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Correlation between Detection of Herpes Simplex Virus in Oral Secretions by PCR and Susceptibility to Experimental UV Radiation-Induced Herpes Labialis

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We examined the oral secretions of 25 patients for herpes simplex virus (HSV) at the time of and following experimental UV radiation (UVR). HSV was detected in one or more oral secretion specimens in 5 of 12 (42%) cases by cell culture and in 8 of 12 (67%) cases by PCR. On the day of UVR, HSV was detected in 1 of 12 (8%) patients who developed a lip lesion and 2 of 16 (13%) patients who did not (the difference is not significant). We conclude that PCR is more sensitive than culture in the detection of HSV and that HSV is not shed with increased frequency from the oral cavity before the development of UVR-induced herpes labialis.

After exposure to experimental UV radiation (UVR), 40 to 70% of susceptible patients develop herpes labialis within 7 days (12). Lesions that arise 3 or more days after irradiation (delayed lesions) are thought to arise by the ganglion trigger mechanism: a stimulus to the trigeminal ganglion (e.g., UV light exposure, neurosurgical manipulation, or fever) is followed by the reactivation of herpes simplex virus (HSV) within the ganglion and the travel of virions down the sensory nerve to epithelial cells where infection is established (2). However, the pathogenesis of lesions that occur within 48 h of exposure to UVR (immediate lesions) is poorly explained by this mechanism because virus reactivation in the ganglion, axonal transport, and skin infection are unlikely to occur so rapidly.

In 1976, Hill and Blythe proposed the skin trigger theory of HSV pathogenesis, which postulates frequent production of virus in the ganglion with transport to the skin and the establishment of microfoci of infection in the epithelial cells of the lip (3). These authors proposed that most of these microfoci are eliminated by host defense mechanisms, but occasionally changes in the resistance of the epithelium, such as those induced by UVR, allow the growth of virus and establishment of a symptomatic lesion. While oral tissue biopsies might detect such microfoci of infection, this would be impractical with human subjects. The detection of HSV in oral secretions prior to UVR-induced herpes labialis would provide both evidence for antecedent peripheral HSV shedding and support for the skin trigger hypothesis.

PCR is a technique ideally suited to examine this issue because of its ability to detect relatively small quantities of viral DNA from viable or nonviable viral particles. Using swabs taken from genital herpes lesions, Cone et al. demonstrated that PCR was more sensitive than viral culture alone in the detection of HSV type 2 (1). Robinson et al. were able to identify 12 of 12 patients with acute herpetic gingivostomatitis by PCR, detecting as few as 45 to 135 virions per sample (8). In addition to enhanced sensitivity, PCR offers other theoretical advantages over cell culture in the detection of noninfectious virus, including incomplete viral particles and viral DNA integrated into the host cell genome. The use of PCR in tandem with cell culture provides a highly sensitive procedure to determine if HSV is excreted into the oral cavity in patients predisposed to experimental UVR-induced herpes labialis.

Twenty-five patients with a history of recurrent herpes labialis following sun exposure were studied. The patients' lips were exposed to UVR as previously described (12). Immediate lesions were defined as those occurring within 48 h of UVR, and delayed lesions were defined as those occurring more than 48 h after UVR. Oral secretion specimens were obtained by swishing 5 ml of sterile Trypticase soy broth with antibiotics in the mouth at the time of exposure to UVR (day 0) and on days 2 and 4 thereafter. Oral secretions were cultured on monolayers of mink lung cells (11). Two hundred microliters of each oral secretion specimen was prepared for PCR by proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. A positive control (1989 HSV type 1 patient specimen) and a negative control (template DNA omitted) were amplified in parallel with unknown oral secretion specimens. Amplification of HSV type 1 DNA was performed in a Perkin-Elmer Cetus thermal cycler with primers (National Bioscience, Hamel, Minn.) from the thymidine kinase gene region corresponding to bp 46620 to 46730 of the genome (5). The presence of amplified product was determined by agarose gel electrophoresis and ethidium bromide staining. Statistical comparisons between groups were made with Fisher's exact test.

Among 28 patients exposed to UVR, 12 (43%) developed herpes labialis within 1 week. Five of these twelve lesions (42%) were apparent within 2 days (immediate lesions), and the remaining lesions (58%) were seen 3 to 7 days following UVR (delayed lesions) (Fig. 1). HSV was cultured after directly swabbing the lesions of 8 of 10 (80%) patients.

Oral excretion of HSV was detected by PCR for 3 of 27 patients on the day of UVR and for 2 of 28 patients 2 days following UVR (day 2) (Table 1). Virus isolation on day 0 was not significantly different among those patients who subsequently developed herpes labialis from virus isolation among those who did not (1 of 11 versus 2 of 16, respectively; $P = 0.44$). On day 4 after UVR, HSV was detected in oral secretions from 8 of 12 patients with herpes labialis and from 1 of 16 patients without herpes labialis ($P = 0.001$).

