

## Pathogenesis of Herpes Simplex Labialis: Correlation of Vesicle Fluid Interferon with Lesion Age and Virus Titer

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Of 51 patients with herpes simplex labialis, 50 had detectable interferon (IFN) in samples of lesion vesicle fluid. The median titer of vesicle fluid IFN was 8,200 U, and the range of values was 400 to 63,600 U. The amount of vesicle fluid IFN was correlated with lesion age ( $r = 0.32$ ,  $P = 0.024$ ) and vesicle fluid virus titer ( $r = 0.59$ ,  $P = 0.00004$ ), but not with the clinical severity of the disease. The presence of vesicle fluid IFN (1,500 to 28,600 U) in 15 lesions less than 12 h old emphasizes the need for early treatment in studies of antiviral agents for herpes simplex labialis.

In a recent report (6), we identified high titers of antiviral activity in vesicle fluid from 18 of 19 otherwise healthy patients experiencing an episode of recurrent herpes simplex labialis. This antiviral activity was neutralized by antibody against virus-induced human leukocyte interferon (IFN- $\alpha$ ) in eight patients studied. The finding of large quantities of IFN in herpes vesicles indicates that endogenous lesion IFN could play a role in the inhibition of viral replication and the natural resolution of the disease. In the present report, we used vesicle fluid IFN determinations in an expanded series of patients to describe the correlation of vesicle fluid IFN titer with patient characteristics, lesion age, and several measures of disease severity.

### MATERIALS AND METHODS

**Patient population.** The study subjects were individuals who visited the University of Utah Herpes Study Clinic. Patients with a history of recurrent, transient episodes of vesicular lesions on the external surface of the lips or peroral area were considered eligible. Many volunteers had been patients in prior studies of topical antiviral therapy (4, 9, 10). A questionnaire was completed on each subject to document the characteristics of the patient's recurrent episodes.

**Collection of herpes labialis vesicle fluid.** Vesicular lesions were broken open with a sterile needle. Vesicle fluid was drawn up by capillary flow into a graduated micropipette, measured, and then diluted in 200  $\mu$ l of tissue culture medium as described previously (6).

**Identification and quantitation of HSV.** HSV was identified in vesicular fluid by the production of the characteristic cytopathic effect after inoculation of transformed guinea pig cells. Quantitation of virus in vesicle fluid was performed by plaque assay as described previously (5). Because of the small volumes of vesicle fluid available and the dilution in medium at the time of collection, amounts of virus less than  $10^4$ -<sup>5</sup>

PFU/ml could not be detected or quantitated. Presence or absence of HSV was determined in some patients by culture of lesion swab specimens.

**IFN assay.** Identification and quantitation of IFN in specimens of diluted vesicle fluid were performed by plaque reduction in microtiter plates as described previously (2, 6). The lowest level of IFN activity which could be detected in the assay was 3 U. The National Institutes of Health reference human IFN, which has a designated titer of 20,000 U, had a titer of 23,800 U in this assay system. Assay results were corrected for the degree of dilution and expressed as units of vesicle fluid IFN.

**Statistics.** The relationship between two variables was examined by calculating the Pearson correlation coefficient ( $r$ ). The probability ( $P$ ) that the observed correlation was nonrandom was also determined. A  $P$  value of less than or equal to 0.05 was considered significant (1).

### RESULTS

Data from a total of 51 patients were analyzed, including the 19 subjects from our earlier report (6). Sixty-nine percent of the patients were women, and all were adult Caucasians. The average age was 31 years. The subjects had experienced recurrent herpes labialis for an average of 18 years, and the median number of recurrent episodes over the past year was 4 (range, 1 to 12). All study subjects had typical vesicular lesions of recurrent herpes labialis on the day vesicle fluid specimens were collected. The volume of vesicle fluid obtained ranged from 0.5 to 15  $\mu$ l.

