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Rapid Identification and Typing of Herpes Simplex Virus Types 1 and 2 by a Direct Immunofluorescence Technique

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An immunofluorescence (FA) technique has been developed which can identify herpes simplex virus (HSV) in clinical specimens and also type the virus directly as type 1 or type 2. This test, first applied to cervicovaginal specimens obtained from 80 mice genitally inoculated with HSV, indicated a sensitivity approaching 80% in comparison to standard viral isolation methods. A similar sensitivity was found when the test was applied to 185 clinical specimens with adequate cells for staining, which were obtained from a variety of sites of patients with suspect herpetic infection. In only 1 of 6 specimens positive by both FA and culture methods was the HSV type wrongly identified by the FA technique. There were also six specimens which were negative by culture methods but positive by the FA test, indicating a specificity of 91%. It is likely that these are not instances of false-positive tests but of other factors which may have resulted in negative viral isolations by culture methods. As more specific reagents become available, it is anticipated that the FA technique will have wider usage in diagnostic laboratories for the identification and typing of HSV types 1 and 2.

Immunofluorescence (FA) techniques have been successfully utilized for the identification of herpes simplex virus (HSV) in various types of clinical specimens, such as skin and eyes (1-4, 11, 13). More recently, the FA technique was adapted as a means of differentiating HSV types 1 and 2 from tissue culture isolates (5). Therefore, it appeared of interest to determine if typing, as well as identification, could be done directly in clinical specimens obtained from patients with suspect HSV infections. The development of such a test would be particularly useful in view of the increased recognition of differences in the clinical manifestations and modes of transmission of HSV types 1 and 2 (7, 8).

MATERIALS AND METHODS

Mouse studies. To evaluate whether the direct typing of HSV by FA techniques could be accomplished from human clinical specimens, experiments were first performed in mice. Random-bred Swiss female mice were inoculated as previously described (9) by the intravaginal route with cotton pellets soaked with HSV type 2 virus (Ellison strain from the cervix). The pellets were removed the following day. At intervals from the 3rd to 5th postinoculation days, each mouse was swabbed intravaginally with sterile swabs premoistened with phosphate-buffered saline (PBS), *p*H 7.2. The first swab was inoculated onto primary rabbit kidney (PRK) tissue culture to determine whether infection in the mouse had been established. The second swab was pressed onto two separate ring areas of a glass slide to obtain cells for FA studies. The cells were air-dried and fixed in prechilled acetone for 15 min at −20 C. After fixation, cells were tested immediately or stored at −70 C for later staining.

Method of immunofluorescence staining. Fluorescein-conjugated anti-HSV type 1 and type 2 antisera, prepared as previously described (5), were used at dilutions which have been found earlier to permit differentiation of the two HSV types from tissue culture isolates. At these dilutions, anti-type 1 conjugated sera reacted only with type 1 HSV-infected cells, whereas anti-type 2 conjugated sera reacted with both type 1- and type 2-infected cells. One drop of anti-1 conjugate, mixed with a 1:64 dilution of rhodamine-labeled normal rabbit serum, was placed on one of the two ring spots on each slide, and one drop of the anti-2 conjugate mixed with rhodamine counterstain was placed on the other spot on the slide. The slides were incubated at room temperature for 20 min in a moist chamber and then given four 10-min washes with PBS. Slides were air-dried and mounted in glycerol buffer and examined with a Leitz Ortholux microscope, with a BG-12 primary filter and OG-1 secondary filter.

TABLE 1. *Culture and FA methods for the detection and typing of mice genitally inoculated with type 2 herpes simplex virus*

FA technique	Culture method		
	Positive	Negative	Total
Positive	24[a]	9[a]	33
Negative	7	40	47
Total	31	49	80

[a] All reactions were consistent with HSV type 2.

Method for isolation and identification by tissue culture. The comparison specimen tubes for tissue culture which were inoculated on PRK in triplicate were examined daily for cytopathic effect. Positive cultures were confirmed as HSV by previously described techniques (5).

Human clinical specimen studies. Specimens were obtained from patients seen in a private dermatological clinic in San Antonio, Tex., and in a municipal hospital or venereal disease clinic in Atlanta, Ga. Tests were done on 213 specimens from a wide variety of body sites, including genitalia, eyes, face, and hands, in patients with suspect HSV infections. Two samples were taken from each lesion: the first, obtained with a swab, was placed in Hanks balanced-salt solution (BSS), and the second sample, obtained by light curettage or with a swab, was placed on each of two ringed areas on a slide. After air-drying of the specimens, the slides were placed in acetone for 15 min at −20 C. In case of specimens obtained in San Antonio, both the Hanks material and the slides were shipped to Atlanta in dry ice, where they were stored at −70 C before processing. Smears obtained in Atlanta were processed the same day or at various intervals after storage at −70 C. The culture isolation methods, and HSV identification and typing in the smears obtained from the human clinical specimens, were done as described for the mouse studies.

RESULTS

Mouse studies. Table 1 summarizes results obtained on 80 mice inoculated with HSV type 2. In 40 of the 80 mice, no virus was isolated by culture techniques, and the virus could not be demonstrated by FA methods. (It is not unusual, from past experience, to obtain approximately a 50 to 80% infectivity rate after attempted intravaginal inoculation with type 2 strains.) Of 31 mice shown to be genitally infected by culture, 7 were not detected by FA methods, indicating a sensitivity of 77% for the FA technique. In 33 instances in which the FA technique gave positive results for HSV, the virus was recoverable by culture in 24 cases (73%). In at least five of the nine culture-negative, FA-positive mice, cultures taken a few days later were positive for HSV. All positive FA smears demonstrated HSV type 2 by the FA typing method. These preliminary results in mice were encouraging enough to attempt application of the FA technique for identification and typing of HSV types 1 and 2 in human clinical samples.