Five of twelve (42%) patients who developed herpes labialis

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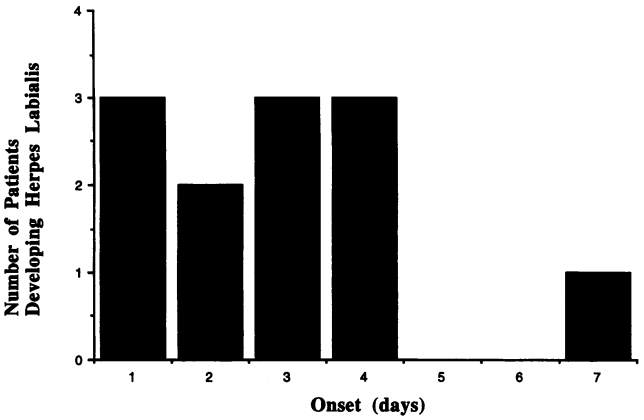

FIG. 1. Onset of herpes labialis lesions following UVR.

following UVR had HSV in oral secretions detectable by both PCR and cell culture, and three additional patients with herpes labialis had HSV in oral secretions detectable only by PCR (8 of 12 [67%]) (Table 2). HSV was detected in oral secretions from two of five (40%) patients with immediate lesions and six of seven (86%) patients with delayed lesions ($P = 0.14$) by PCR. Four of twelve (33%) patients who developed herpes labialis did not have HSV detectable in oral secretions by either cell culture or PCR, although HSV was isolated in tissue culture from swabs taken directly from lesions in three of these cases. Among the 16 patients who did not develop herpes labialis following UVR, 3 (19%) had HSV detected in oral secretions, 2 by PCR only and 1 by PCR and cell culture.

TABLE 1. Detection of HSV in oral secretions following UVR exposure

Patient	Herpes labialis lesion	Result					
		Day 0		Day 2		Day 4	
		PCR	Culture	PCR	Culture	PCR	Culture
B01	Yes	−	−	−	−	+	−
B03	Yes	−	−	−	−	−	−
B05	Yes	−	−	−	−	+	+
B06	Yes	−	−	+	+	+	+
B07	Yes	−	−	−	−	−	−
B09	Yes	−	−	−	−	+	−
B12	Yes	−	−	−	−	−	−
B17	Yes	−	−	−	−	+	+
B21	Yes	−	−	−	−	+	+
B23	Yes	+	−	−	−	+	−
B25	Yes	QNS[a]	QNS	−	−	−	−
B26	Yes	−	−	−	−	+	+
B02	No	−	−	−	−	−	−
B04	No	−	−	−	−	−	−
B08	No	+	−	−	−	−	−
B10	No	−	−	−	−	−	−
B11	No	−	−	−	−	−	−
B14	No	−	−	−	−	−	−
B15	No	−	−	−	−	−	−
B18	No	−	−	−	−	+	−
B19	No	−	−	−	−	−	−
B20	No	−	−	−	−	−	−
B22	No	−	−	−	−	−	−
B24	No	+	+	+	+	−	−
B27	No	−	−	−	−	−	−
B28	No	−	−	−	−	−	−
B29	No	−	−	−	−	−	−
B30	No	−	−	−	−	−	−

[a] QNS, quantity not sufficient for PCR assay.

TABLE 2. Summary of HSV detection results

Group (*n*)	No. of patients (%)			
	+ PCR, − culture	− PCR, + culture	+ PCR, + culture	− PCR, − culture
Developed lesions (12)	3 (25)	0 (0)	5 (42)	4 (33)
No lesions (16)	2 (13)	0 (0)	1 (6)	13 (81)

This study showed that the development of experimental UVR-induced herpes labialis is seldom preceded by demonstrable virus in the oral cavity. At the time of UVR, HSV was detected in only 1 of 11 patients who went on to develop herpes labialis and in 2 of 16 patients who did not. The use of PCR to demonstrate the presence of HSV had enhanced sensitivity over that of tissue culture alone, allowing the detection of virus in 8 of 12 (67%) patients by PCR versus only 5 of 12 (42%) patients by cell culture. In each instance in which HSV was detected by culture, it was also detectable by PCR. The rates of HSV detection in oral secretions among patients who developed immediate and delayed lesions were similar (40 and 75%, respectively; the difference is not significant).

This study confirms the occurrence of rapidly developing or immediate herpes labialis lesions within 48 h of experimental UVR. The proportion of immediate lesions in this study was 42%, compared with 26 and 14% in previous studies using this model (9, 12). In contrast, in a study of UVR-induced genital herpes recurrences by Rooney et al., no site-specific lesions were seen until 3 days after irradiation (10). This observation may be due to differences in the mechanisms of reactivation of the viruses (HSV type 1 versus HSV type 2) or physiologic differences between the two body sites, such as greater distance from the sacral ganglia to the skin than from the trigeminal ganglion to the lip.

The high frequency of virus in oral secretions during the presence of delayed lesions (on day 4, six of seven cases were positive) is consistent with concurrent, parallel viral seeding of the oral cavity and lips from a neural source. These results are similar to those obtained by culturing oral secretions after trigeminal nerve surgery (6, 7). The detection of HSV in oral secretions at the time of herpes labialis may also be explained by the inoculation of saliva by lip lesions. In contrast, virus was not commonly isolated from oral secretions at the time of immediate lesion onset (on day 2, one of five cases), suggesting that another mechanism may be involved in the pathogenesis of this lesion subset.

We consider the results of this study to weigh against the skin trigger hypothesis of herpes recurrences since HSV was not detected in oral secretions with increased frequency on day 0 among those patients who subsequently developed UVR-induced herpes labialis. These conclusions are tempered by the possibility that extremely small amounts of virus may have been present at levels below the detection threshold of our PCR assay.

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