

Rapid Detection of Herpes Simplex Virus in Clinical Samples by Flow Cytometry after Amplification in Tissue Culture

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Murine monoclonal antibodies (MAbs) against herpes simplex virus type 1 and 2 (HSV-1 and -2, respectively) nuclear antigens were used to identify cells infected with HSV-1 or -2 by indirect immunofluorescence in conjunction with flow cytometry after virus amplification of MRC-5 cell monolayers. The results indicate that MAbs Q1, Q2, and H-640 detect HSV-1- and HSV-2-infected cells, MAb SD-1 detects HSV-2- but not HSV-1-infected cells, and MAb 58-S detects HSV-1- but not HSV-2-infected cells. MAb Q1, which detects HSV-1- as well as HSV-2-infected cells, was used to detect HSV-infected cells after inoculation and overnight (16- to 20-h) incubation of MRC-5 monolayers with clinical samples suspected of containing HSV. In comparing the efficiency of flow cytometry with cytopathic effect (CPE) in tissue culture for detecting HSV in clinical samples, HSV was detected by flow cytometry in 77% of the cases where HSV was detected by CPE in tissue culture. In many cases, flow cytometry detected HSV from 1 to 3 days before HSV was detected by CPE.

Indirect immunofluorescence in conjunction with flow cytometry is a rapid, sensitive, and quantitative method for detecting virus-infected cells from tissue culture and clinical specimens. We have previously demonstrated the use of this technology for detection and quantitation of cytomegalovirus-infected cells in bronchoalveolar lavage specimens (3) and human immunodeficiency virus-infected cells in peripheral blood (7). However, all clinical specimens do not contain cell-associated virus or sufficient numbers of virus-infected cells to be detected by flow cytometry. To overcome these limitations, it is possible to use tissue culture to amplify the small number of infected cells or to make cell-free virus into cell-associated virus. By using monoclonal antibodies (MAbs) specific for viral antigens and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin G F(ab')₂ antibodies, these infected tissue culture cells can be examined by flow cytometry for the presence of viral proteins that are expressed early after virus infection. In this report, we present evidence to demonstrate that herpes simplex virus (HSV) can be detected and quantitated by flow cytometry after amplification in tissue culture cells. When clinical specimens were amplified overnight in tissue culture and then assayed for HSV by flow cytometric analysis, there was good agreement between the flow cytometric assay and the standard tissue culture assays.

MRC-5 monolayer cultures in tissue culture tubes were infected with HSV type 1 (HSV-1) or HSV-2 at a multiplicity of infection of approximately 10. Mock-infected MRC-5 cells served as controls. After overnight incubation at 37°C, the infected cells were removed with 0.05% trypsin-EDTA, washed with phosphate-buffered saline without Ca²⁺ and Mg²⁺, fixed with 90% methanol, incubated with the primary MAb to virus-specific antigen, washed, and incubated with FITC-conjugated goat anti-mouse immunoglobulin G F(ab')₂ antibody. After a final wash, the antibody-treated cells were incubated with RNase and propidium diiodide to determine the DNA content of the infected cells. The doubly labeled cells were analyzed for two-color fluorescence by

flow cytometry as previously described (3, 5-7). MAbs were obtained from the following sources: the H-640 MAb was a generous gift of L. Pereira (1); MAbs Q1, Q2, and SD-1 were a generous gift of K. Powell (2, 9); and the 58-S MAB was a generous gift of M. Zweig.

For quantitation of the percentage of cells that are stained with each MAb, a gate was set such that approximately 1% of the uninfected MRC-5 cells are above the gate. This gate was used to measure the percentage of HSV-infected cells in the HSV-1- and HSV-2-infected MRC-5 cell populations. The data in Table 1 show that at dilutions of the H-640 monoclonal antibody from 1/20 to 1/640 there was no significant fluorescence in the uninfected, control MRC-5 cells whereas the various dilutions of the H-640 MAB detected from 98 to 99% of the HSV-1-infected MRC-5 cells and from 36 to 92% of the HSV-2-infected MRC-5 cells. The specific activity of the H-640 MAB approached 99% for HSV-1-infected cells and 94% for HSV-2-infected cells. The significant difference in the percentage of HSV-2-infected cells detected by flow cytometry is due to a less-efficient infection of the MRC-5 cells by the HSV-2 stock. These results indicate that the H-640 MAB can detect and quantitate both HSV-1- and HSV-2-infected MRC-5 cells. Similar results were found with the Q1 and Q2 MAbs, which also recognize HSV-1- and HSV-2-infected cells (2, 9). The data in Table 2, which were derived from flow cytometric analysis of SD-1 MAB-treated HSV-1- and HSV-2-infected MRC-5 cells, show that the SD-1 MAB detected only HSV-2-infected cells. At the various dilutions tested, the MAB detected approximately 93% of the infected cells with a specific activity approaching 98%. The data in Table 3 show that the 58-S MAB detects only HSV-1-infected MRC-5 cells, of which approximately 90% of the cells were detected with a specific activity approaching 98%. The results of these experiments show that, using MAbs specific for HSV-1 and HSV-2 in conjunction with flow cytometry, HSV-infected MRC-5 cells can be detected, quantitated, and typed by this method.

