiol Rev 16:96–113.

Kumaki S, Villa A, Asada H, Kawai S, Ohashi Y, Takahashi M, Hakozaki I, Nitanai E, Minegishi M, Tsuchiya S. 2001. Identification of anti-herpes simplex virus antibody-producing B cells in a patient with an atypical RAG1 immunodeficiency. Blood 98:1464–1468.

Lafferty WE, Coombs RW, Benedetti J, Critchlow C, Corey L. 1987. Recurrences after oral and genital herpes simplex virus infection: Influence of site of infection and viral type. N Engl J Med 316:1444–1449.

Liljeqvist J-Å, Svennerholm B, Bergström T. 1999. Herpes simplex virus type 2 glycoprotein G-negative clinical isolates are generated by single frameshift mutations. J Virol 73:9796–9802.

Maertzdorf J, Remeijer L, Van Der Lelij A, Buitenwerf J, Niesters HGM, Osterhaus ADME, Verjans GMGM. 1999. Amplification of reiterated sequences of herpes simplex virus type 1 (HSV-1) genome to discriminate between clinical HSV-1 isolates. J Clin Microbiol 37:3518–3523.

McGeoch DJ, Dolan A, Donald S, Rixon FJ. 1985. Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. J Mol Biol 181:1–13.

McGeoch DJ, Dalrymple MA, Davison AJ, Dolan A, Frame MC, McNab D, Perry LJ, Scott JE, Taylor P. 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J Gen Virol 69:1531–1574.

Norberg P, Bergström T, Rekabdar E, Lindh M, Liljqvist J-Å. 2004. Phylogenetic analysis of clinical herpes simplex virus type 1 isolates identified three genetic groups and recombinant viruses. J Virol 78:10755–10764.

Norberg P, Kasubi MJ, Haarr L, Bergström T, Liljqvist J-Å. 2007. Divergence and recombination of clinical herpes simplex virus type 2 isolates. J Virol 81:13158–13167.

Obara Y, Furuta Y, Takasu T, Suzuki S, Suzuki H, Matsukawa S, Fujioka Y, Takahashi H, Kurata T, Nagashima K. 1997. Distribution of herpes simplex virus types 1 and 2 genomes in human spinal ganglia studied by PCR and in situ hybridization. J Med Virol 52:136–142.

Ramachandran S, Kinchington PR. 2007. Potential prophylactic and therapeutic vaccines for HSV infections. Curr Pharm Des 13:1965–1973.

Ratner JJ, Sanford BA, Smith KO. 1980. A serological study of herpes simplex virus type 1 antibody over a 13-year period. Dermatology 161:227–232.

Reeves WC, Corey L, Adams HG, Vontver LA, Holmes KK. 1981. Risk of recurrence after first episodes of genital herpes: Relation to HSV type and antibody response. N Engl J Med 305:315–319.

Remeijer L, Maertzdorf J, Doornenbal P, Verjans GMGM, Osterhaus ADME. 2001. Herpes simplex virus 1 transmission through corneal transplantation. Lancet 357:442.

Remeijer L, Maertzdorf J, Buitenwerf J, Osterhaus ADME, Verjans GMGM. 2002. Corneal herpes simplex virus type 1 superinfection in patients with recrudescent herpetic keratitis. Invest Ophthalmol Vis Sci 43:358–363.

Roest RW, Carman WF, Maertzdorf J, Scoular A, Harvey J, Kant M, van der Meijden WI, Verjans GMGM, Osterhaus ADME. 2004. Genotypic analysis of sequential genital herpes simplex virus type 1 (HSV-1) isolates of patients with recurrent HSV-1 associated genital herpes. J Med Virol 73:601–604.

Sacks SL, Griffiths PD, Corey L, Cohen C, Cunningham A, Dusheiko GM, Self S, Spruance S, Stanberry LR, Wald A, Whitley RJ. 2004. Introduction: Is viral shedding a surrogate marker for transmission of genital herpes? Antiviral Res 63S1:S3–S10.

Sakaoka H, Kurita K, Iida Y, Takada S, Umene K, Kim YT, Ren CS, Nahmias AJ. 1994. Quantitative analysis of genomic polymorphism of herpes simplex virus type 1 strains from six countries: Studies of molecular evolution and molecular epidemiology of the virus. J Gen Virol 75:513–527.

Sakaoka H, Aomori T, Gouro T, Kumamoto Y. 1995. Demonstration of either endogenous recurrence or exogenous reinfection by restriction endonuclease cleavage analysis of herpes simplex virus from patients with recrudescent genital herpes. J Med Virol 46:387–396.

Spruance SL, Evans TG, McKeough MB, Thai L, Araneo BA, Daynes RA, Mishkin EM, Abramovitz AS. 1995. Th1/Th2-like immunity and resistance to herpes labialis. Antiviral Res 28:39–55.

