Antibodies to Cytomegalovirus-Induced Pre-Early Nuclear Antigen in the Anticomplement-Immunofluorescent Test in Comparison to IgG and IgM Antibodies in the Indirect and Direct Enzyme-Linked Immunosorbent Assay in Diagnosing Cytomegalovirus Infections

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Summary

Development of antibody to pre-early nuclear antigen (anti-PENA) in persons with primary cytomegalovirus (CMV) infection was tested in serial serum specimens of four renal transplant patients and four patients undergoing open heart surgery using the anticomplement-immunofluorescent test (ACIF). In patients undergoing open heart surgery seroconversion of anti-PENA was mostly concomitant with the rise of IgG or IgM antibodies determined in the indirect or direct enzyme-linked immunosorbent assay (ELISA) whereas in all renal transplant patients developing anti-PENA a delayed rise of this antibody compared to IgG and IgM antibodies was observed. Significant rise of anti-PENA accompanied by an increase of IgG and IgM antibodies in indirect and direct ELISA was also found in three patients undergoing open heart surgery with recurrent CMV-infection. Anti-PENA was shown to persist longer than IgM antibody. Moreover, anti-PENA was present in the serum of nearly two-thirds of 30 persons with IgG antibody but without IgM antibody.

It is concluded that antibody determination to PENA can serve as an additional means of diagnosing primary and recurrent CMV infections. Because of its long persistence only seroconversion or significant rise of this antibody may be considered evidence of infection. In some patients a delayed development of anti-PENA must be taken into consideration.
Introduction

Determinations of antibody to pre-early nuclear antigen (anti-PENA) using the anticomplement-immunofluorescent test (ACIF) have been carried out by different authors (1, 2, 3, 5). It has been found that seroconversion of anti-PENA follows primary and recurrent CMV infection. However, different results were obtained regarding the timing of seroconversion of anti-PENA in comparison to other CMV specific antibodies and regarding the presence of anti-PENA in CMV seropositive persons without evidence of current or recent CMV infection (1, 2, 3, 5).

Therefore, the present study was undertaken to investigate anti-PENA by ACIF in groups of patients with primary and with recurrent CMV infections, and in a group of individuals without evidence of recent CMV infection. The presence of anti-PENA was compared with studies of CMV-specific IgG and IgM as tested by indirect and direct enzyme-linked immunosorbent assay (ELISA), respectively, and CMV-specific IgM also by the indirect immunofluorescent test (IF). The diagnostic value of the anti-PENA test was also considered.

Materials and Methods

Sera

Serial serum specimens taken before and at different times after onset of CMV infection were obtained from four renal transplant patients with primary infection and from seven patients undergoing open heart surgery with primary or recurrent infection. Furthermore, sera from 30 patients obtained before open heart surgery were also investigated.

Virus

The AD 169 strain of CMV was used to prepare the antigens for ACIF, ELISA and IF. The virus was propagated in human embryonic fibroblasts.

ACIF

PENA-positive nuclei of human embryonic fibroblasts were prepared by inoculating the cells with CMV at a multiplicity of 0.5 to 1 plaque-forming unit/cell. After virus adsorption for 2 hours the infected cells were incubated for 2 hours at 37°C. Further preparations were carried out essentially according to Mintz et al. (4). Briefly, trypsinated CMV-infected cells were washed three to four times in distilled water containing 2 per cent fetal calf serum. Then the nuclear pellet was resuspended in washing solution and adjusted at a concentration of 10^6 cells per ml. Drops of nuclei suspension were placed on glass slides, air dried, fixed in acetone:methanol (1:1 by volume) for 10 minutes at -20°C, and stored at -70°C until use.

The antibody determination was performed in three steps. Smears of PENA positive nuclei were covered with different dilutions of serum and incubated at 37°C for 30 minutes. Then the preparations were washed in PBS, covered with human complement (1:5 diluted in BSS: 0.8 per cent NaCl, 0.014 per cent CaCl₂, 0.04 per cent KCl, 0.02 per cent MgSO₄ · 7H₂O, 0.06 per cent KH₂PO₄, 0.06 per cent Na₂HPO₄ · 2H₂O) and incubated at 37°C for 30 minutes. After washing in PBS the preparations were covered with an FITC-conjugated anti-human-complement (1:20 diluted in BSS;