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Studies on Human Cytomegalovirus Strain Variations by Membrane-Fluorescence

Brief Report

By

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It is well documented that the human cytomegaloviruses (CMV) share antigens to a wide extent. Using sensitive serological methods, antigenic differences can, however, be demonstrated among various strains (1—3, 6).

A new CMV antigen located on the cell membrane of CMV-infected human fibroblasts has recently been detected (5). The aim of the present study was to investigate whether membrane antigens induced by 3 laboratory strains of CMV possess strain specificity.

The CMV strains Ad169, Davis and C87 together with the techniques for producing CMV hyperimmune sera in rabbits with these strains have been described previously (1, 2).

Fibroblast antibodies were removed by absorbing the hyperimmune sera on washed, noninfected human fibroblasts (8×10^7 cells/0.5 ml serum, diluted 1:5) for 1 hour at room temperature and then at 4° C overnight. In addition the sera were absorbed twice with the same amount of freeze-thawed, disrupted cells for 1 hour at room temperature and then at 4° C overnight.

Finally the sera were spun at 10,000 r.p.m. for 30 minutes and stored at —20° C. All sera were studied in a dilution of 1:10 in the membrane-fluorescence test against noninfected fibroblasts and found negative.

Coverslip cultures were prepared in 6 cm Petri dishes seeded with 7.5×10^5 human fibroblasts and incubated at 37° C for 2 days. They were inoculated with 1—5 p.f.u. virus/cell and incubated for 4 days, after which 80—90 per cent of the cells exhibited cytopathic changes. The indirect membrane-immuno-fluorescence test was performed on the washed, nonfixed coverslip cultures by treating with diluted hyperimmune serum for 30 minutes at room temperature. After washing thrice with buffered saline the preparations were covered for the same period of time with FITC-conjugated antirabbit IgG globulin diluted 1:60. After three further washes the preparations were mounted in buffered glycerol.

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The antibody titre of antisera was determined using twofold dilutions, starting 1:10.

The results are summarized in Table 1. It was found that all three strains shared common antigens on the cell membrane, but as seen from the table some antigenic diversity among the strains was also found. The highest titres in all cases were found against the homologous strain; these titres were 8—16 times higher than the titres to at least one of the other strains.

Table 1. *Cross Testing of CMV Membrane Antigens*

	Membrane—antibody titres to strains		
Hyperimmuneserum	Ad169	C87	Davis
Ad 169	640	40	40
C87	80	80	10
Davis	80	10	160
Control[a]	<10	<10	<10

[a] Serum from rabbit immunized with the culture medium of noninfected human fibroblasts. Some nonspecific immunofluorescence was seen with this serum, infected and noninfected cells showed the same weak fluorescence.

The results suggest that CMV membrane antigens are partly strain specific, as has been reported for the two types of *herpesvirus hominis* (4).

The CMV hyperimmune sera used in this study have previously been used in cross neutralization studies (2) in which some antigenic differences between the 3 strains were found. However, the antigenic variations found in the latter study were less marked than those in the present one. This suggests that either the CMV membrane antigens are different from neutralization antigens or that the membrane fluorescence test is more sensitive than the neutralization test for measuring strain differences.

CMV strain variations have been detected using intranuclear antigens and human convalescent sera (3). The hyperimmune sera used in this study have also been tested for these antigens in fixed preparations, but they did not react with intranuclear CMV antigens. This may be due to the fact that our hyperimmune rabbit sera did not contain capsid-antibodies, as the rabbits were immunized with CMV harvested from the medium of infected cultures.

In summary, it has been demonstrated that the cell membrane antigens induced by the Ad169, Davis and C87 strains of CMV exhibit some strain specific character.

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