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Use of Immunoperoxidase for Rapid Diagnosis of Mucocutaneous Herpes Simplex Virus Infection

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The indirect immunoperoxidase method was applied to the rapid diagnosis of mucocutaneous herpes simplex virus infection. Specimens were obtained directly from lesions at various stages in their evolution. The immunoperoxidase method identified herpes simplex virus-infected cells in 91% of vesicular lesions which were positive by standard virological culture. At later stages, the number of cases that could be diagnosed decreased appreciably. The immunoperoxidase method appears to be a rapid and reliable method for the diagnosis of early herpes simplex virus infection.

The introduction of the immunoenzyme technique for the immunological identification of many viral antigens has broadened our capability for rapid viral diagnosis. The majority of studies have involved tissue culture isolates, but the ultimate aim is to identify viral antigens directly in clinical samples. The rising incidence of genital herpesvirus infection and the possibility of future treatment modalities requires a rapid and relatively simple method for accurate laboratory diagnosis.

Immunofluorescence has been used very successfully by some investigators for the recognition of herpes simplex virus antigen directly in clinical samples (4, 5, 9). However, the attractive advantages of an immunoenzymatic method (2) prompted our studies. The indirect immunoperoxidase (IP) method has already proved satisfactory for the detection of herpes simplex virus antigen in brain tissue in cases of encephalitis (3). The practicality of the method has also been demonstrated in mucocutaneous infections in a small number of cases (8). This present study investigates the applicability of the same method for the routine diagnosis of mucocutaneous herpes simplex virus infection in a clinical setting.

MATERIALS AND METHODS

Patients and source of specimens. Specimens were collected from mucocutaneous lesions from patients attending the Genital Herpes Clinic, Harborview Medical Center, Seattle, Wash.; in-house patients at Children's Orthopedic Hospital and Medical Center, Seattle, Wash.; and patients referred to the Clinical Virology Laboratory, Department of Laboratory Medicine, University of Washington, Seattle. Cervical lesions were not included in this study.

Many different individuals were involved in specimen collection, and no attempt was made to standardize the procedure. The only instruction was to scrape the base of the lesion with a calcium alginate swab, as vesicular fluid seldom contains sufficient cells. These swabs were found to be an efficient method for obtaining cells from the base of the lesion.

Specimen processing. Smears of the lesion scrapings were placed in three or four wells on a single fluorocarbon-coated slide and immediately fixed in acetone for 5 to 10 min. Following air drying, the slides were stored at room temperature for up to 1 month prior to performance of the immunoassay.

Routine viral cultures were obtained at the same time. Swabs were transported in veal infusion broth and inoculated on W1-38 cells or human embryonic tonsil diploid fibroblasts. The standard laboratory procedure for identifying herpes simplex viruses was employed. Following the development of the typical cytopathic effect, the agent was identified by neutralization with typing antisera obtained from the Center for Disease Control (Atlanta, Ga).

Antisera and peroxidase conjugates. The antisera (anti-herpes simplex virus types 1 and 2) were obtained from the Center for Disease Control. These rabbit antisera were absorbed with bovine liver powder for 1 h at 35°C and used at a dilution of 1:40 to 1:80 in phosphate-buffered saline (PBS). The antisera demonstrated no cross-reactivity with uninfected fibroblasts, fibroblasts infected with varicella-zoster or cytomegalovirus, adenovirus-infected primary monkey kidney cells, cells infected with paramyxovirus types 1, 2, and 3, vaccinia, or group B coxsackieviruses. Anti-rabbit gamma globulins (Antibodies Inc., Davis, Calif.) were conjugated with horseradish peroxidase, type VI (Sigma Chemical Co., St. Louis, Mo.), using the gluteraldehyde-coupling method of Avrameas and Ternyck (1) and were used at a dilution of 1:100 in PBS with 1% bovine serum albumin.

IP method. Each slide was placed in acid alcohol for 5 min to eliminate any endogenous peroxidase activity, followed by a PBS rinse for 5 min. After gently blotting dry, a drop of PBS or normal rabbit

serum was placed in the first well to serve as a control. Drops of appropriately diluted types 1 and 2 antisera were placed in the second and third wells. Slides were incubated in a moist chamber for 45 min at 35°C and then rinsed in PBS for 15 min. Drops of anti-rabbit peroxidase conjugate were placed in each well and reincubated at 35°C for 45 min, followed by another 15-min PBS rinse. The slides were then immediately stained with Kaplow medium (6) for 60 s, dehydrated through alcohol to xylene, and mounted in Permount. With this stain, peroxidase activity produces bright blue granules in cytoplasm and on the cell membrane, whereas the safranin counterstain results in a pink or red nuclear and cytoplasmic staining. Slides were examined with a light microscope.

