

Rapid Typing of Herpes Simplex Virus Strains Using the Indirect Immunoperoxidase Method

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Received for publication 5 August 1974

The indirect immunoperoxidase technique with type-specific antisera was compared to kinetic neutralization for the typing of herpes simplex virus strains. There was complete agreement between the results obtained by kinetic neutralization and indirect immunoperoxidase. The indirect immunoperoxidase assay was far simpler to perform and interpret than the kinetic neutralization tests, and it offered a rapid means for the routine antigenic typing of herpes simplex isolates.

Since the recognition of two groups of herpes simplex viruses, a wide variety of markers have been utilized to separate clinical isolates into these two, apparently distinct types (11). A number of studies have compared certain biological characteristics of these groups with antigenic structure. Whereas some of the biological markers correlate with antigenic structure, others appear to vary independently (13, 15, 17). Apart from deoxyribonucleic acid density and base composition differences (4, 6), serological typing offers the best method of distinguishing the two types. Kinetic neutralization (KN) is the most commonly employed reference for typing herpes simplex strains; however, this technique is time consuming and cumbersome. Immunofluorescence of surface antigens has been used to differentiate the two groups, using tissue culture isolates and by using it directly on clinical specimens (8, 9, 10).

The indirect immunoperoxidase (IP) method has been successfully employed in our laboratory for the typing of human myxo- and paramyxoviruses isolated in tissue culture (2). It is simple, rapid, and reproducible, apparently offering a number of advantages over standard neutralization tests and immunofluorescence. The epidemiological importance of typing herpesvirus strains prompted us to develop the IP method for use in this situation and to compare it with KN.

MATERIALS AND METHODS

Viruses. The 60 herpes simplex virus strains and the type-specific guinea pig antisera were supplied by B. B. Wentworth and E. Alexander, School of Public Health and Community Medicine, University of Washington, Seattle. Details of the origin of these

isolates have been previously published (17). All were stored at -70°C and had been passaged from four to seven times. They were inoculated onto human diploid fibroblasts in culture tubes (16 by 125 mm) and used for the IP assay when there was 2 to 4+ cytopathic effect. Other cell cultures used included primary pigtail monkey kidney cells and a continuous line of heteroploid cells of human origin.

KN test. Thirty-three isolates were typed by KN. The technique for this assay has been reported (16).

Antisera. Three groups of antisera were employed in this study. (i) Type-specific antisera produced in guinea pigs had homologous K values of 31.5 (type 1) and 14.3 (type 2), with heterologous K values of 5.53 (type 1) and 4.7 (type 2). The immunization schedule and antigens-used have been previously recorded (17). The antisera were absorbed with bovine liver powder for one h at 35°C and used at a dilution of 1:60 (type 1) and 1:80 (type 2) in phosphate-buffered saline (PBS). Bovine liver powder removed most nonspecific activity from the sera, and the optimal dilutions were established by a series of titration experiments to give maximal specific staining without any background staining.

(ii) Cerebrospinal fluid (CSF) from a patient with herpes simplex encephalitis was found to contain immunoglobulin G anti-herpes type 1 antibodies. At a dilution of 1:8 in PBS, this CSF demonstrated a strong reaction with herpes type 1 (American Type Culture Collection, strain MacIntyre) by the IP assay but no reaction with type 2 (American Type Culture Collection, strain MS).

(iii) Type-specific antisera prepared in rabbits were obtained from the Center for Disease Control (Atlanta, Ga.). After a 1-h absorption with bovine liver powder at 35°C , they were employed at a dilution of 1:60 in PBS.

Preparation of conjugates and slides. Conjugates of anti-rabbit gamma globulin, anti-guinea pig gamma globulin, and anti-human immunoglobulin G were prepared by the glutaraldehyde coupling method of Avrameas (1) with commercial antisera (Antibodies

Inc., Calif.) and horseradish peroxidase, type VI (Sigma Chemical Co., St. Louis, Mo.).

Fluorocarbon-coated slides (Fluoroglide, film bonding grade, Chemplast, Inc.) with 40 wells per slide were prepared according to Goldman (3). Infected cell monolayers were scraped with a Pasteur pipette and centrifuged at $800 \times g$ for 5 min. Then the cells and cell debris were suspended in 0.2 ml of PBS. One drop of cell suspension was placed in each well (10 wells per culture), air-dried, fixed in acetone at 4 C for 10 min, and allowed to dry.