Sucato G, Wald A, Wakabayashi E, Vieria J, Corey L. 1998. Evidence of latency and reactivation of both herpes simplex virus (HSV)-1 and HSV-2 in the genital region. J Infect Dis 177:1069–1072.

Umene K. 1998a. Herpesvirus: Genetic variability and recombination. Fukuoka: Touka Shobo, 345 p.

Umene K. 1998b. Molecular epidemiology of herpes simplex virus type 1. Rev Med Microbiol 9:217–224.

Umene K, Kawana T. 2000. Molecular epidemiology of herpes simplex virus type 1 genital infection in association with clinical manifestations. Arch Virol 145:505–522.

Umene K, Kawana T. 2003. Divergence of reiterated sequences in a series of genital isolates of herpes simplex virus type 1 from individual patients. J Gen Virol 84:917–923.

Umene K, Yoshida M. 1989. Reiterated sequences of herpes simplex virus type 1 (HSV-1) genome can serve as physical markers for the differentiation of HSV-1 strains. Arch Virol 106:281–299.

Umene K, Eto T, Mori R, Takagi Y, Enquist LW. 1984. Herpes simplex virus type 1 restriction fragment polymorphism determined using Southern hybridization. Arch Virol 80:275–290.

Umene K, Yamanaka F, Oohashi S, Koga C, Kameyama T. 2007. Detection of differences in genomic profiles between herpes simplex virus type 1 isolates sequentially separated from the saliva of the same individual. J Clin Virol 39:266–270.

Whitley RJ, Miller RL. 2001. Immunologic approach to herpes simplex virus. Viral Immunol 14:111–118.

Zweerink HJ, Stanton LW. 1981. Immune response to herpes simplex virus infections: Virus-specific antibodies in sera from patients with recurrent facial infections. Infect Immun 31:624–630.

Japanese Journal of Sexually Transmitted Infections

性器ヘルペスウイルス感染症（性器ヘルペス）

川名　尚

日本性感染症学会誌
Vol.20,No.1

性器ヘルペスウイルス感染症（性器ヘルペス）

Issues included in surveillance systems for genital herpes

帝京大学医学部附属溝口病院産婦人科
Department of Obstetrics & Gyrecology, Mizonokuchi Hospital, Teikyo University
帝京平成看護短期大学
Teikyo Heisei Nursing Junior College
川名　尚
Takashi KAWANA

Two issues included in current surveillance systems for genital herpes are proposed. First, it seems improper to exclude recurrent cases from a report of genital herpes in the light of the pathogenesis of the genital herpes simplex virus infection. I insist on registering recurrent cases as well as initial cases in the surveillance report form of genital herpes. Second, it is essential to make an accurate diagnosis for the surveillance report of genital herpes. It is urgent to develop a method with excellent sensitivity and specificity to detect HSV-DNA. For this purpose the LAMP method is proper and under development.

Key words : Genital herpes, Recurrent cases, Surveillance system

はじめに

現在の性器ヘルペスウイルス感染症（以下、性器ヘルペスとする）のサーベイランスにおける筆者の感じている二つの問題点を述べたい。性器の単純ヘルペスウイルス（Herpes Simplex Virus, HSV）感染は他の性感染症と異なる独特の感染病理を有する。即ち、HSV は初感染後速やかに知覚神経節に潜伏感染するが潜伏感染しているHSV がしばしば再活性化されて再発する。初感染時に症状がなく免疫の低下によって初めて発症することもある。このような感染病態を認識した上で本疾患の動向をみるべきである。2006 年より届出基準から再発例が除かれたが果たしてこれで良かったのか疑問を抱いている。もう一つの問題が診断である。性器ヘルペスは多彩な症状を呈し、臨床的に診断は難しい場合がある。一方、性器ヘルペスと紛らわしい疾患が多くある。このような状況で現在保険で行える蛍光抗体法による HSV 感染細胞の検出は特異度は高いが感度が非常に悪い。この点クラミジア感染症や淋菌感染症には鋭敏な核酸増幅法が日常臨床に用いられており、診断の精度が高いばかりでなく時には妊婦など無症候の例についても検査が行われ疾患の掘りおこしさえ行われている。この点、性器ヘルペスにはこのような精度の高い検査法がなく大変たち遅れている。

本稿では、現在の発生動向調査から見えてくる性器ヘルペスの動態を述べたのち、これらの問題点について筆者の私見を述べたいと思う。

Ⅰ．定点調査からみた性器ヘルペスの動向

性器ヘルペスは、STD の定点把握の一つとして 1987 年からその動向調査が行われてきた。1987～2006 年までの経時的トレンドをみると男性は 0.7 から 0.4 とやや減少傾向にあるが、女性は 0.25 から 0.5 と増加傾向を示している。その結果、最近では女性の方が男性の 1.5 倍と多くなっている。