To determine whether these MAbs can be used in conjunction with flow cytometry to detect HSV in clinical

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TABLE 1. Results for H-640 MAb

Sample	Antibody dilution	% Positive cells	Mean Y^a	Sp act (%) ^b
Control	1/20	1.13	0.16	
	1/40	0.9	0.36	
	1/80	0.9	0.09	
	1/160	1.0	0.08	
	1/320	1.0	0.06	
	1/640	0.9	0.06	
HSV-1	1/20	99.4	6.03	97
	1/40	98.7	5.77	94
	1/80	99.4	5.92	98
	1/160	99.6	4.33	98
	1/320	99.6	4.21	99
	1/640	97.6	4.16	99
HSV-2	1/20	92.7	2.11	92
	1/40	36.1	1.55	77
	1/80	75.5	1.25	93
	1/160	86.7	1.17	93
	1/320	80.1	0.85	93
	1/640	73.1	0.93	94

^a Mean Y is obtained by gating on the population of interest and calculating the mean fluorescence of the cells in the gate (5, 6).

^b Specific activity = mean Y infected - mean Y uninfected/mean Y infected \times 100.

samples after amplification in tissue culture, 171 consecutive clinical samples sent to the Clinical Microbiology Laboratory at the Albany Medical Center Hospital were inoculated into tubes containing MRC-5 cell monolayers. After overnight incubation (16 to 20 h) at 37°C, cells from one tube were removed, fixed, treated with the Q1 MAb at a 1/500 dilution, and processed for flow cytometry. The remaining tubes were observed for cytopathic effect (CPE) for 5 days after inoculation. Results for tubes showing CPE suggestive of HSV infection were confirmed by the latex particle agglutination (LPA) test specific for HSV which, in our experience, has 100% specificity and 100% sensitivity (4, 8). Twenty percent (35 of 171) of the samples were positive for HSV on the basis of the LPA test. Seventy-seven percent (27 of 35) of the LPA-positive samples were identified as positive for HSV antigen by the flow cytometric assay after overnight amplification in tissue culture cells. All of the samples judged to be negative on the basis of tissue culture after 5 days of incubation were judged to be negative by flow

TABLE 2. Results for SD-1 MAb

Sample	Antibody dilution	% Positive cells	Mean Y^a	Sp act (%) ^b
Control	1/20	0.90	0.07	
	1/40	0.22	0.05	
	1/80	0.01	0.05	
	1/160	0.01	0.05	
HSV-1	1/20	2.69	0.11	36
	1/40	0.23	0.06	17
	1/80	0.05	0.05	0
	1/160	0.02	0.05	0
HSV-2	1/20	93.53	2.33	97
	1/40	93.68	2.06	98
	1/80	93.46	2.90	98
	1/160	92.75	3.02	98

^a See Table 1, footnote *a*.

^b See Table 1, footnote *b*.

TABLE 3. Results for 58-S MAb

Sample	Antibody dilution	% Positive cells	Mean Y^a	Sp act (%) ^b
Control	1/20	4.8	0.27	
	1/40	3.4	0.18	
	1/80	0.4	0.06	
	1/160	1.4	0.09	
	1/320	1.1	0.17	
HSV-1	1/20	81.8	2.29	88
	1/40	93.9	4.41	96
	1/80	93.6	3.88	98
	1/160	92.5	2.40	96
	1/320	85.1	1.74	90
HSV-2	1/20	1.53	0.30	0
	1/40	1.01	0.15	0
	1/80	0.05	0.15	0
	1/160	0.05	0.15	0
	1/320	0.02	0.15	0

^a See Table 1, footnote *a*.

^b See Table 1, footnote *b*.

cytometric assay. The samples with greater than 90% antigen-positive cells by flow cytometry were detected within 24 h by CPE; however, samples having 3 to 24% positive cells by flow cytometry took up to 5 days to be detected by the standard tissue culture assay. These results suggest that overnight amplification of virus in tissue culture, followed by incubation with HSV-specific MAbs and FITC-conjugated goat anti-mouse immunoglobulin G F(ab')₂ antiserum and detection by flow cytometry, may be a useful procedure for the rapid diagnosis of HSV infections. Since not all HSV-positive samples were detected by flow cytometry, observation of CPE in tissue culture will remain the "gold standard" for detection of infectious virus. However, this rapid, quantitative flow cytometric technique will reduce the time to detect HSV in the majority of clinical specimens.

We have demonstrated that HSV-1- and HSV-2-infected MRC-5 cells can be detected and quantitated by flow cytometry and that flow cytometry can be used as a rapid diagnostic technique for detecting HSV in clinical samples after amplification in tissue culture. By using the tissue culture amplification system in conjunction with direct immunofluorescence involving FITC-labeled antibody to HSV-1 and phycoerythrin-labeled antibody to HSV-2, it will be possible to detect, quantitate, and type HSV by flow cytometry in a single procedure. This demonstration expands the role of flow cytometry in rapid viral diagnosis from direct specimen detection of cell-associated virus, as has been previously demonstrated by us for cytomegalovirus in bronchoalveolar lavage specimens (3) and human immunodeficiency virus in peripheral blood mononuclear cells (7), to detection of cell-free virus after amplification in tissue culture cells. Thus, all viruses that infect tissue culture cells and to which MAbs are available can be detected and quantitated by flow cytometry. Furthermore, the use of MAbs to different viruses may allow distinction of multiple viruses in the same clinical sample.

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