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Elimination of Fc Receptor Binding of Human Immunoglobulin G in Immunofluorescence Assays for Herpes Simplex Virus Antibodies

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The binding of human immunoglobulin G by Fc receptors in herpes simplex virus (HSV)-infected cells can cause false-positive interpretations in the immunofluorescence test for HSV antibody. When the infected cell smears were treated with 10% glacial acetic acid for 5 min and rinsed in phosphate-buffered saline before the immunofluorescence test was performed, the Fc receptors were completely inactivated, resulting in a reliable method for HSV antibody detection.

The indirect immunofluorescence (IF) test is a relatively simple method for measuring antibodies to herpes simplex virus (HSV) and cytomegalovirus (CMV), and IF kits for performing these tests are available from several manufacturers. HSV- and CMV-infected cells produce Fc receptors for immunoglobulin G (IgG) of human and certain other animal species (5). These receptors result in fluorescent staining of the infected cells in the IF test regardless of the presence of specific viral antibody in the serum specimen. The Fc receptors in CMV-infected cells are concentrated in the perinuclear region of the cytoplasm, and to avoid false-positive interpretations in the IF test, only nuclear fluorescence is attributed to the presence of CMV antibody. The Fc receptors in HSV-infected cells appear to be distributed throughout the cytoplasmic membrane, and the staining pattern from nonspecific binding of IgG to these Fc receptors in the HSV IF test cannot be differentiated morphologically from specific HSV antibody reactions.

Because of the problems we encountered with interpretation of the HSV IF test, the anticomplement immunofluorescence test (ACIF), which is not affected by Fc binding of IgG, is used in this laboratory for IF determinations of HSV antibody (3). However, this method requires complement, Veronal buffer, and anti-C3 conjugate, and because ACIF kits for HSV antibody measurement are not commercially available, this test is not in general use.

In the IF test for HSV antibody, false-positive interpretations owing to Fc binding of human IgG are a real possibility, and the elimination of this problem would greatly improve the specificity of this method. We found that pretreatment of the acetone-fixed HSV cell smears used in the HSV IF test with certain solutions (pH 2.3) prevented Fc binding without affecting the sensitivity of the antigen.

Fifty serum samples previously tested for HSV antibodies by ACIF and by an enzyme immunoassay (EIA) method developed in this laboratory (1) were selected for this study. Forty-one of these samples were negative for HSV antibody by both tests, and one was ACIF positive and close to the cutoff but negative by EIA. To evaluate the effects of low pH on Fc binding of human IgG to HSV-infected cells, acetone-fixed cell smears prepared as previously described (2) and stored at −60°C were brought to room temperature, and various concentrations of glacial acetic acid in distilled water were added to the smears for different time intervals. The slides were then dipped in 0.01 M phosphate-buffered saline (PBS) (pH 7.3), the moisture was aspirated from the smears, and the IF test was performed (4). The slides were read on an epifluorescence microscope (Carl Zeiss, Inc., New York, N.Y.) at a magnification of ×250, and the reactions were graded in staining intensity from 0 to 4+.

The effects of exposure of HSV type 1 (HSV-1)-infected cell smears to various concentrations of acetic acid for various lengths of time are shown in Table 1. Acid (3%) was added to the smears for 5, 30, and 60 min, the slides were rinsed in PBS, and the IF test was performed with one HSV-positive and six HSV-negative serum specimens. Negative samples could not be differentiated from the positive specimen on the untreated slides. Nonspecific staining of the HSV-infected cells with the negative specimens was reduced after a 5-min exposure to 3% acetic acid and was absent after a 60-min exposure. To determine if a higher concentration of acid would reduce the exposure time required, we reacted cell smears with 10, 30, and 50% acetic acid for 5 min. The addition of 10% acetic acid (pH 2.3) to the smears for 5 min eliminated the staining of HSV-1-infected cells with the negative serum specimens without diminishing the staining intensity of the positive specimen, but higher concentrations of acid apparently denatured the antigen. Exposures (1 and 3 min) to 10% acetic acid did not completely remove nonspecific reactions with the negative serum samples.

HSV-1- and HSV type 2 (HSV-2)-infected cell smears were then reacted with 10% glacial acetic acid for 5 min, rinsed in PBS, and used to determine the presence of HSV IF antibody in 50 serum samples. In addition, 34 of these specimens were tested with a commercial HSV IF kit (Electro-Nucleonics, Inc., Columbia, Md.) according to the directions of the manufacturer, except that one set of slides was pretreated with acid. IF reactions of 20 of these serum samples are shown in Table 2.

The eight serum specimens positive for HSV antibody by ACIF and EIA were positive by IF on the acid-treated slides. Specimen no. 9 was ACIF and IF positive and borderline negative by EIA. Staining reactions were more intense on our HSV-1 slides than on HSV-2 or acid-treated commercially prepared HSV-1 slides. Serum samples negative by ACIF and EIA were negative by IF with the acid-treated antigens, but the 25 HSV-negative specimens tested on the untreated commercial slides stained the HSV-infected cells at 2+ brightness, and according to the kit directions, this reaction should be interpreted as positive.