最近の 7 年間では男女合わせてみると性器ヘルペスは上昇傾向にある。性器クラミジア感染症や淋菌感染症が 2002 年をピークとして減少に転じているがウイルス性の性器ヘルペスや尖圭コンジローマは増加している点は注目すべきである（**図 1**）。

前述のように、2006 年より性器ヘルペスの届出基準が変わり「明らかに再発であるもの及び血清抗体のみ陽性のものは除外する」ことになった。その影響がどのように出ているかをみた(**図 2**)。2005 年と 2006 年は報告数はほとんど同じであり再発を除くという変更が周知徹底していなかったようだが 2007 年に至り報告数が次第に減少し前年度の 88%になった。おそらく今後もっ

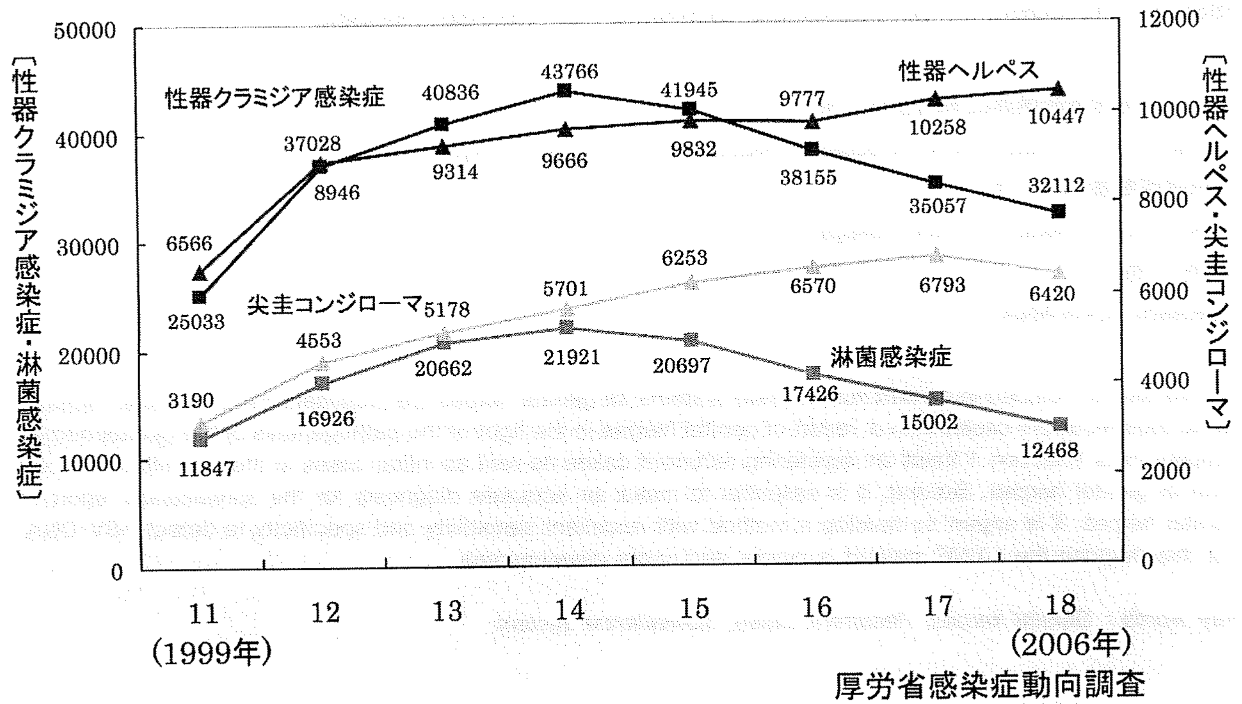

図１　性感染症報告数　平成 11 年～平成 18 年

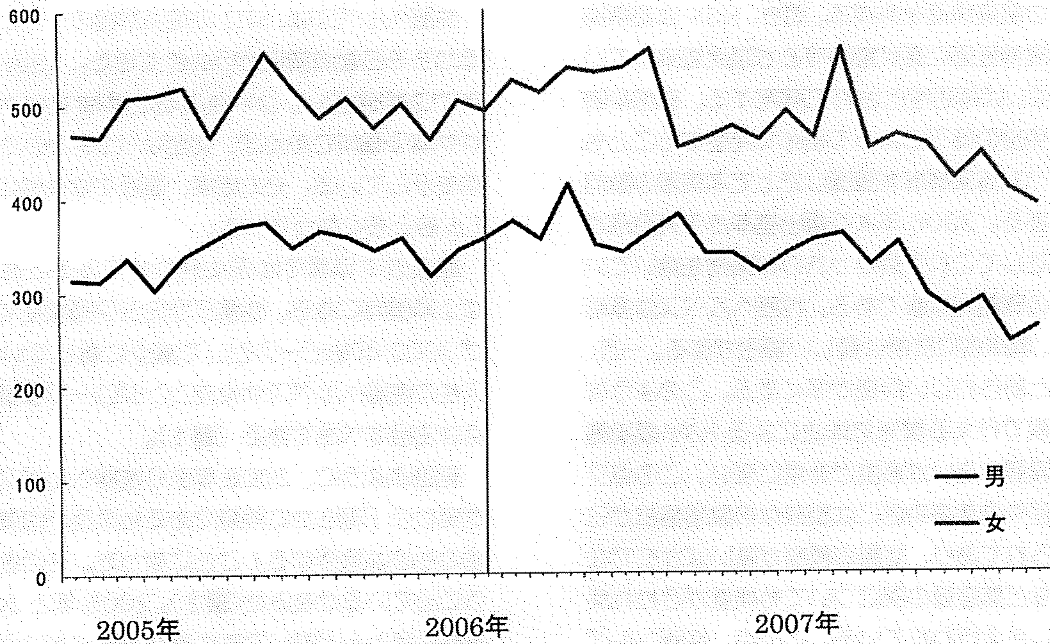

図２　性器ヘルペス報告数
2005～2007.11　経時的データ

46

と減少することになろうが、これは性器ヘルペスが減少したと錯覚してはなるまい。後述するように性器ヘルペスは治癒することはなく再発をくり返すので社会全体における症例数は増加すると考えられるからである。

年令分布について

性器ヘルペスの年令分布が性器クラミジア感染症や淋菌感染症などと異なるのは、後者が20代をピークとして年令が高くなるに従って減少し40代には男女とも報告数がほとんどなくなるのに対し性器ヘルペスでは40代〜60代にもかなりの症例数が報告されている点である。高齢者の報告例の多くはHSVの再活性化による再発例ではないかと考えられている。