Of the 51 patients, 50 had demonstrable IFN in vesicle fluid specimens. The median vesicle fluid IFN titer in IFN-positive specimens was 8,200 U, and the range was 400 to 63,600 U. Of the 51 patients, 45 had HSV in their lesions at

the time of vesicle fluid IFN determination; HSV could be quantitated by serial dilution of vesicle fluid in 42 subjects and was identified qualitatively from a lesion swab specimen in three others. We were unable to confirm the diagnosis in six patients, one of whom was the subject without detectable vesicle fluid IFN. This latter individual had a lesion which lasted 7 days and had experienced two episodes per year for six years.

Pearson correlation coefficients were determined to evaluate the relationship between vesicle fluid IFN levels and patient or lesion characteristics. As shown in Table 1, no significant correlations were obtained between vesicle fluid IFN titers, patient age, the frequency of herpes labialis episodes, the size of the lesion at the time of sampling, and the lesion healing time. In contrast, significant positive correlations were obtained between  $\log_{10}$  vesicle fluid IFN levels and the age of the lesion at the time of sampling and  $\log_{10}$  vesicle fluid IFN titers and the corresponding vesicle fluid virus titers (Table 1 and Fig. 1 and 2). Fifteen subjects had their vesicle fluid sampled within 12 h of lesion onset; all specimens had demonstrable IFN, ranging from 1,500 to 28,600 U (Fig. 1). Men and women had comparable titers.

## DISCUSSION

This study confirms and extends our initial observation that patients with herpes labialis

TABLE 1. Correlation between vesicle fluid IFN titer and other selected patient and lesion variables

Correlation between $\log_{10}$ vesicle fluid IFN titer and:	No. of subjects <sup>a</sup>	Correlation statistics	
		<i>r</i>	<i>P</i>
Patient age	47	0.14	0.36
No. of episodes/yr	47	-0.09	0.54
Characteristics of the lesions:			
Size when sampled	47	0.01	0.97
Healing time	49	0.08	0.57
Age when sampled <sup>b</sup>	50	0.32	0.024
$\log_{10}$ vesicle fluid virus titer	42	0.59	0.00004

<sup>a</sup> The one subject without detectable vesicle fluid IFN was not included in this analysis. In addition, some patient or lesion data were not available in a small number of cases. Only instances where HSV could be quantitated in the vesicle fluid ( $\geq 10^{4.5}$  PFU/ml of HSV in vesicle fluid) were used to correlate IFN and virus titer.

<sup>b</sup> From onset of lesion induration.

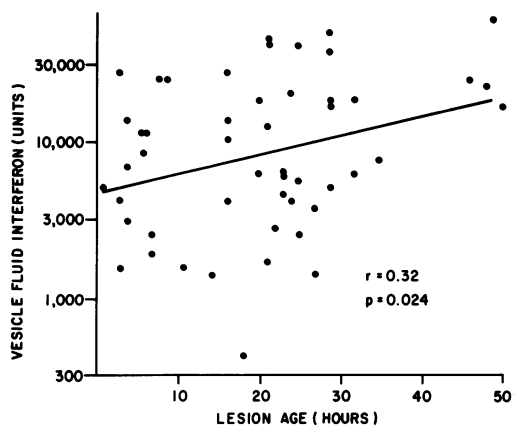


FIG. 1.  $\log_{10}$  plot of vesicle fluid IFN units versus lesion age for the 50 patients with detectable vesicle fluid IFN; *r* is the Pearson correlation coefficient, and *P* is the probability that this relationship is nonrandom.

have high titers of IFN in vesicle fluid (6). We have now examined the relationship of vesicle fluid IFN to patient characteristics, lesion age, and different measures of severity of herpes labialis. Vesicle fluid IFN was found in subjects with early lesions which had been present for 12 h or less. Vesicle fluid IFN significantly increased with lesion age and was strongly correlated with the quantity of virus in vesicle fluid. No correlations were observed between vesicle fluid IFN and any clinical aspect of herpes labialis, including frequency of episodes and size or healing time of the lesion sampled.

