Evidence for Early Nuclear Antigens in Cytomegalovirus-Infected Cells

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SUMMARY

Human cytomegalovirus (CMV) induces nuclear antigens resembling the Epstein–Barr nuclear antigen (EBNA) as early as 3 h after infection. These early antigens can be detected only with the anti-complement immunofluorescence staining (ACIF) technique. Synthesis of these new antigens is not influenced by cytosine arabinoside (ara-C).

It has been reported that a period of 6 h to 4 d is necessary to detect early nuclear antigens in human fibroblasts after infection with cytomegalovirus (CMV; Rapp, Rasmussen & Benyesh-Melnick, 1963; Jack & Wark, 1972; Laing, 1974). Recently, however, naked capsids have been demonstrated within the nucleus as early as 5 min after initiation of penetration when high multiplicity of infection is used (Smith & de Harven, 1974). The synthesis of late virus proteins in different cell-virus systems can be inhibited by cytosine arabinoside (ara-C), but the production of early antigens is not affected (Pope & Rowe, 1964; Rapp et al. 1964; Sabin & Koch, 1964; Geder et al. 1966/67; Ward & Stevens, 1975). Thus, it is possible to distinguish between early nuclear antigens and late proteins.

Albrecht & Rapp (1973) have reported that malignant transformation of hamster embryo fibroblasts occurs following exposure to CMV irradiated with ultraviolet light. Hamsters bearing tumours after injection of these transformed cells have antibodies which react with the cytoplasmic membrane and the cytoplasm of CMV-infected cells. The same kind of reaction develops when CMV-transformed cells are reacted with CMV-immune serum. However, no intranuclear reactivity has been found in these two systems using the indirect immunofluorescence (IF) test.

This paper presents evidence that human CMV induces nuclear antigens in cells as early as 3 h post infection (p.i.). These antigens can be detected only with the anti-complement immunofluorescence (ACIF) test; synthesis of these new antigens is not influenced by ara-C. The staining pattern and early appearance of these nuclear antigens is similar to that of the Epstein–Barr nuclear antigen (EBNA; Reedman & Klein, 1973).

Monolayers of human embryo lung (HEL) cells were grown on coverslips in medium 199 supplemented with 10 % foetal calf serum. The AD-169 strain of CMV was used for inoculation at a multiplicity of 0.02 p.f.u./cell. Coverslip cultures were incubated with the inoculum for 1 h at 37 °C, after which the unadsorbed virus was removed and replaced with medium. Incubation at 37 °C followed. Some coverslip cultures were pre-incubated with 50 μg/ml of ara-C for 24 h before inoculation. The medium which replaced the inoculum contained the same amount of DNA inhibitor. Medium was changed at daily intervals. Coverslips were removed at 1, 2, 3, 6, 24, 48 and 96 h p.i. The cell sheets were washed three times with phosphate buffer (pH 7.4), air dried for 30 min, and then fixed with a mixture of equal amounts of methanol and acetone for 5 min at 4 °C.

Human antisera to CMV were obtained from hospital patients and staff members of the Department. Immune sera ‘W’ and ‘T’ had immunofluorescent antibody titres to
CMV-infected HEL cells of 1:128 and 1:256 respectively. Serum 'W' had titres of 1:64 to HSV-1-infected HEL cells and 1:32 to HSV-2-infected HEL cells. Serum 'T' reacted with HSV-1-infected cells at a dilution of 1:64 and with HSV-2-infected cells at a dilution of 1:32. HSV-2-specific hamster serum had an immunofluorescent antibody titre to HSV-2-infected HEL cells of 1:32, and PARA (defective SV40)-adenovirus 7 (PARA-7) tumour-bearing hamster serum reacted with homologous tumour cells at a titre of 1:8 in the IF test.

