Diagnosis of Herpes Simplex Virus Infection by Immunofluorescence

LARRY H. TABER,[1] * FRANCOISE BRASIER,[2] ROBERT B. COUCH,[2] STEPHEN B. GREENBERG,[2] DAN JONES,[3] AND VERNON KNIGHT[2]

Virus Diagnostic Laboratory, The Methodist Hospital, Houston, Texas 77030

The utility of the indirect immunofluorescent antibody (IFA) technique for diagnosis of herpes simplex virus (HSV) infection was examined by testing specimens for this agent from 31 patients with encephalitis or meningitis, 17 with conjunctivitis, 19 with genital disease, and 1 with genital disease and meningitis. Brain biopsy tissue from four patients with encephalitis was positive by IFA and virus culture for HSV. Leukocytes in cerebrospinal fluid from these four patients and one with HSV meningitis were also positive by IFA, but virus isolation attempts on the fluid were all negative. Conjunctival scrapings from two patients with conjunctivitis were positive for HSV by both IFA and virus culture. Eleven of 12 culture-positive lesions of herpes progenitalis were positive by IFA, and 1 dark field-positive syphilitic chancre was also positive for HSV by both IFA and culture. Evidence for specificity of the results was provided by internal controls in each test and negative results from patients with other diagnoses. Thus, the IFA technique constituted a rapid, sensitive, and specific diagnostic method for the diagnosis of HSV infections.

Immunofluorescent antibody techniques have been used to identify various viral antigens in tissue culture (15), to detect viral antibodies in sera (17), and to a lesser extent to diagnose viral illness by examination of clinical specimens (7). Both the direct and indirect fluorescent antibody (IFA) techniques have been used to examine specimens from a variety of sites, for herpes simplex viral antigens, including the cornea, brain, skin, and genitalia (1, 4–6, 9, 12, 14, 16, 17). Recently, Dayan and Stokes (2) reported the application of the IFA technique for the detection of herpes simplex viral antigen in cerebrospinal fluid (CSF) leukocytes from patients with herpes simplex virus (HSV) encephalitis.

In the present study, we compared the specificity and sensitivity of the IFA technique with that of virus isolation in tissue culture for the diagnosis of HSV infections of the central nervous system, eye, and genital tract.

MATERIALS AND METHODS

Patients and source of specimens. Specimens were collected as appropriate from the central nerv-

[1] Present address: Department of Pediatrics, Baylor College of Medicine, Houston, Tex. 77030.

[2] Present address: Department of Microbiology and Immunology, Baylor College of Medicine, and The Methodist Hospital, Houston, Tex. 77030.

[3] Present address: Department of Ophthalmology, Baylor College of Medicine, Houston, Tex. 77030.

ous system, eye, or genital tract of patients with a clinical diagnosis of HSV infection (results of testing of specimens from 12 of these patients were included in an earlier report [10]). Specimens were also collected from the same sites of patients with other diseases. Central nervous system specimens consisted of brain biopsy tissue, CSF leukocytes, or both; specimens from the eye were conjunctival scrapings; genital specimens were either vesicular fluid or scrapings of lesions. All specimens were processed immediately.

Processing of specimens for IFA tests. CSF leukocytes were obtained from centrifuged sediments. These cells, brain tissue, conjunctival scrapings, or vesicular fluid were applied directly onto glass slides. The slides were air-dried, fixed in cold acetone (−20 C) at least 10 min, and air-dried again. Fixed preparations were stored no longer than 24 h at −70 C before IFA tests were performed.

Preparation of antiserum and conjugate. Hyperimmune HSV antiserum prepared in guinea pigs with the type 1 McIntyre strain was obtained from Microbiological Associates (lot no. 6978). The HSV antiserum was absorbed with an equal volume of a 15% suspension of baby beef brain in phosphate-buffered saline (PBS) at room temperature for 40 min. To determine the specificity of the HSV antiserum, uninfected tissue culture cell lines, including human embryonic lung fibroblast (WI-38), African green monkey kidney (Vero), and human embryonic kidney, were harvested, affixed to microscope slides, and tested with the HSV antiserum plus the conjugate at a 1:40 dilution. These uninfected cell lines showed no fluorescence when tested with the HSV

antiserum at dilutions of 1:8, 1:16, 1:32, 1:64, and 1:128.

Tissue culture cell lines including human embryonic kidney and WI-38 were infected with the following viruses: coxsackievirus type B5, echovirus type 4, adenovirus (untyped), and varicella-zoster. These cells were then harvested and affixed to microscope slides for testing with the HSV antiserum (1:64) plus conjugate (1:40). None of the infected cells showed any fluorescence. Absorbed antiserum was stored in 0.2-ml volumes at −20 C.

