Comparison of Monoclonal and Polyclonal Antibody for Confirmation of Cytomegalovirus Isolates by Fluorescent Staining

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A fluorescein isothiocyanate-labeled monoclonal cytomegalovirus antibody reagent incorporating Evans blue (Syva/Genetic Systems, Palo Alto, Calif.) was compared to a fluorescein isothiocyanate-labeled polyclonal antibody (Dynatech Diagnostics, Inc., South Windham, Maine) for identification of cytomegalovirus in tissue culture. A total of 191 cultures, 86 positive and 105 negative for cytomegalovirus by cytopathic effect in WI38 cells, were stained in parallel and examined by fluorescent microscopy. No false-negative results were noted with either reagent; however, the polyclonal antibody-stained slides required the use of a separate counterstain to decrease nonspecific fluorescence. The polyclonal reagent gave a false-positive reaction with one varicella-zoster virus isolate and four simian cytomegalovirus isolates. No false-positive results were noted with the monoclonal reagent.

Cytomegalovirus (CMV) has been acknowledged as a leading infectious cause of congenital abnormalities and a common opportunistic pathogen (1, 6, 8). Recently, CMV has been associated with acquired immune deficiency syndrome and Kaposi’s sarcoma (3, 5, 10, 12).

CMV isolates have traditionally been identified on the subjective basis of typical cytopathic effect (CPE) in tissue cultures (9). In a clinical setting in which a wide variety of strains and differences in the quantity of virus present in a specimen may occur, differentiation of human CMV CPE from that of nonhuman CMV, adenovirus, herpes simplex virus and other viral agents causing CPE can be difficult. The use of specific antisera is therefore necessary to ensure greater accuracy and control over the quality of the result produced. With the increasing number of smaller clinical laboratories that are beginning viral diagnosis, reliable reagents for the identification of isolates become even more important. The use of monoclonal antibody for the identification of CMV-infected cells in tissue specimens has been previously reported (4, 13). In this report we compare monoclonal and polyclonal antibodies for the confirmation of tissue culture isolates of CMV.

A total of 58 recent and 28 frozen CMV isolates from patient samples were cultured by standard methods (9) in human embryonic lung fibroblast (WI38) monolayers (Flow Laboratories, Inc., McLean, Va.). WI38 tubes infected with the following viral isolates were also used to check reagent specificity: 6 of varicella-zoster virus, 20 of herpes simplex virus, 4 of simian CMV, 5 of adenovirus, and 1 of respiratory syncytial virus. All isolates were subpassaged by trypsinization of infected cells (9) until ca. 20% of the monolayer appeared infected by microscopic observation of CPE. Cultures were maintained on Eagle modified minimal essential medium supplemented with 2% fetal bovine serum and antibiotics (penicillin, 80 U/ml; streptomycin, 80 μg/ml; kanamycin, 40 μg/ml; and nystatin, 2 U/ml). Negative tissue cultures tested were obtained from 69 WI38 monolayers negative for viral CPE 6 weeks after inoculation with patient specimens. Uninoculated tissue cultures from concurrent lots of WI38 cells were used as negative controls. Positive control smears were made from WI38 cells infected with the AD-169 or Davis strain of human CMV (Syva/Genetics Systems, Palo Alto, Calif.).

Fluorescein-conjugated monoclonal antibody specific for a late CMV antigen (4) was contained in a protein-stabilized buffer solution containing Evans blue (Syva/Genetic Systems). The polyclonal CMV antibody (goat) used was conjugated to fluorescein-isothiocyanate in a carbonate buffer, pH 9.5 (Dynatech Diagnostics, Inc., South Windham, Maine). The dilution (1:32) used was determined by titration against infected cells to maximize fluorescence and to minimize nonspecific background staining. Evans blue was incorporated into the polyclonal antibody-staining procedure to reduce nonspecific background fluorescence. Without the use of this counterstain, interpretation of negative results was difficult and time consuming.