性活動が衰えてくるこの年令の性器ヘルペスは性行為で感染したものではなく潜伏していたHSVの再活性化によるものではないかという考えである。性感染症を「性行為により感染した病原体により間なくして発症した疾患」と定義すればこのような例は動向調査から除外するべきという考えも出てくる。一方、性行為により感染したHSVが長い潜伏期の後に発症した疾患とも言える。従って筆者は除外すべきではないと考えている。

II. 性器ヘルペスの感染病理

(1) 初発と再発の分布

性器ヘルペスは臨床的に初発と再発に分けられている。初発とは初めて発症したものであり、再発とは以前に発症した経験が一度でもある場合を言う。では初診時における初発と再発の分布はどのようになっているのであろうか。

2006年11月に行われた千葉県の性感染症の全数調査（厚生労働省研究班「性感染症に関する特定感染症予防指針の推進に関する研究」主任研究者 小野寺昭一氏）によれば、届出された性器ヘルペスの約60%が初発、約40%が再発となっている。一方、平成5〜7年における大阪府の性感染症動態調査では初発が約30%、再発が約70%となっている。筆者の1970年から今日までに経験した初診の症例では約65%が初発、約35%が再発であった。これらを勘案するとおよそ40〜50%は再発例と考えられ、もし再発例を除外すると届出数もこの程度に減少する可能性はある。

(2) 初発の感染病理

初発は感染病理学的には初感染初発と非初感染初発に分けられる。前者は初感染であるが、後者は既に感染し

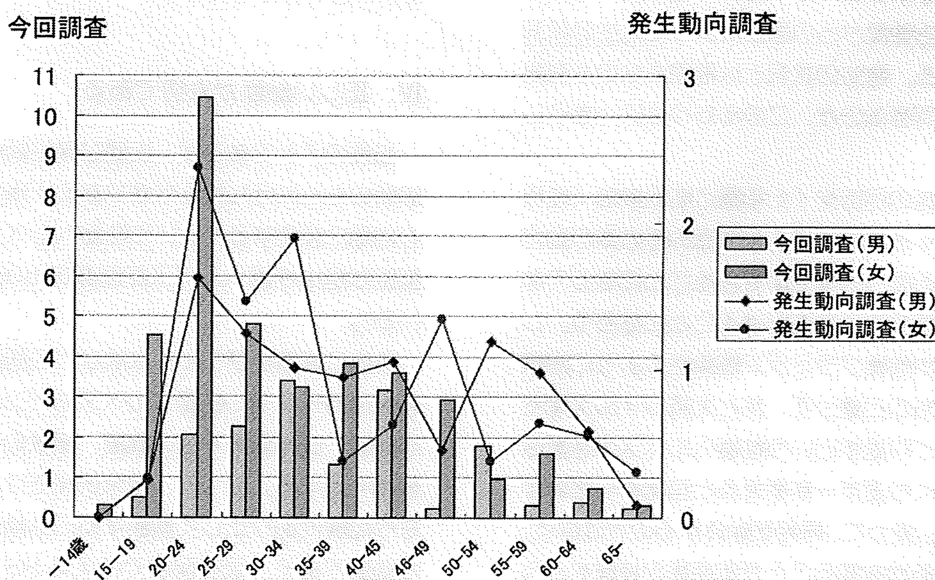

図3 性器ヘルペスウイルス感染症
(初発あるいは初感染)
(4県合計)(人口10万人あたり)