All immune sera used in the IF and ACIF tests were adsorbed with 10⁶ HEL cells/ml of undiluted serum for 1 h at 37 °C and then overnight at 4 °C before use. One sample of immune serum 'T' was adsorbed with sonicated CMV-infected HEL cells (10⁶ cells/0.5 ml of undiluted immune serum, m.o.i. 0.3 p.f.u./cell). The cells were pre-incubated with ara-C for 24 h before virus inoculation. The medium which replaced the inoculum contained the same amount of DNA inhibitor (50 µg/ml). The cells were harvested for adsorption 48 h after virus inoculation.

Anti-human and anti-hamster IgG fluorescent conjugate (rabbit) and fluorescent conjugates for anti-human complement staining (goat) were purchased from Cappel Laboratories, Downingtown, Pennsylvania. Fluorescein isothiocyanate (FITC) conjugates were adsorbed with 10⁶ target cells/ml before use. CMV- and HSV-negative human sera were used as complement in the ACIF tests.

Standard procedures for the indirect IF test were used. The preparations were incubated with the immune serum for 30 min at 37 °C and were then incubated with the anti-human IgG fluorescent conjugate for the same period. After repeated washings, the cells were mounted on glass slides in a mixture of phosphate buffer and glycerol (1:9).

In the ACIF tests, coverslip cultures were covered with heated anti-CMV serum and incubated at 37 °C for 45 min. After repeated washings, four units of human complement were added for 45 min at 37 °C. The cells were washed again with phosphate buffered solution and covered with anti-human complement goat serum labelled with FITC for 45 min at 37 °C. After final washings, the cells were mounted on slides in a mixture of phosphate buffer and glycerol (1:9).

Routine anti-complement staining procedures were modified when negative results were obtained in the first test. The preparations were reacted with immune serum first at 37 °C for 30 min and then at 4 °C overnight, followed by 45 min of treatment at 37 °C with complement and anti-complement FITC, respectively.

The development of fluorescence in CMV-infected cells always commenced in the nucleus. In contrast to previous reports, diffusely distributed intranuclear antigens were detected in the cells as early as 3 h after initiation of the infection when the ACIF test was used (Fig 1 a). These cells were negative when tested with the indirect IF method. With indirect immunofluorescence, very faint, fine, granular intranuclear fluorescence was first noticed at 6 h p.i. (Fig 1b). The ACIF test produced very strong nuclear fluorescence in these cells. The treatment of the cells with ara-C did not influence the localization or the pattern of the reaction. Cytoplasmic staining of the infected cells was seen only in cells which were not first treated with ara-C at 24 h p.i.

In cells which were treated with 50 µg/ml of ara-C, no cytoplasmic antigens developed. The antigens were localized in the nucleus with strong diffuse fluorescence as late as 4 d p.i. (Fig 1c). The antigens were easily detectable with both methods (Table 1).

The human convalescent sera did not react with uninfected ara-C-treated control HEL cells (Fig 1d) in the ACIF test. Human convalescent serum 'T' lost its reactivity with the early nuclear antigens of CMV-infected HEL cells after adsorption with ara-C-treated, CMV-infected HEL cells. The reactivity of the adsorbed serum with HSV antigens remained
Fig. 1. (a) Fluorescence photomicrograph of nuclear antigens detected in CMV-infected HEL cells 3 h p.i. CMV-specific human convalescent serum was used with human complement and fluorescein isothiocyanate-conjugated anti-human complement goat serum in ACIF tests. The cells were grown in medium containing 50 µg/ml of ara-C. Magnification × 1250. (b) Fluorescence photomicrograph of nuclear fluorescence detected in CMV-infected HEL cells 6 h p.i. CMV-specific human convalescent serum was used with fluorescein isothiocyanate-conjugate anti-human rabbit serum in indirect immunofluorescence tests. The cells were grown in medium containing 50 µg/ml of ara-C. Magnification × 1250. (c) Fluorescence photomicrograph of nuclear antigens detected in CMV-infected HEL cells 4 d p.i. CMV-specific human immune serum was used with human complement and fluorescein isothiocyanate-conjugated anti-human complement goat serum in ACIF tests. The cells were grown in medium containing 50 µg/ml of ara-C. Magnification × 1250. (d) Photomicrograph of ara-C-treated uninfected human embryo lung cells reacted with CMV convalescent human serum used for the detection of early antigens in CMV-infected HEL cells. Human complement and fluorescein isothiocyanate-conjugated anti-human complement goat serum were used in ACIF tests. Magnification × 1250.

unaffected. Anti-HSV-2-immune and PARA-7 tumour-bearing hamster sera did not show fluorescence with the antigens of CMV-infected cells (Table 1).