Later in the course of the study, the staining reagent was changed to aminoethyl carbozole, and the slides were mounted in glycerol. This substrate produces a red-brown precipitate at the site of peroxidase activity (7).

A positive control slide was included with each batch of clinical samples assayed. This contained diploid fibroblasts infected with herpes simplex virus types 1 and 2 and one well containing uninfected fibroblasts.

RESULTS

A total of 258 specimens were examined; 39 were judged inadequate for evaluation due to a paucity or absence of cells. Most of these instances occurred early in the study and were from older lesions which had crusted over.

The 219 specimens which contained sufficient cells were divided into four clinical groups, based on the gross morphology of the mucocutaneous lesion. There were 67 vesicular lesions, 40 pustules, 59 ulcers, and 52 dry, flat, crusted lesions. The results of viral culture and IP are illustrated in Table 1.

In two instances, viral antigen was demonstrated with IP, but was not cultured. These cases most probably represent culture failures rather than false negative staining reactions, but this could not be proven in this study. No difference was found between the ability of types 1 and 2 antisera to detect viral antigen. Since there is considerable cross-reactivity between those two antisera, most positive specimens stained with both, so that direct typing of the herpes simplex virus was not possible with the antisera employed.

TABLE 1. *Comparison of tissue culture and IP for diagnosis of mucocutaneous herpes simplex virus infection*

Clinical sample		Culture	IP	
Type	Total no.		Positive	Negative
Vesicle	68	Positive	50	5
		Negative	1	12
Pustule	40	Positive	13	13
		Negative	0	14
Ulcer	59	Positive	17	12
		Negative	1	29
Crusted	52	Positive	4	14
		Negative	0	34

Ninety-one percent of the vesicular lesions that yielded herpes simplex virus in tissue culture had easily demonstrable antigen by IP. This decreased to around 50% for the pustular and ulcerated lesions and down to 22% for the dry-crusted lesions. There was no difference between oral and genital lesions in the number of positive specimens that were detected with the IP method.

DISCUSSION

One feature of many of the immunofluorescent studies is that specimens are obtained by a single investigator who is able to optimize the conditions of collection as well as patient selection. However, this has little clinical reality, and so it is not surprising that similar results seldom can be reproduced in a clinical setting.

In this study, we have attempted to use a method without regard to the clinical stage of the lesion and without any elaborate or specific collection procedures. Our results clearly demonstrate that there is an optimal time during which the indirect IP method is highly satisfactory for the rapid diagnosis of herpes simplex virus infection. After approximately 10 days, or when the lesion becomes crusted over, the chance of finding specifically infected cells decreases to the point of unreliability, although virus may still be cultured. An additional problem at this late stage is that few cells are detachable, so that the preparations are frequently unsuitable for evaluation.

There is also little doubt that the method of obtaining the clinical sample is crucial in determining the success of the method. The more vigorously the base of the lesion is scraped, the greater the probability of detecting infected cells. This is especially important during the vesicular phase, when relatively few cells are in the fluid itself. With simple instructions, most people are able to obtain satisfactory specimens.

The IP procedure has a number of advantages over routine tissue culture. Whereas tissue culture requires at least 24 to 48 h before the cytopathic effect of herpes simplex virus becomes apparent, the IP method can be performed in approximately 4 h. Multiple samples can be examined at the same time, so that a considerable saving in technologist time can be effected. In addition, the cost of reagents and complexity of equipment required for the routine diagnosis of herpes simplex virus infections is markedly decreased. The problems associated

with fluorescent microscopy are avoided in large part, and the diagnostic sensitivity is far greater than routine cytological preparations. This has been previously demonstrated by Morisset et al. (8). In their study of 50 cases of conjunctival, corneal, oral, vulvar, and cervical lesions which were identified with the IP method, only a minority had a concomitantly positive Tzanck preparation. Because of the sensitivity of the method in detecting herpes simplex virus-infected cells in the early stage of infection, we recommend that the IP method be limited to these cases. At the time of sampling the lesion for the IP technique, a swab should be placed in an appropriate viral transport medium. If the IP test is negative or if the preparations are unsatisfactory, then virus culture should be undertaken. If the IP test is positive, there appears to be no indication to proceed with any further confirmatory procedures. Because of the rather poor sensitivity of the test later in the disease process, the best laboratory diagnostic test remains culture.

Although many cases of mucocutaneous herpes infections are diagnosed clinically without culture or antigen confirmation, the increasing incidence of genital infection and future development of antiviral agents requires a simple, inexpensive method for specific viral diagnosis. This is especially so when the lesions are clinically atypical. In view of the results of this study, the IP method can be recommended as a rapid, reliable, and reproducible technique. Its optimal use appears to be in the early diagnosis of vesicular lesions due to herpes simplex virus infection.

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