潜伏していたHSVが再活性化され初めて発症したもので、感染はかなり以前におきていることになる。

筆者は初発性器ヘルペスについて発症時に抗体の有無を調べてみた。もし原因となったHSVの型と同型の型の抗体が存在すればそれはおそらく再活性化ということになり非初感染初発となる。HSV-1による初発例では約20%がHSV-2の初発例では約40%が平均すると約30%が非初感染初発と同定された[1]。

前述の厚労省の研究で行われた全数調査において4県の症例をまとめたものであるが、初発例の年令分布をみると40才以降にかなりの症例が報告されている（**図3**）が、非初感染初発もかなり含まれていると思う。

III．再発を除いたことの問題点

前述のように2006年から届出基準から再発例を除くことになった。除くことになった経緯について厚生労働省健康局結核感染症課情報管理係に問合せたところ、必ずしも明確な答は得られなかったが、同一人が再発のたび毎に毎回届出すると症例数が本来の数より多く報告されることになるという理由のようであった。また、高齢者の届出数が多くこれらは本来の届出対象ではないとの認識を持っているようであった。発生動向調査の目的は何であろうか。感染症法には「法第十四条第一項の規定に基づき指定届出機関からの届出によって発生の状況を把握する」とある。発生の状況とは罹患率なのか有病率なのかあるいは両者なのか、このあたりははっきりしていない。

性感染症動向調査の目的を「1年間に性的接触（性行為）によって伝播する病原体による疾患の患者数の動向をみる」と定義するならば罹患率をみることになるであろう。とすれば初感染のみを届出することになろう。しかし、淋菌感染症や性器クラミジア感染症のように治療により完治できるものと違って、たとえ抗ウイルス剤で治療しても再発をくり返すという性器ヘルペスの特異な感染病理ー再発をくり返すーを考えると有病率も考慮すべきと思っている。従って、再発を除外するのではなく、どの位再発例があるかを知ることが本疾患の特質をとらえた発生動向調査となると思う。

再発性器ヘルペス患者が定点診療所を受診した時は届出されないことになっているが、前医が定点診療所でなければ（おそらく大部分はこのような状況と考えられる）完全にこのような例は届出数にのってこないことになり過少評価されることになる。

再発例は初発例と同じように感染性があるばかりでなく再発性器ヘルペス患者のQOLは著しく損なわれるなど社会的には大きな問題でもある。

以上より、性器ヘルペスについては有病率も大切な「発生の状況」と言わざるを得ない。諸外国で再発を除いている報告はみられないのもこのような観点からではないか。そこで次のように提言したい。

前述のように、非初感染初発は潜伏しているHSVの再活性化により発症しているが、これは再発の感染病理と同じである。現在は非初感染初発を届出しているのであるから再発を届出しないというのは矛盾することになりはしまいか、ただし、同一人の再発を来院時毎に届出するのは過大評価になるので初診時のみ届出したら良いのではないか。

提　言

「定点を受診した性器ヘルペス患者は、初発であれ再発であれ初診時に1回届出する。この際、初発と再発を区別できるような項目を設ける。」

なお、再発の届出は年1回とし、翌年再発したら年1回は届出することにする。

IV．正しい診断が大切である

当然のことであるが、正確な発生動向調査には正しい診断のもとに行われるべきである。「浅い潰瘍性病変が左右対称に多発する」という性器ヘルペスの教科書的な典型的な例は性器ヘルペス症例数の50%以下と言われている[2]。

外陰に潰瘍やびらんや水疱などの病変を呈する疾患は多数ある[3]。一方、性器ヘルペスもピンホールのような微小病変、左右対称でない潰瘍、線状のびらんなど多彩な様相を呈する。従って、臨床所見だけで診断するのはかなり危険であり正しく診断するには精度の高い病原診断が必須である。現在保険で行えるHSV感染細胞の蛍光抗体法による検出は、性器ヘルペスのような小さい病変の多いHSV感染症では感度が非常に悪い。諸外国では感度と特異性が非常に良い培養法や核酸増幅法が用いら

