Specific Identification of Human Cytomegalovirus Isolates by Anti-Complement Immunofluorescence with Immune Hamster Sera

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An anti-complement immunofluorescence test utilizing cytomegalovirus immune hamster sera specifically identified cytomegalovirus isolates showing an early, typical cytopathic effect. Inclusion of a control conjugate against adenovirus group antigen permitted correct identification, as adenoviruses, of a few isolates initially suspected of being cytomegalovirus on the basis of cytopathic effect.

There is a continuing need for methods to specifically identify human cytomegalovirus (CMV) isolates recovered in cell cultures. Many laboratories rely solely upon the typical viral cytopathic effect (CPE), and in some cases host cell range, for a presumptive identification of this important virus. However, other viruses, particularly adenoviruses, may produce a CPE which can be mistaken for that of CMV, even by workers highly experienced in interpreting viral CPE. Some laboratories use direct or indirect immunofluorescence (IF) or immunoperoxidase staining with antisera of human origin for CMV identification; however, pitfalls associated with using human sera of uncertain antibody content for virus identification are well recognized. Although sera may be selected which are free from antibodies to other human herpesviruses, they still are likely to contain antibodies to adenoviruses or other human viruses which may be recovered from the same sites as CMV.

In contrast to CMV immune sera from animals immunized with unpurified virus, those from guinea pigs (5) and hamsters (1) immunized with purified virus have been sufficiently free from antibodies to host proteins to permit their use for specific virus identification by IF and radioimmunoassay procedures. Another problem, however, in attempting to identify CMV isolates by direct or indirect IF or immunoperoxidase staining is the nonspecific cytoplasmic staining caused by Fc-immunoglobulin G (IgG) receptors produced in CMV-infected cells (2, 6). This may interfere with detection and interpretation of the virus-specific nuclear staining of CMV-infected cells.

The anti-complement IF (ACIF) test, based upon binding of complement to viral antigen-antibody complexes in CMV-infected cells and detection of the bound complement with fluorescein-labeled antibodies to the C3 component, possesses certain advantages over direct and indirect IF staining. With optimal concentrations of reagents, nonspecific staining caused by binding of IgG to Fc receptors is avoided. Furthermore, ACIF is more sensitive than direct or indirect IF staining, due to amplification of the antigen-antibody complex by the additional layer of complement, and ACIF is capable of detecting certain nuclear and early herpesvirus antigens which are not detectable by direct or indirect IF (3, 9).

This report describes the use of highly specific CMV antisera produced in hamsters (1), together with an ACIF method (7) using guinea pig, rather than human, complement for specific identification of CMV isolates from cell cultures.

Specimens suspected of containing CMV were inoculated into tube cultures of a diploid line of human fetal lung (HFDL) cells maintained on Eagle minimum essential medium with 2% fetal bovine serum. Cultures were incubated in a roller drum at 36°C and refed at weekly intervals for a total observation period of 55 to 60 days. When focal areas of CPE resembling that of CMV were observed, a preliminary report of "probable CMV" was made, and specific identification was then attempted by ACIF. Cultures tested by ACIF contained at least six or seven focal areas of CPE per tube. Trypsinized cells from the infected cultures were washed in 0.01 M phosphate-buffered saline, pH 7.2, with 2% fetal bovine serum by centrifugation at 2,000 rpm for 5 min and resuspended in approximately 0.05 ml of phosphate-buffered saline with 2% fetal bovine serum, and three smears approximately 5 mm in diameter were made on each of three microscope slides. Smears of uninfected cells from the same lot were prepared in the same manner. After drying at room temperature,
smears were fixed in acetone for 10 min at room temperature.

Guinea pig complement for the ACIF test (pretested for absence of antibodies to certain human viral, rickettsial, and chlamydial agents) was from Microbiological Associates, Bethesda, Md. Fluorescein-labeled immune globulin to guinea pig complement was prepared as described previously (7) from the IgG fraction of a commercial (Cappel Laboratories, Inc., Downingtown, Pa.) goat antiserum to guinea pig complement C3 (B1/B1A). A fluorescein-labeled immune reagent to guinea pig complement C3 from Cappel Laboratories was equally satisfactory. CMV immune hamster serum was produced by intraperitoneal inoculation of hamsters with purified virus, as described by Forghani et al. (1). The serum had a CMV complement-fixing antibody titer of 1:1024, a neutralizing antibody titer of 1:512, an indirect IF titer of 1:1024, and an ACIF titer of 1:1024.