The results presented here demonstrate, by the sensitive ACIF method, the presence of intranuclear early antigens in CMV-infected cells as early as 3 h p.i. This method will be used to detect CMV-specific antigens in the nuclei of CMV-transformed hamster embryo cells; it may be that the antigens have eluded prior detection due to the insensitivity of the indirect IF test (Albrecht & Rapp, 1973). Selection of proper immune sera also seems to be an important factor, for it has been reported that more human sera are reactive with cytoplasmic antigens than with nuclear antigens (Jack & Wark, 1972). This may also be the case with the tumour-immune sera of the hamsters.
Table 1. Compartmentalisation of antigens in CMV-infected cells with and without ara-C treatment in different phases of the infection

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Specificity of antisera</th>
<th>CMV ‘T’*</th>
<th>CMV ‘Ta’†</th>
<th>HSV-2‡</th>
<th>SV4o ‘T’ antigen§</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>IF</td>
<td>ACIF</td>
<td>IF</td>
<td>ACIF</td>
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<tr>
<td>HEL control + ara-C</td>
<td></td>
<td>—</td>
<td>—</td>
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<td>—</td>
</tr>
<tr>
<td>HEL + CMV</td>
<td></td>
<td>3 h</td>
<td></td>
<td>NF†</td>
<td>—</td>
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<tr>
<td>HEL + CMV</td>
<td></td>
<td>6 h</td>
<td></td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>HEL + CMV 24-96 h</td>
<td></td>
<td>NF</td>
<td>ND**</td>
<td>ND</td>
<td>—</td>
</tr>
<tr>
<td>HEL + ara-C + CMV 24-96 h</td>
<td></td>
<td>NF</td>
<td>ND</td>
<td>ND</td>
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</tr>
<tr>
<td>HEL + HSV-1 24 h</td>
<td></td>
<td>+</td>
<td>+</td>
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<tr>
<td>HEL + HSV-2 48 h</td>
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<tr>
<td>ARA-7</td>
<td></td>
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</tbody>
</table>

† Immune serum: human convalescent ‘T’ adsorbed with ara-C-treated, CMV-infected HEL cells.
‡ Serum of hamster immunized with HSV-2.
§ Serum of PARA (defective SV4o)-adenovirus 7 tumour-bearing hamster.
|| HEL cells infected with CMV, both with and without ara-C treatment.
¶ Nuclear fluorescence.
** Not done.
†† Cytoplasmic fluorescence.

We have reported recently (Geder et al. 1976) that persistent infection of human embryonic lung fibroblasts with a genital isolate of CMV (Rapp et al. 1975) resulted in oncogenic transformation of these cells. Immunofluorescence techniques detected virus-specific antigens and microcytotoxicity tests established that the transformed cells share a membrane antigen with hamster cells transformed by inactivated cytomegalovirus. The transformed human cells induced progressively growing tumours in weanling athymic, nude mice. The human CMV-convalescent sera which were used in ACIF tests for the localization of early antigens in CMV-infected cells reacted with EBNA-like nuclear antigens in these CMV-transformed human cells when the ACIF method was used. HSV-2-immune and PARA-7 tumour-bearing hamster sera did not react with these cells.

Thus, these antigens may be CMV-specific T antigens of CMV-transformed cells. Further tests for the CMV-specificity of these nuclear antigens and for their antigenic relationship with the EBNA-like early antigens of CMV-infected cells are in progress.

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REFERENCES


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