Immunoperoxidase method. Each isolate was tested in duplicate against all five antisera. One drop of diluted antiserum was placed into each well, incubated at 35 C for 45 min in a moist chamber, rinsed in distilled water, and washed in PBS for 20 min. Slides were gently blotted dry and one drop of the appropriate anti-species enzyme conjugate was added to each well and incubated at 35 C for 45 min. The 20-min wash in PBS was repeated.

The slides were immediately stained with Kaplows medium for 60 s, dehydrated through alcohol to xylene, and mounted in Permount (5). Peroxidase activity was evidenced by bright blue granules. This reaction product is stable unless exposed to direct sunlight. The background stain was red.

The intensity of staining was graded from 0 to 4+, and the slides were coded and read independently by two observers without knowledge of the KN results.

Controls. The antisera were tested by the IP method against varicella-zoster, cytomegalovirus, vaccinia, parainfluenza viruses (types 1, 2, and 3), uninfected fibroblasts, human heteroploid cells, and pigtail monkey kidney cells. There was no cross reactivity or nonspecific staining. Controls using PBS in place of specific antisera were similarly negative.

RESULTS

Serial 10-fold dilutions made with two strains were inoculated into cell cultures to examine the influence of the degree of cytopathic effect on the intensity of staining. There was no detectable difference in individual cell staining from the cell cultures manifesting varying amount of cytopathic effect from 1 to 4+. One isolate was cultured in fibroblasts, human heteroploid cells, and pigtail monkey kidney cells to determine whether the cell isolation system altered antigenic expression. Once again, no difference in staining intensity was noted.

Figure 1 illustrates the staining reactions of all 60 isolates with the guinea pig antisera. Two groups of viruses were clearly differentiated. With the rabbit antisera (Fig. 2), however, this difference was not discernable. As it was impossible to differentiate many strains, results with the rabbit antisera were not used further when attempting to assign a virus to a given type by the IP assay.

An isolate was determined to be type 2 when there was 1+ or greater difference in the stain-

ing reaction with the guinea pig type 2 antiserum compared to the type 1, and when there was no reaction with the human CSF containing herpes simplex type 1 antibodies (Fig. 3). Of the 26 strains determined to be type 2, 24 demonstrated 2+ or greater difference in staining intensity with the guinea pig antisera.

All isolates reacting with the CSF containing herpes simplex type 1 antibodies, regardless of the intensity of staining, were assigned to type 1 (Fig. 3). Of the 34 strains that reacted, 24 demonstrated 2+ or greater difference in stain-

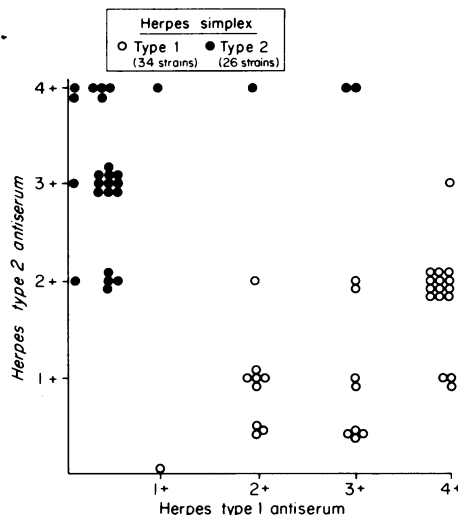


FIG. 1. Relative staining reactions of 60 herpes simplex isolates with type-specific antisera prepared in guinea pigs.

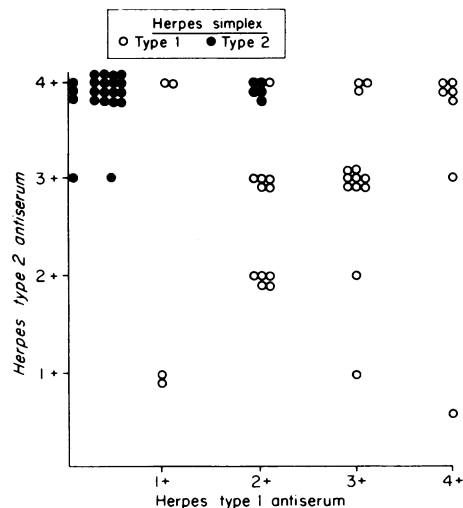


FIG. 2. Relative staining reactions of 60 herpes simplex isolates with type-specific antisera prepared in rabbits.