れている。培養は時間と費用がかかる。また、現在本邦で確立された核酸増幅法はまだない。核酸増幅法としてPCR 法や LAMP 法が開発中である。LAMP 法（Loop-Mediated Isothermal Amplification）は本邦で開発された核酸増幅法で、微量の HSV-1、HSV-2 DNA を増幅することができる。筆者らの検討では感度・特異度共に培養法とほぼ同等であった。特に本法は 2 時間という短時間のうちに結果が出せる上に温度が一定で良いので反応に用いる加熱器は小型な簡易装置で良い点が利点である。臨床の現場でも手軽に使用できるようになる可能性があるので大いに期待している。

おわりに

現行の性器ヘルペスの動向調査について二つの問題を提起した。

(1) 現行の届出基準を「初発も再発も初診時に 1 回届出する。この際、初発と再発を分ける。ただし、再発は年 1 回届出する。」に改めること。

(2) 届出のためには正しい診断が必須であり、そのための感度・特異度の良い病原診断法の開発が緊急の課題である。

最後に、再発を届出しないという現行の動向調査が徹底してくると性器ヘルペスの症例数が減少することになるが、これをもって性器ヘルペスが減少していると決して誤解してはならないことを言及しておきたい。

文 献

1）川名 尚：初発性器ヘルペスの感染病態．日本産科婦人科学会千葉地方部会会誌，2008；1：10-12．

2）性感染症 診断・治療ガイドライン 2008；病状とその鑑別診断；潰瘍性病変．日性感染症会誌，2008；19(1)(supp)：18-23．

3）Lautenschlager, S, Eichmann, A：The heterogeneous clinical spectrum of genital herpes. Dermatology. 2001；202(3)：211-219.

4）塚越静香，川名 尚，西澤美香ほか：Loop-mediated isothermal amplification（LAMP）法による性器ヘルペス迅速診断．日性感染症会誌，2006；17(1)：104-109．

新しい単純ヘルペスウイルス型特異抗体検出キットの評価

西澤美香、川名　尚、西井　修

日本性感染症学会誌
Vol.20,No.1

新しい単純ヘルペスウイルス型特異抗体検出キットの評価

Evaluation of a new test kit for the measurement of herpes simplex virus type-specific antibodies

西澤美香[1)]　川名　尚[1),2)]　西井　修[1)]
Mika NISHIZAWA　Takashi KAWANA　Osamu NISHII

単純ヘルペスウイルス（HSV）の1型（HSV-1）と2型（HSV-2）の感染はglycoprotein Gを使った抗体検出によって血清学的に型別が可能になった。最近開発された型特異的抗体検出キットPlatelia HSVについて世界的に既に用いられてきたHerpeSelectと比較して評価を行った。HSV-1による性器ヘルペス患者から採取した30例の血清について検討した所HerpeSelectはすべてHSV-1抗体のみであったがPlateliaは29例にHSV-1抗体を検出し1例に低力価のHSV-2抗体を検出した。HSV-2を分離した30例から得た血清ではHerpeSelectとPlateliaともにすべてHSV-2抗体が検出され分離HSVの型と一致した。初感染の陽転率は第3週目において、HSV-1感染例ではPlatelia 77%、HerpeSelect 41%、HSV-2感染例ではPlatelia 100%、HerpeSelect 83%となりPlateliaの方がやや優れていた。Platelia HSV-1、Platelia HSV-2は特異度・感度共に優れたキットである。

The test based on the type-specific protein glycoprotein gG of HSV has been shown to accurately differentiate between antibodies to HSV-1 and those to HSV-2. A new test kit, Platelia HSV, for the measurement of type-specific antibodies, was compared with the HerpeSelect test kit. In 30 sera obtained from patients with genital HSV-1 infection, the HSV-1 antibody was detected in 30 sera by HerpeSelect and in 29 by Platelia respectively. The HSV-2 antibody was detected by Platelia in one of these sera though at very low titer. In 30 sera obtained from patients with genital HSV-2 infection, The HSV-2 antibody was detected in all 30 sera by HerpeSelect and by Platelia. The seroconversion rates at three weeks of infection in patients with primary HSV-1 infection were 77% by Platelia and 41% by HerpeSelect respectively. The seroconversion rates at three weeks of infection in patients with primary HSV-2 infection were 100% by Platelia and 83% by HerpeSelect respectively. This study indicated the usefulness of the Platelia kit for the detection of type-specific antibodies to HSV.