For slide preparation, maintenance medium was removed from the WI38 monolayers. 0.1 ml of Dulbecco phosphate-buffered saline, pH 7.4, was added, and each tube was agitated on a Vortex apparatus for 5 to 10 s. Two 5-mm wells on each of two acetone-cleaned glass slides (Carlson Scientific, Inc., Peotone, Ill.) were filled with ca. 0.03 ml of the cell suspension. Smears were air dried, fixed in cold acetone for 10 min, and stained immediately.

To stain the smears, a 0.03-ml volume of the monoclonal or polyclonal antibody reagent was added to duplicate wells. Slides were incubated at 37°C for 30 min and rinsed for 10 s in distilled water. Slides stained with polyclonal antibody were further incubated with 0.03 ml of Evans blue counterstain (Bartels Immunodiagnostic Supplies, Bellevue, Wash.) for 15 min at 37°C in a moist chamber and again rinsed for 10 s in distilled water. All slides were air dried, mounted with buffered glycerol, pH 9.0, and cover slipped. Stained slides were read at ×250 and ×400 magnification on an epifluorescent Zeiss microscope equipped with an HBO 50-W mercury lamp, H 485/20 excitation filter, FT 510 dichromatic beam splitter, and LP 520 barrier filter. Each smear was evaluated in terms of fluorescent intensity (negative to 4+), and the percentage of infected cells was recorded. Slides were read under code.

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On all culture-proven CMV isolates, confirmatory agreement by fluorescent-antibody staining was 100% (86 of 86) with both monoclonal and polyclonal antibody. No false-negative results were recorded when cell monolayers with ≥20% CPE were used. A typical granular staining of the cytoplasm occurred with the monoclonal antibody as opposed to the general, overall fluorescence which occurred with the polyclonal antibody (Fig. 1). Nonspecific trapping occasionally occurred in large clumps of cells with either the monoclonal or polyclonal reagents, but this effect was easily distinguishable from a specific cellular positive fluorescence and did not cause problems in interpretation.

The monoclonal reagent was 100% specific; however, of the 36 viral isolates tested that were not identified as CMV, there were five instances of false-positive fluorescence with the polyclonal antibody. Four of these were simian CMV, which is a common endogenous contaminant in African green monkey tissue cultures (2, 7, 11) and which is antigenically related to human CMV. Their fluorescent staining pattern was indistinguishable from that of the human CMV isolates. This agent may be inadvertently transferred to human fibroblast cell lines and can be confused on the basis of CPE with other human viruses such as CMV or adenovirus. The fifth cross-reaction occurred with a varicella-zoster virus isolate that exhibited an aberrant punctate-appearing fluorescence on highly red-staining enlarged cells that was not characteristic of the fluorescence seen with any of the CMV isolates.

The fact that neither background nonspecificity nor any cross-reactions were seen with the monoclonal antibody and the simplicity of the staining technique with a single 30-min incubation were advantages of the use of this reagent. Although the polyclonal antibody had brighter fluorescent intensity (4+) which allowed the reader to quickly pick up a positive cell, the monoclonal reagent had a distinct staining pattern and sufficient fluorescent intensity (3+) to provide more confidence in defining a CMV-infected cell.

A major disadvantage of this technique in routine laboratory diagnosis was the necessity of having a monolayer demonstrate ≥20 to 25% CPE before identification of the isolate. In clinical specimens, especially from transplantation patients, the quantity of virus present may be minimal, with only one or two foci of CPE observed on initial isolation. This requires extensive passaging of a rather slow-growing virus to build the viral titer to an acceptable level. Problems were encountered with either negative results or the necessity of extensive examination of wells for small numbers of infected cells when monolayers with <20% CPE were used. One possible solution might be trypsinization of the monolayer into a cell suspension, which would alleviate cellular clumping and increase the probability of detecting positive cells. This alternative is now being evaluated in our laboratory. Since a monoclonal antibody to a late CMV antigen was used in this study, further evaluation of monoclonal antibodies against early antigens of CMV may increase the likelihood of earlier detection of virus antigen without the requirement for significant CPE in monolayers.

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