The conjugate, rabbit antiserum to guinea pig 7S gamma globulin, was obtained from Hyland Laboratories (lot no. 2130X001A1). Before use, it was absorbed with WI-38 cells in the following manner. Tube cultures of WI-38 cells were washed with PBS and all the fluid was removed. A volume of 0.2 ml of conjugate was added to each washed tube. Each tube was shaken at 37 C for 2 h and then allowed to refrigerate overnight. The conjugate was then centrifuged lightly to remove any free cells. The conjugate was then diluted in a 1:200 dilution of rhodamine in PBS. Noninfected tissue cultures including WI-38 and human embryonic kidney were harvested and affixed to slides. These cells were then tested with the conjugate, and no fluorescence was seen at a dilution of 1:20 or greater. Only minimal nonspecific fluorescence was seen at 1:5 or 1:10 dilutions of the conjugate. To determine the appropriate dilutions in PBS of antiserum and conjugate for testing of specimens, grid titrations were performed using cover slip preparations of WI-38 cells that had been infected with a type 1 strain of HSV. The highest dilutions of the antiserum (1:64) and conjugate (1:40) that gave a 4+ apple green fluorescence, which was readily distinguishable from the nonspecific fluorescence seen at the lower dilutions, were the dilutions chosen for the testing of clinical specimens. Normal guinea pig serum that lacked complement-fixing and neutralizing antibody against HSV was used along with the conjugate in the same manner as the HSV antiserum as a further determination of any nonspecific fluorescence, since this normal serum was used as a control for each clinical specimen tested.

IFA testing. Slides for IFA tests were placed in a tray lined with wet paper towels. Each specimen was covered with antiserum. The tray was then covered with foil and placed in an incubator at 37 C for 30 min. Specimens were washed three times for 10 min each with PBS (pH 7.4), followed by one quick wash with distilled water. The slides were air-dried, and the specimens were covered with conjugate diluted 1:40 in a 1:200 dilution of rhodamine in PBS. The slides were incubated for 30 min, and the washing procedure was repeated. After air drying, the slides were covered with buffered glycerol and examined for fluorescence. Control slides were prepared with each set of specimens, using normal guinea pig serum that lacked complement-fixing and neutralizing antibody against HSV.

All slides were examined for fluorescence, employing a Leitz Ortholux microscope with a Ploem illuminator, by at least two of us, and one of us (FB) examined the slides without knowledge of the clinical diagnosis.

HSV isolation was attempted in either primary rabbit kidney or WI-38 cell cultures by previously described procedures (3). All isolates were identified as HSV by neutralization of 32 to 100 50% tissue culture infectious doses by 20 antibody units of the HSV antiserum described above. Specific typing of each HSV isolate was not performed.

RESULTS

Results of IFA and virus isolation attempts are shown in Table 1. In 17 of 18 patients where HSV was the probable cause of active disease, the IFA test indicated the presence of HSV antigen. In one patient with clinically diagnosed herpes progenitalis, virus was cultured from the lesion but the IFA test was negative. However, in an additional patient with a dark field-positive syphilitic chancre, both the IFA and culture tests revealed the presence of HSV in scrapings from the chancre. There were no instances of a positive IFA and/or positive culture on specimens from the other 49 patients. Moreover, it seems likely that, in the patient with primary syphilis, two infections were occurring simultaneously.

Five patients proved to have HSV infection of the central nervous system, four with encephalitis and one with herpes progenitalis, cutaneous lesions, and meningitis. Although viral cultures of the CSF were negative, HSV was recovered from the genital and skin lesions in the latter case. As noted in the table, brain biopsy tissue (temporal lobe) from those patients with a clinical diagnosis of HSV encephalitis was positive for HSV in both IFA and culture tests. In all cases fluorescing cells were easily recognizable in the brain tissue so that no difficulty in the interpretation of the IFA test was encountered. In the four patients with encephalitis of unknown etiology, HSV was initially considered a possibility, but in each instance the typical clinical findings were not present (8). Histology of brain tissue, culture for viruses and other microorganisms, and numerous other diagnostic tests did not provide a diagnosis in those patients.