Optimal dilutions of CMV antiserum, complement, and conjugate for use in the ACIF test were determined by box titrations of varying dilutions of each reagent against CMV-infected and uninfected cells. The CMV immune serum was used at a working dilution of 1:400, the complement was used at a dilution of 1:20, and the conjugate was used at a dilution of 1:20. The ACIF test was performed as described previously (7) with 20-min incubation periods for the antiserum and conjugate and a 45-min period for the complement. Care was taken to avoid drying of the slides between washing off the complement and adding the conjugate. For heterologous controls, isolates were stained by direct IF with conjugates to adenovirus group antigen and to varicella-zoster virus. Other controls were uninfected cells reacted with the ACIF reagents and with the heterologous conjugates. The former would detect possible C3 receptors on the host cells; these were not seen with the HFDL cells used in this study.

Fifteen viral isolates presumptively identified as CMV on the basis of a typical CPE were specifically identified by ACIF staining; 13 of the isolates were from urine frozen in 35% sorbitol; 1 was from fresh, unfrozen urine; and 1 was from lung tissue. Three isolates from urine specimens which showed a CPE considered to be typical of CMV were identified as adeno viruses by positive staining with an adenovirus conjugate. Figure 1 shows typical nuclear staining of CMV by ACIF and of adenovirus by direct IF. A commercially available adenovirus conjugate (Microbiological Associates) was found to be as satisfactory as the conjugate prepared in this laboratory for group-specific identification of adenovirus isolates. To further confirm the specificity of ACIF for CMV identification, the technique was applied to HFDL cells infected with herpes simplex virus type 1 and with varicella-zoster virus and to Epstein-Barr virus propagated in P3HR-1 cells; all gave negative staining.

The ACIF method proved to be a much more satisfactory approach to specific identification of CMV than was direct or indirect IF staining. In our experience, commercial conjugates for CMV gave very poor specific nuclear staining of viral antigen and also showed cytoplasmic staining of Fc receptors. Indirect IF staining with CMV immune hamster serum gave strong cytoplasmic staining of virus-infected cells, making it difficult to identify virus-specific nuclear staining. The use of guinea pig, rather than human, serum as a source of complement for ACIF is a distinct advantage over other ACIF systems described for CMV (3, 4, 6, 10).

Although CMV immune animal sera for the ACIF method are not now available commercially, preparation of antisera such as those used in the present study is well within the competence of virus laboratories which have facilities for virus propagation and purification. The fact that the antisera can be used at high dilutions makes it economically feasible to produce them in a small animal species. Three different lots of CMV immune hamster serum have reacted satisfactorily and similarly by ACIF. On the other hand, immunization of rabbits with the same purified CMV preparation used for immunizing hamsters produced antisera with low specific ACIF titers for CMV, and which showed nonspecific reactivity to almost the same levels. We also considered the possibility of producing CMV antiserum by immunization of young goats with purified virus, but abandoned the idea because preimmunization bleedings from most of the animals tested gave nuclear staining of CMV-infected cells by ACIF.

In the present study, specific identification was not attempted until the isolates showed a clear-cut CPE resembling that of CMV, because our overall recovery rate of CMV isolates was too low to warrant testing of specimens failing to show a CPE. However, other workers have indicated that ACIF can be used to detect CMV antigen in infected cells before CPE is apparent (3, 8) and also to detect CMV antigen directly in infected tissues (5, 10). Laboratories dealing with larger numbers of CMV-positive clinical specimens might effectively use ACIF to detect early CMV antigens which appear in infected cells before CPE is evident.

These studies confirmed the need for specific
identification of suspected CMV isolates and the value of using a conjugate for adenovirus group antigen as a heterologous control, since a few isolates considered to be CMV on the basis of CPE were in fact adenoviruses.

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LITERATURE CITED