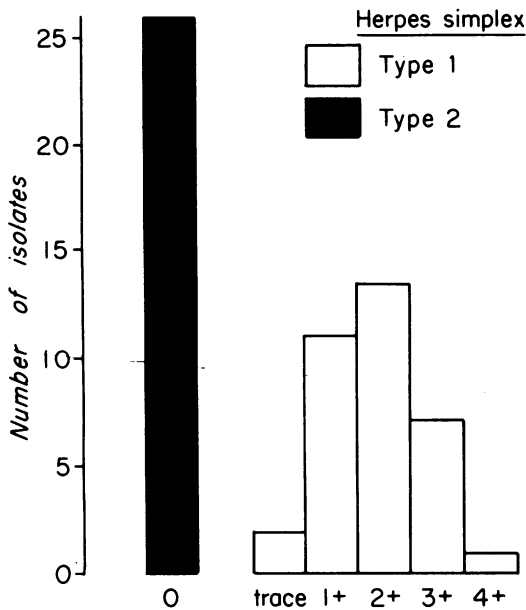


FIG. 3. Relative staining reactions with human CSF containing herpes simplex type 1 antibodies.

ing with the guinea pig type 1 antiserum compared to the type 2 (Fig. 1). Nine type 1 isolates showed only 1+ difference, and one isolate demonstrated identical reactions with both type 1 and 2 antisera.

Utilizing the two guinea pig antisera and the CSF containing herpes simplex type 1 antibodies, the reactions were always sufficiently clear-cut to allow confidence in assigning an isolate to one of the two types.

Thirty-three strains typed by KN are compared to the results obtained by the IP assay in Table 1. There was complete agreement in typing between these two methods.

DISCUSSION

The IP assay proved easy to interpret, and no difficulty was encountered in assigning an isolate to a given type. For those isolates that demonstrated only 1+ difference in staining reactions with the guinea pig antisera, the results with the CSF containing herpes simplex type 1 antibodies resolved the issue. The results clearly demonstrate that the IP method allows herpes simplex virus strains to be divided into two distinct groups, correlating accurately with KN tests.

The success of the IP method depends primarily on the quality of the type-specific antiserum. Both the rabbit and guinea pig type 1 antisera were relatively specific, whereas the

TABLE 1. Comparison of typing 33 isolates with IP and KN

IP	KN	
	Type 1	Type 2
Type 1	22	0
Type 2	0	11

guinea pig type 2 antiserum was far more satisfactory than that prepared in rabbits. For this reason the rabbit antisera were not used in typing. On the other hand, the immunoglobulin G antibodies in the human CSF used in this study appeared to be completely specific for herpes simplex type 1. Previous observations have noted that type 1 antiserum is usually more specific than type 2 (8). Absorption with heterologous virus has provided improved specificity (10), especially for type 2 antisera. However, this study illustrates that it is not essential for adequate serological typing.

The diversity in staining reactions suggests some variability in antigenic relatedness of individual isolates to one another. A similar spectrum has been observed with other serological assays in addition to certain biological markers. The mechanism underlying this variation is not clearly understood.

From a technical standpoint, the IP assay is simple to perform, rapid (results are obtained within 4 h), reproducible, and may be performed on initial isolation of the virus. This avoids the need for further passage which may conceivably alter antigenic expression. Glycoproteins, which determine the immunologic specificity of the virus (14), become variable in laboratory-adapted strains, either disappearing or being replaced by different glycoproteins (12). Although this did not present a problem with the antisera used in this study, it may become extremely important if specific antisera are employed against more selected antigenic determinants. The IP assay circumvents the problems associated with the more complex KN tests. Immunochemical methods offer a number of advantages over immunofluorescent methods, including easier interpretation of results, simpler methodology, and a permanent, stable record. A number of recent studies have demonstrated the advantages of IP over immunofluorescence (7, 18).

ACKNOWLEDGMENTS

I am indebted to C. George Ray of the Departments of Pediatrics, Laboratory Medicine, and Clinical Microbiology, University Hospital and Children's Orthopedic Hospital and Medical Center, University of Washington, Seattle, for his

constant advice and helpful criticism. My thanks to E. R. Alexander, Department of Epidemiology and International Health, University of Washington, for reviewing this manuscript and to Patricia Skahen, Linda French, and Mary Sondsmo for excellent technical assistance.

This research was supported by Public Health Service general research support grant 5-501-RR05655-04 to the Children's Orthopedic Hospital and Medical Center.

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