ACIF and IF HSV-1 and HSV-2 antibody titers were determined for five of the positive serum specimens with

TABLE 1. Effect of pretreatment of HSV-1-infected cell smears with various concentrations of acetic acid and exposure times upon the IF test for HSV antibody

Specimen	ACIF[a]	EIA (OD)[b]	Degree of fluorescence by the following acetic acid concn and exposure time[c]:								
			0% and 0 min	3% and 5 min	3% and 30 min	3% and 60 min	10% and 1 min	10% and 3 min	10% and 5 min	30% and 5 min	50% and 5 min
1	≥8	8.9	4	4	4	4	4	4	4	3	0
2	<8	<0.5	3	1–2	0	0	0	0	0	0	0
3	<8	<0.5	3	1–2	0	0	1–2	±	0	0	0
4	<8	<0.5	3	1–2	0	0	0	0	0	0	0
5	<8	<0.5	3	1–2	0	0	+	0	0	0	0
6	<8	<0.5	3	1–2	0	0	+	0	0	0	0
7	<8	<0.5	3	1–2	1–2	0	2–3	+	0	0	0

[a] ≥8 = positive.
[b] ≥1 = positive. OD, Optical density.
[c] At 1:8 serum dilution. Staining intensity was graded on a scale from 0 to 4+. 0, No staining; ±, inconclusive; 1 to 4, positive.

untreated cell smears for ACIF and with acid-treated antigens for the IF test. Titers were higher on HSV-1 than on HSV-2 smears, and the IF titers were equal to or higher than those obtained by the ACIF method.

The Fc receptors in HSV-infected cells appeared to be irreversibly inactivated when exposed to a low pH. Slides treated for 5 min with a sodium acetate-hydrochloride buffer and a citric acid-Na_2HPO_4 buffer, both adjusted to pH 2.3, gave similar results to 10% acetic-acid-treated antigens, suggesting that the pH of the buffer and not the particular reagent was responsible for this reaction. To determine if the inhibition of the binding of serum IgG to the Fc receptors was due to residual acid on the cell smears, serum specimens were reacted in duplicate on acid-treated slides dipped briefly in PBS and on slides placed in PBS and agitated on a shaker for 30 min. Identical results were obtained with both sets of slides, indicating that a low pH when the serum samples were added was not a factor.

Pretreatment of acetone-fixed HSV-infected cell smears with 10% glacial acetic acid for 5 min eliminated nonspecific IF staining reactions due to Fc binding of human IgG and did not alter the sensitivity of the antigen. The acid-treated commercial HSV-1 slides gave specific results but did not stain quite as brightly as did the HSV-1 slides prepared in this laboratory. According to the package insert, these commercial slides had been pretreated to block Fc binding. However, we found the Fc receptors to still be active, as evidenced by low-level staining of the HSV-infected cells with all the negative serum specimens tested. An attempt to find an acetic acid concentration and exposure time that would eliminate Fc binding in CMV-infected cells was not successful. This may be because CMV Fc receptors are concentrated in the perinuclear region of the cytoplasm. Conditions which eliminated these receptors also denatured the antigen.

No staining with positive sera was detected when freshly prepared HSV slides were acid treated and stored at −60°C for 2 months before use, so this method cannot be recom-

TABLE 2. Effect of pretreatment of cell smears with 10% glacial acetic acid for 5 min upon the IF test for HSV antibody determinations

Specimen	ACIF[a]	EIA (OD)[b]	Degree of fluorescence by pretreatment of slides[c]			
			In house acid treated		ENI[d]	
			HSV-1	HSV-2	Untreated HSV-1	Acid treated HSV-1
1	128	9.5	4	3	4	3
2	128	8.9	4	3	4	3
3	32	7.2	4	3	4	3
4	8	3.0	3	2	3–4	2–3
5	32	2.8	3	2	4	+
6	8	1.4	+	±	2	±
7	8	1.4	+	0	2	±
8	8	1.2	2	+	2	+
9	32	0.84	2	0	2	±
10	<8	<0.5	0	0	2	0
11	<8	<0.5	0	0	2	0
12	<8	<0.5	0	0	2	0
13	<8	<0.5	0	0	2	0
14	<8	<0.5	0	0	2	0
15	<8	<0.5	0	0	2	0
16	<8	<0.5	0	0	2	0
17	<8	<0.5	0	0	2	0
18	<8	<0.5	0	0	2	0
19	<8	<0.5	0	0	2	0
20	<8	<0.5	0	0	2	0

[a] ≥8 = positive.
[b] ≥1 = positive. OD, Optical density.
[c] At 1:8 serum dilution. Staining intensity was graded on a scale from 0 to 4+. 0, No staining; ±, inconclusive; 1 to 4, positive.
[d] Prepared commercially by Electro-Nucleonics, Inc.

mended for commercial HSV antigens. However, a 5-min slide treatment with 10% acetic acid and a brief PBS rinse can easily be performed by any laboratory and appear to greatly improve the specificity of the IF test for HSV antibody detection.

The performance of the EIAs by the serology unit is greatly appreciated.

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