Of particular interest is the occurrence in five patients of a positive IFA test of CSF leukocytes despite negative culture results for HSV. Four of these five patients subsequently had brain biopsies that were positive for HSV by IFA and virus isolation; in the fifth patient, specimens of genital and cutaneous lesions were also subsequently positive for HSV. The IFA staining pattern included both nuclear and cytoplasmic fluorescence also seen in cells from other sites. In some instances the CSF was centrifuged for cells and the supernatant fluid was used for inoculation of tissue cultures, but some speci-

TABLE 1. *Detection of HSV in clinical specimens by IFA and virus culture*

Type of specimen	Final diagnosis	No. of patients	No. of IFA positive	No. of culture positive
Brain tissue	HSV encephalitis	4	4	4
	Encephalitis of unknown origin[a]	4	0	0
Spinal fluid cells	HSV encephalitis[b]	4	4[c]	0
	Other viral encephalitis[d]	10	0	0
	HSV meningitis[e]	1	1	0
	Bacterial and fungal meningitis[f]	10	0	0
	Noninfectious encephalitis[g]	3	0	0
Conjunctival scrapings	HSV conjunctivitis	2	2	2
	Adenoviral conjunctivitis[h]	7	0	0
	Other cases of inflammatory conjunctivitis[i]	8	0	0
Genital scrapings or vesicle fluid	Herpes progenitalis[j]	12	11	12
	Primary syphilis (dark field positive)	6	1	1
	Chancroid and drug eruption	2	0	0

[a] Final results of all tests did not provide an etiological diagnosis, although HSV encephalitis was initially considered a possibility in each case.

[b] Same patients from whom brain tissue was obtained.

[c] Includes one patient who was negative by IFA initially but positive at a later date.

[d] Encephalitis in association with varicella-zoster, congenital cytomegalovirus, or enterovirus; subacute sclerosing panencephalitis, Reyes syndrome, and postviral encephalitis.

[e] Virus recovered from genital and skin lesions.

[f] Includes two patients with brain abscess.

[g] Leukemia, collagen disease, and lead encephalopathy.

[h] Adenovirus isolated from all specimens.

[i] Chlamydia, allergy, drug reaction, two patients with non-HSV corneal ulcers.

[j] Includes the patient listed above with HSV meningitis.

mens contained cells and these were also negative when tested in tissue culture. This failure to recover HSV from the CSF of patients with HSV encephalitis has been noted earlier, although CSF cultures apparently may be positive when the patient has meningitis caused by HSV type 2 (13).

Conjunctival scrapings from two patients with unilateral follicular conjunctivitis were positive for HSV by both IFA and virus culture. One of these subsequently developed HSV epithelial keratitis.

DISCUSSION

The present study has shown the IFA technique to be as sensitive as virus isolation in tissue culture for the detection of HSV. Specificity of the IFA test in diagnosis of HSV infection is indicated by the occurrence of positive results in all but one of the patients from whom virus was recovered and the absence of positive results in patients who had other diagnoses proven. These findings, in combination with the brief time of 3 to 4 h required to perform a test, recommend this method for diagnosis of HSV infection.

Critical to the use of IFA is the need to obtain suitable specimens for testing. The specimens from lesions must contain cells in adequate numbers for visualization. However, even with good specimens, it is possible that virus cultures might be more sensitive than IFA in later stages of HSV-induced disease. All specimens in the present study were tested as soon as possible after the patient presented them to the physicians.

The availability of an immediate method for definitive diagnosis is an asset in the management of acute follicular conjunctivitis, most commonly caused by HSV, adenovirus, or chlamydial agent. Herpetic follicular conjunctivitis without cutaneous or corneal involvement cannot be distinguished from early adenoviral conjunctivitis. Because of the potential intensification of HSV infection by topical corticosteroids, caution against their injudicious use should be exercised. The two IFA and culture-positive patients in this series were both initially suspected of having adenoviral conjunctivitis. Prompt identification of HSV by IFA permitted specific antiviral therapy.

Most cases of primary syphilis do not present

a problem in diagnosis, since a high proportion of chancres are dark field-positive for treponemes. However, HSV is a more common cause of genital lesions than *Treponema pallidum,* and atypical syphilitic chancres and other non-syphilitic "soft ulcer" lesions are commonly encountered. The availability of immediate diagnostic methods for HSV should aid in the treatment and subsequent evaluation of such patients.

Of particular interest is the confirmation of the results of Dayan and Stokes on the testing of CSF cells from cases of HSV encephalitis by the IFA method (2). They obtained evidence of HSV antigen in CSF leukocytes from 11 of 12 patients with this illness, and we obtained similar results in our 4 patients. Only three of our four patients were positive at initial testing, a finding that may be attributable to differences in duration of disease, since Dayan and Stokes indicated that increasing duration of disease increased the likelihood of obtaining a positive IFA test with CSF cells. Although they did not report results of virus cultures on the same specimens, our failure to recover virus from CSF specimens confirms previous reports (11) and suggests that this dichotomy of positive IFA and negative virus recovery may be a characteristic of HSV encephalitis.

ACKNOWLEDGMENTS

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