Key words : HSV-1, HSV-2, Genital herpes, ELISA, Type-specific antibody

緒　言

単純ヘルペスウイルス（HSV）には1型（HSV-1）と2型（HSV-2）があり、それぞれ感染の疫学や臨床的な意義が異なる。即ち、HSV-1は幼少時に主に口腔内に感染し三叉神経節に潜伏感染し、しばしば口唇などに再発することがあり、また口腔内にHSV-1を排泄する。脳、眼、口唇などに感染するHSVはほとんどがHSV-1である[1)]。上半身のHSV感染はHSV-1によるがHSV-1は性器にも感染することがあることが判明した[2)]。一方、

1）帝京大学医学部附属溝口病院産婦人科：Department of Obstetrics & Gynecology, Mizonokuchi Hospital, Teikyo University
2）帝京平成看護短期大学：Teikyo Heisei Nursing Junior College
平成21年3月2日受付、平成21年4月13日掲載決定
（〒213-8507）神奈川県川崎市高津区溝口3-8-3　帝京大学医学部附属溝口病院産婦人科　西澤美香

HSV-2は発見当初から性器などの下半身に感染することがわかっており、その伝播は性行為感染とされている[3]。従って性器にはHSV-1とHSV-2の両方が感染する。性器のHSV-2感染例ではHSV-1感染例に比べて再発の頻度が高く、またHSV-2はHSV-1に比べて神経向性が強いというように、その生物学的性格の違いもみられている[4]。HSV-2は性的接触によって伝播することから、ある集団のHSV-2に対する抗体保有率はその集団の性的活動の指標と考えられている[5]。このように、感染しているHSVの型を決めることは臨床的、疫学的に重要である。感染しているHSVの型は病変部からHSVを分離培養するかHSV DNAを核酸増幅法で検出することによって行われている。しかし病原診断が困難な場合には血清学的診断によらなくてはならない。その際に注意すべきことはHSV-1とHSV-2には共通抗原があるため交差反応がおこる点である[1]。最もHSVに特異的といわれている中和抗体法を用いた場合でも、HSV-1抗体はHSV-1に対する抗体価の4分の1程度にHSV-2に対しても中和抗体価を示し、またHSV-2抗体はHSV-1に対してもHSV-2とほぼ同程度の中和抗体価を示す[6]。従って、従来から用いられてきた感染細胞を抗原とする補体結合法、蛍光抗体法、中和抗体法、ELISA法などでは正しく型特異的に抗体を検出することはできない。しかし、HSV粒子の表面にあるglycoprotein G (gG)はHSV-1とHSV-2で異なることがわかり[7]、これを抗原としたELISA法による型特異的抗体の測定法が開発された。2000年に米国のFDAにより最初に認可されたものがHerpeSelect (Focus Diagnostics, Inc.)であった。HerpeSelectはその感度と特異性が高いことから最も信頼されてきたキットである[8]。しかし、HSV-2抗体検出のgold standardといわれているワシントン大学で行われているウエスタンブロット法でみるとHerpeSelectの陽性検体の84%しか真の陽性ではないとの報告がある[9]。特に抗体値が1.1～3.0の弱陽性群に疑陽性が多いという。さらに筆者らが検討した所、HerpeSelectは型特異的に抗体の測定が可能な優れたキットではあるが、HSV-1抗体の検出感度が低く、感染後の陽転率も50～60%と低いことが問題であった[10]。今回、Bio-Rad社から新しくPlatelia HSV IgGが開発され検討する機会を得たが、HerpeSelectの欠点を補うことができるのではないかとの期待を持ってHerpeSelectと比較しながら本キットの評価を行った。

対象と方法

1. 血清

東京大学病院分院産婦人科および帝京大学医学部附属溝口病院産婦人科を受診しHSVを分離して性器ヘルペスと診断された93例から得た血清、および健康人20例から得た血清を用いた。93例のうちの60例（60検体）はキットの型特異性の検討に使用し、検討しやすくするために抗体価が低値から高値に分布する血清を選択した。内訳はHSV-1を分離した30例(初発15例、再発15例)とHSV-2を分離した30例(初発7例、再発23例）である。残る33例（91検体）は初回来院時に抗体が陰性で初回来院から8週目までの間に2回から4回（平均2.8回）の検体が採取されているもので、抗体の陽転時期の検討に用いた。その内訳はHSV-1を分離した21例から得られた60検体と、HSV-2を分離した12例から得られた31検体である。血清は測定まで−30℃で保存され、使用時に溶解して測定に用いた。

2. ウイルス分離と同定

性器の病変から擦過して得た検体をVeroまたはR-66細胞に接種して37℃、5% CO_2インキュベーターで培養した。細胞変性効果が出現した感染細胞について、FITC標識抗HSVモノクローナル抗体（ヘルペス1・2 FA試薬「生研」(デンカ生研株式会社)または、MicroTrak Herpes（シバ社））を用いて同定と型の決定を行った。

3. 血清抗体の検出

上記の血清について3種類の間接法によるELISA法のキットを用いて検討した。方法はすべて添付文書に従い、測定は同じ検体について同様の測定を2回行った。キット間で判定が一致しなかった検体についてウエスタンブロットならびに中和法を用いて確認試験を行った。