If the amount of IFN produced plays a major role in recovering from herpes labialis or reduc-

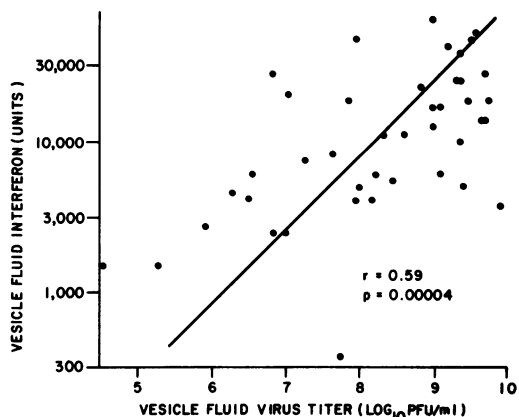


FIG. 2.  $\log_{10}$  plot of vesicle fluid IFN units versus vesicle fluid virus titer for 42 patients wherein HSV could be quantitated in vesicle fluid; *r* and *P* are as for Fig. 1.

ing the frequency of recurrent episodes, one might expect patients with the highest titers of IFN in vesicle fluid to have the smallest lesions, the shortest healing time, and the lowest frequency of recurrent episodes. This was not found, and IFN titers paralleled the quantity of HSV, suggesting that IFN production was reflecting the amount of viral replication rather than inhibiting it. Inability to identify a relationship between lesion IFN content and severity of recurrent disease was not entirely unexpected. Despite more than 20 years of intensive experimentation, the role of IFN in the resolution of acute infection is predominantly inferential (13). A lack of association between the clinical severity of herpes labialis and lesion IFN content can be interpreted in several ways: (i) lesion IFN has no role; (ii) production of lesion IFN is a significant variable early in lesion formation but is not accurately reflected by the quantity of IFN in vesicle fluid; or (iii) lesion IFN is protective and prevents progressive lesion enlargement, but production of IFN occurs to an equal extent relative to virus titers in healthy, normal subjects, and other factors are responsible for the characteristic variable severity of the disease. Variable lesion size in normal subjects could be determined largely by the quantity of virus released from nerve endings and the number of neurons excreting virus, and lesion IFN might serve to maintain the lesion within borders which already have been defined by the distribution of infected neurons.

There are multiple previous observations supportive of the concept that local IFN production has a role in recovery from herpes labialis. Titers of IFN in vesicle fluid are considerably higher than that required to inhibit replication of HSV in human cell cultures (7). In addition, exogenously administered human leukocyte IFN reduced the intensity of reactivated oral HSV disease after trigeminal nerve root surgery (8). In immunosuppressed patients with herpes zoster, continued progression of vesicular lesions was associated with absence of detectable IFN in vesicle fluid, whereas resolution of the lesions was accompanied by the appearance of IFN in vesicle fluid (12). Presumably, the same phenomenon may occur in immunosuppressed patients with progressive herpes labialis. Several investigations in experimental animals provide evidence that IFN is important in recovery from primary HSV infections. Treatment of mice with anti-mouse IFN antibody markedly enhances susceptibility to HSV infection (3). Mice that are genetically susceptible to HSV infection produce very low levels of IFN in the serum after intraperitoneal challenge with the virus, and their spleen cells produce very low levels of IFN after exposure to inactivated HSV

in vitro. In contrast, genetically resistant mice produce high levels of serum and spleen cell IFN in response to HSV (14).

The identification of 1,500 to 28,600 U of IFN in vesicle fluid in lesions less than 12 h of age provides additional evidence of the rapid natural evolution of herpes labialis (11). If antiviral treatment regimens are to curtail lesion development more effectively than that which will be accomplished by normal host defense mechanisms, chemotherapy clearly must be given before the time that high-titered endogenous IFN activity appears. Herpes labialis treatment protocols should target prodromal and erythema-stage lesions, and this can be accomplished by prospectively dispensing medication to patients and beginning treatment at home (Spruance and Crumpacker, *Am. J. Med.*, in press).

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