Human clinical specimen studies. Table 2 presents the correlation between FA and culture results in 185 clinical specimens obtained from a variety of sites; not included in this comparison are 28 additional specimens with inadequate cells for FA staining. About half of the 185 specimens originated from genital sites and half from nongenital sites. Culture and FA tests were positive for HSV type 2 in 39 cases and for HSV type 1 in 20 cases. There were 103 specimens with negative culture and FA results. One specimen from a

TABLE 2. *Culture and FA methods for the detection and typing of HSV in specimens obtained from patients with suspect HSV type 1 or type 2 infections*

Culture/FA results	Penis or urethra	Cervix or vulva	Skin below waist (thighs, legs, buttocks)	Skin above waist (chest, face, or hands)	Mouth or lips	Eyes	Total
Type 2 HSV/type 2 HSV	17	12	10				39
Type 1 HSV/type 1 HSV				10	8	2	20
Type 1 HSV/type 2 HSV					1		1
Negative for HSV/negative for HSV	41	20	9	20	9	4	103
Type 2 HSV/negative	9	1					10
Type 1 HSV/negative	1			1	4		6
Negative/type 2 HSV	1	1					2
Negative/type 1 HSV				2	2		4
Total	69	34	19	33	24	6	185[a]

[a] Twenty-eight additional specimens with inadequate cells for FA staining are not included.

TABLE 3. *Summary of culture and FA methods for the detection and typing of HSV in specimens obtained from patients with suspect HSV type 1 or type 2 infections*

FA technique	Culture method		
	Positive	Negative	Total
Positive.....	60[a]	6	66
Negative.....	16	103	119
Total	76	109	185

[a] In 59 cases, HSV type was correlated by both FA and culture methods.

lip lesion, positive by both culture and FA methods for HSV, was found to be type 1 by the culture method and type 2 by the direct FA technique. There were 16 cases with positive HSV isolation by culture but with negative FA tests; 10 were type 2 and 6 were type 1. There were also six cases with negative viral isolations but positive FA tests for HSV; two were type 2 and four were type 1.

Table 3 summarizes the number of cases in which FA and cultures were both positive or both negative, as well as instances in which one technique yielded positive and the other yielded negative results, or vice versa. Of 76 cases positive by culture methods, 16 were negative by FA, indicating a sensitivity of 80%. It should be noted, however, that of 28 specimens with inadequate cells for staining, 16 were positive by culture; if these are included, the sensitivity of the FA test would be 65%. Of the 60 FA-positive cases, only 1 disagreed with culture results regarding HSV type. There were also 6 specimens which were negative by culture of 66 which were found to be positive with the FA technique, indicating a specificity of 91%.

DISCUSSION

Any method attempting to demonstrate HSV-induced changes in cells, such as cytological or immunofluorescence techniques, is beset with the problem of obtaining adequate smears. This problem is not only one of having a sufficient number of cells of any kind on the slide but also of having enough virus-infected cells on the smears to be examined. In previous studies attempting to isolate HSV from clinical specimens by culture methods, we have found that the most adequate specimens are those obtained from fresh herpetic vesicles before their ulceration (7). With immunofluorescence methods, it becomes additionally important to obtain cells which contain detectable virus (around 6 to 40 hr after initial infection of the cell).

It is difficult to know, therefore, how many of the 16 specimens with positive cultures, but negative FA results, contained a sufficient number of cells at the right stage of infection (Table 3). It should be noted that 28 specimens, of which 16 were positive by culture, in addition to the 185 recorded in Table 2, had been received with such low numbers of cells that it was not possible to perform an adequate FA test. The interval between the time of obtaining the clinical specimen and performing the FA test may have also influenced the results. In several instances, in both the mouse and human specimens, the culture-positive, FA-negative slides included cases in which the FA test was performed more than 1 month after collection of the original specimen.

The mouse data also suggest that the FA technique may detect HSV in specimens with negative culture isolations. In a corollary study (10) which compared shipment of clinical specimens in Hanks BSS in dry ice with three other transport media shipped at ambient temperature, virus could not be isolated from about 10% of specimens obtained in Hanks which were positive in one or more of the other media. In the present study, five of the six specimens with positive FA tests and negative cultures (Table 2) were obtained from cases with typical herpetic infections. It should also be noted that the virus type in the six specimens was that which would be anticipated on the basis of site of infection (5, 6). Thus, the two type 2 FA-positive, culture-negative specimens originated from penile or cervical lesions, and the four type 1 FA-positive, culture-negative specimens originated from mouth or lips and skin above the waist. The 1 case of 60 in which the FA typing result did not corroborate the culture results may be attributed to the problem that two areas on one slide may have different numbers of infected cells. The recent availability of more specific conjugates (5*a*) for type 1 and type 2 herpes might preclude this difficulty in the future.

The increasing need for ready availability of laboratory confirmation and typing of HSV from skin, genital, and other sites is based on recent clinical differentiation between the two HSV types (6, 8). The expanding role of genital herpes as a venereal disease and as a possible cause of fetal and newborn infections and its possible association to cervical cancer have increased the need for such improved laboratory tests. The advent of possible antiviral chemotherapy for serious HSV infections (4*a*) and the possible difference in the susceptibility of the two HSV types to some of the antiviral drugs (14) have also stimulated efforts to obtain rapid HSV diagnosis and typing. Such laboratory capability would be considerably augmented by relatively simple and rapid methods

of identification and typing. The results of this study suggest that the FA technique may provide a possible answer to this need.

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