1）ヘルペスIgG EIA「生研」(デンカ生研株式会社）はHSVに対するIgG抗体を検出するキットで、HSV-1の感染細胞より調製したものを抗原としているため抗原性が強いが型特異性のないgB、gDなどに対する抗

体が検出される。型特異的な検出はできないが体外診断薬として本邦で広く用いられている。

2）型特異的に検出するキットとしてHerpeSelect（Focus Diagnostics, Inc.）（以下HerpeSelect）とPlatelia HSV IgG（Bio-Rad）（以下Platelia）を用いた。これらはウイルス表面にある型特異的な蛋白であるHSV-1はgG-1、HSV-2はgG-2の抗原がプレートに固相され、HSVのIgG抗体を型別に検出することができる。型特異性の検討では、すべての検体についてHSV-1抗体とHSV-2抗体を測定した。

① HerpeSelectの抗原はgG-1、gG-2ともにリコンビナント抗原を使用している。抗体の測定は、血清を添付の希釈液で101倍に希釈したものをプレートに100μl入れて室温で1時間反応させた後、希釈した添付の洗浄液で3回洗浄後ペルオキシダーゼ標識ヤギ抗ヒトIgGを100μl入れて室温で30分反応させた。3回洗浄後テトラメチルベンチヂンを100μl入れて遮光して室温で10分反応させ、1M硫酸を100μl入れて反応を停止し450nmで吸光度を測定した。添付のカットオフ用血清を同時に3回測定し、検体の吸光度をカットオフ用血清の平均吸光度で割った値を抗体指数（Index Value）とした。1.11以上を陽性、0.90未満を陰性、0.90以上1.10以下を判定保留とした。

② Plateliaの抗原はgG-1はリコンビナント抗原、gG-2は合成ペプチドを用いている。測定は、添付の説明文書に従って血清を希釈液で21倍に希釈し、プレートに200μl入れて37°Cで1時間反応させた後、希釈した添付の洗浄液で4回洗浄後51倍希釈したペルオキシダーゼ標識ヤギポリクローナル抗ヒトγ鎖抗体を200μl入れて37°Cで1時間反応させた。4回洗浄後テトラメチルベンチヂンを200μl入れて遮光して室温で30分反応させ、1N硫酸を100μl入れて反応を停止し450nmで吸光度を測定した。添付のカットオフ用血清を同時に2回測定し、検体吸光度をカットオフ用血清の平均吸光度で割った値を算出した。1.10以上を陽性、0.90未満を陰性、0.90から1.09を判定保留とした。

3）ウェスタンブロット法はHerpeSelect 1 and 2 Immunoblot IgG（Focus Diagnostics, Inc.）を使用した。抗原はgG-1は分子量35から45キロダルトンのリコンビナント抗原、gG-2は分子量80から110キロダルトンのリコンビナント抗原を使用している。測定は、添付の説明文書に従って血清を4つの抗原（抗ヒト血清、ヘルペス共通抗原、gG-1、gG-2）がバンド状に付いたニトロセルロース膜のストリップと反応させ、抗体が結合したバンドをアルカリホスファターゼ標識ヤギ抗ヒトIgGおよび基質（ブロムクロロインドールリン酸とニトロブルーテトラゾリウム）と反応させて発色させ、バンドの有無を目視で判定した。

4）中和抗体測定は、非働化し4倍から128倍まで倍数希釈した血清25μlにHSV-2標準株として筆者らが用いている新鮮分離株であるTHH-54の100TCID$_{50}$を25μl、10単位補体25μlを96ウエルマイクロプレート内で混合し、37°C、CO_2インキュベーターで1時間反応させた後、5×10^5/mlに調整したR-66細胞25μlを添加して5日間培養した。2系列を用い細胞変性効果を阻止した最高希釈倍数を中和抗体価とした。

4．抗原抗体陽転時期の検討方法

初診日を起点とし、第1週目、第2週目、第3週目、第4週～5週目、第6週～7週目、第8週目以降の6つの期間に分け陽転率を検討した。性器ヘルペス患者は毎週採血している訳ではなく血清が採取されていない期間がある。その場合は一度陽性になったら以降も陽性とし、陰性と陰性の間の期間は陰性とした。採血検体がなく、判定できない期間はこれらを除いて発症後の週における陽性率を計算した。

結　果

1．型特異性の検討

1）HSV-1分離症例から得た血清30例（初発15例、再発15例）のHerpeSelect HSV-1の抗体指数は1.18～8.73（平均4.84）に分布してすべて陽性となり、同検体に対してHerpeSelect HSV-2では0.02～0.55に分布してすべて陰性となった（Fig. 1 左○）。分離されたHSVの型と血清抗体の型がすべて一致した。これらの血清についてPlateliaで測定したところ、