NOTES

Detection of Antibody to Cytomegalovirus-Induced Early Antigens and Comparison with Four Serologic Assays and Presence of Viruria in Blood Donors

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Five hundred blood donors were evaluated for cytomegalovirus (CMV) viruria, antibody to CMV early antigens (EA-ab), CMV seropositivity by two screening assays, and CMV-specific immunoglobulin M by two methods. Three donors were viruric, EA-ab positive, and seropositive; two viruric donors were immunoglobulin M positive.

Selection of cytomegalovirus (CMV)-seronegative blood is an accepted method for providing blood with a reduced risk of CMV transmission (1, 18). However, use of only CMV-seronegative blood products would greatly reduce the available blood supply since the worldwide prevalence of CMV antibody is 40 to 100% (1, 10). Two probable mechanisms for explaining CMV transmission by blood are (i) the transfusion of infectious virus in the blood from an actively infected donor and (ii) the activation of the CMV genome present in transfused cells (2). If actively infected donors are able to transmit CMV, then an assay for a serologic marker specifically associated with viral shedding would be useful for screening blood donors. Current CMV serologic assays detect antibodies (mainly immunoglobulin G [IgG]) induced by late CMV antigens, which develop during a primary infection and usually persist for a lifetime (15, 17); these assays cannot separate persistent from recurrent infections. The presence of IgG to CMV early antigens frequently has been associated with recent or active infections, and the level declines after virus excretion ceases (5, 6, 12, 13, 19, 20).

We cultured urine to assess the prevalence of active CMV infection in 500 blood donors, since CMV is more likely to be isolated from urine or respiratory secretions than from blood of healthy individuals (1, 15). To assess which serologic markers were associated with viruria, we tested serum for the presence of antibody to early antigens (EA-ab), CMV seropositivity by two assays, and CMV-specific IgM by two assays. Statistics were determined by commonly used methods (8). *P* values were determined by chi-square derived from a two-variable contingency table.

Urine specimens were cultured on human foreskin fibroblasts (HFF) by standard tissue culture procedures (4). Sera were tested for CMV seropositivity by an enzyme-linked immunosorbent assay (ELISA; Litton Bionetics, Charleston, S.C.) and latex agglutination (Hynson, Westcott, and Dunning, Baltimore, Md.) in accordance with the guidelines

of the manufacturers. CMV-induced IgM was determined by an indirect immunofluorescence assay (IFA; Electro-Nucleonics, Inc., Columbia, Md.) and enzyme-labeled antigen assay (ELA), which was made available for research purposes by Medac (Hamburg, Federal Republic of Germany). The ELA is an ELISA technique in which anti-human IgM is attached to the solid phase. Human anti-CMV IgM in the serum sample then binds and can be detected by subsequent binding of peroxidase-labeled CMV antigen.

The EA-ab assay was prepared in our laboratory. HFF monolayers were inoculated with a cell-free suspension of CMV AD169 (ATCC VR-538) and incubated at 37°C for 72 h in minimal essential medium containing 3% fetal bovine serum and 75 µg of cytosine arabinoside per ml (13). An IFA for IgG (11) was performed with a 1:10 serum dilution and goat anti-human IgG F(ab')₂ conjugated to fluorescein isothiocyanate (Organon Teknika, Malvern, Pa.). The positive EA-ab reaction was defined as diffuse, granular fluorescence of infected cell nuclei with an intensity of 1+ or greater (Fig. 1). A negative reaction was defined as no nuclear fluorescence. The presence of early antigens and absence of late antigens was confirmed with monoclonal antibodies to early and late antigens of strain AD169 (Biotech Research Laboratories, Inc., Rockville, Md.). The positive reaction with the monoclonal antibody to early antigen was diffuse, homogeneous fluorescence in infected cell nuclei (Fig. 2). The monoclonal antibody to late antigen showed no fluorescence. Inoculation of HFF with the supernatant from the cell cultures resulted in no development of cytopathic effect, demonstrating that viral replication had been inhibited. Sera from patients with other herpesvirus infections were assayed for EA-ab, and none cross-reacted. Sera containing antinuclear antibody reacted with the nuclei of infected and uninfected cells yielding an indeterminate result.

Of 500 donors, 3 (0.6%) had positive urine cultures and were positive by the EA-ab assay, ELISA, and latex agglutination. The IFA identified one viruric donor, and the ELA identified two viruric donors. Thirty-five percent of the sera were positive by ELISA (174 of 500) and latex agglutination (176 of 500), with a concordance between the two assays of

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99.6%. The prevalence of EA-ab was 16% (78 of 500); one specimen was indeterminate due to antinuclear antibody. All EA-ab-positive specimens were positive by ELISA and latex agglutination ($P < 0.001$). Thirteen percent of the sera were positive by IFA (65 of 500), and 3% were positive by ELA (15 of 500). The concordance between the assays for CMV-specific IgM was 85% ($P = 0.4184$). The concordance of the IFA was 72% with the EA-ab assay ($P = 0.001$), 57% with ELISA ($P = 0.1129$), and 56.6% with latex agglutination ($P = 0.0979$). The concordance of the ELA was 85% with the EA-ab assay ($P < 0.001$), 66.2% with ELISA ($P = 0.008$), and 65.8% with latex agglutination ($P = 0.009$).

The prevalence of CMV viruria in our study (0.6%) was lower than that in a study of blood donors (2.7%) by Kane et al. in 1975 (9). However, both of these values for CMV shedding are close to the estimated 1 to 2% of seropositive healthy adults shedding CMV at any given time (15). The prevalence of CMV seropositivity in our donor group was relatively low compared with that in other regions of the United States and other parts of the world (1, 10). However, the prevalence of EA-ab (16%) in our donor group was similar to that (10%) in a study of 500 Italian blood donors, in which the seropositivity prevalence was 76% (14). In both groups, the EA-ab-positive donors represented a subset of the seropositive donors, in which proportionately more females than males were EA-ab positive.

CMV-specific IgM was not consistently present in our viruric donors, which is in agreement with other reports that IgM is not a reliable marker for active CMV infection in various populations (7, 16). Because the IFA was used as a rapid screening assay in our study, it was necessary to include weakly positive and indeterminate results with positive results. Although the prevalence of CMV-specific IgM by IFA (13%) in our blood donors was higher than the 4% CMV-specific IgM prevalence in the study of blood donors by Beneke et al. (3), our IgM results by ELA (3%) were comparable to theirs.

Although the mechanisms of transmission of CMV by

FIG. 1. Positive EA-ab reaction of target cell nucleus with human serum. Other cells in the field are uninfected HFF. Magnification, $\times 400$.

FIG. 2. Positive EA-ab reaction of target cell nucleus with mouse monoclonal antibody to CMV early antigen. Other cells in the field are uninfected HFF. Magnification, $\times 400$.

blood transfusion remain unclear, blood products from an actively infected donor are likely to carry a high risk of infectivity. Our results indicate that the prevalence of CMV viruria in blood donors is low and that screening for CMV seropositivity or EA-ab, but not for CMV-specific IgM, will identify these donors. Further studies are needed to determine whether EA-ab is associated with the presence of the CMV genome in donor blood cells. If EA-ab-positive donors are the subset of CMV-seropositive donors who are more likely to transmit infectious virus or the latent CMV genome, then prospective randomized studies of transfusion recipients of EA-ab-screened blood could indicate whether EA-ab is a marker of infectivity.

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