Large-Scale Serological Screening for Cytomegalovirus Antibodies in Homosexual Males by Enzyme-Linked Immunosorbent Assay

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We compared an enzyme-linked immunosorbent assay (ELISA) with complement fixation and radioimmunoassay in determining the presence of immunoglobulin antibodies to cytomegalovirus. Of an initial 93 serum samples tested, the correlation between ELISA and radioimmunoassay was 98.9% and that between ELISA and complement fixation was 96.1%. ELISA was used to screen 1,123 homosexual men in San Francisco. Of 479 men attending a homosexual health fair, 35 (7%) lacked cytomegalovirus antibodies by ELISA. Only 15 (2%) of 644 homosexual men attending a municipal sexually transmitted disease clinic were found to be seronegative. All but one of the seronegatives detected by ELISA were also seronegative by radioimmunoassay and complement fixation. We conclude that ELISA can be used to reliably perform large-scale screening for the presence of immunoglobulin G antibodies to cytomegalovirus.

There have been numerous studies documenting the ability of enzyme-linked immunosorbent assays (ELISA) to detect the presence of antibodies to cytomegalovirus (CMV) (1–3, 7, 9, 11, 12). A comparison with other methodologies has shown that ELISA is more sensitive than complement fixation (CF), anticomplement immunofluorescence, and passive hemagglutination techniques and is equivalent to radioimmunoassay (RIA) for the detection of immunoglobulin G (IgG) antibodies to CMV (2). The advantages of ELISA include the lack of interference from anticomplementary serum factors and the absence of radioisotopes. Because of these considerations, ELISA is ideally suited for large-scale serological screening.

There has been a great deal of interest in understanding the pathogenesis of acquired immunodeficiency syndrome (AIDS) (10). The majority of people afflicted with AIDS have come from the homosexual community. We were particularly interested in identifying a group of CMV-seropositive homosexual males and prospectively studying their immunological parameters. By also studying a comparable seropositive group, we hoped to learn more of the epidemiology of CMV in homosexuals and the role, if any, of the virus in AIDS.

A prior study with a patient population similar to ours with a different technique showed that 93.5% of homosexual males had detectable IgG antibodies to CMV (4). Large-scale screening of the homosexual community is therefore necessary to identify a significant number of seropositive individuals.

We describe our experience with ELISA to screen for IgG antibodies to CMV with a single serum dilution. Using this method, we were able to screen 1,123 homosexual men to ascertain the prevalence of prior CMV exposure.

MATERIALS AND METHODS

Sera. For determining the optimal serum dilution for the proposed screening program, we used serum that had been tested by CF and RIA to CMV in a previous study and was then kept frozen at −20°C. Included were patients with CMV mononucleosis, cardiac transplant recipients, and healthy laboratory personnel.

For the mass screening, sera were obtained in San Francisco from 479 homosexual males attending a homosexual health fair and from 644 homosexual males attending a municipal sexually transmitted disease clinic. Males were asked to identify themselves as either homosexual, bisexual, or heterosexual at the time of blood drawing. No further questioning was undertaken.

CF for CMV. For CF IgG, antibodies to CMV were measured by standard microtiter methods (5). The CMV antigen used was from the same lot as that used in ELISA (described below). Titers greater than or equal to 1:8 were considered positive.

RIA for CMV. For RIA, IgG antibodies to CMV were measured by a previously described method (8). In this assay, titers greater than or equal to 1:3,000 were considered positive.

ELISA for CMV. Ninety-six-well, flat-bottom Immulon 1 plates (Dynatech Laboratories, Alexandria, Va.) were coated with 50 μl of a 1:100 dilution in phosphate-buffered saline (PBS) of either CMV CF antigen (lot 40613040, Flow Laboratories, McLean, Va.) or human embryonic lung (HEL) cell control antigen (lot 41613029, Flow Laboratories). The optimum antigen dilution was determined by titration against a positive control serum. The first two vertical columns received the viral antigen, and the next two were filled with the control antigen. This alternating pattern was continued for the entire plate so that columns 1, 2, 5, 6, 9, and 10 contained CMV and the others contained the control antigen. The plates were then sealed with Parafilm and kept at 4°C for a maximum of 2 weeks before use.

On the day of use, the plates were manually washed three times in PBS containing 0.05% Tween 20 (PBS-T) with multichannel dispenser (Cooke Lab Products). Approximately 200 μl of PBS with 1% bovine serum albumin (PBS-BSA) was added to each well, and the plates were sealed. After 1.5 to 2 h at room temperature, the plates were aspirated, and the appropriate sera were added. For determining the antibody titer, 100 μl of a 1:500 serum dilution in PBS-BSA was added to four wells in the top row (two CMV and two HEL). Thus, three serum samples could be tested on each plate. The remainder of the wells were filled with 50
μl of PBS-BSA, using a multichannel pipettor (Titertek, Flow Laboratories, Finland). Serial twofold dilutions were made with an automated diluter (Mindiluter, Cooke Lab Products). For screening with a single dilution, 50 μl of a 1:1,000 serum dilution in PBS-BSA was added manually to each of two CMV and HEL antigen-containing wells. The top row of each plate contained PBS-BSA (i.e., no serum), positive, and negative serum pool controls, respectively. After overnight incubation at room temperature, the plates were washed four times with PBS-T. Then 50 μl of a 1:3,000 dilution of horseradish peroxidase conjugated to goat anti-human IgG (Tago, Inc., Burlingame, Calif.) was added to each well. The plates were sealed with Parafilm and placed on a rocker platform at 37°C for 1.5 to 2 h. They were then washed 25 times in running tap water, and 0.1 ml of a 1:100 dilution of H₂O₂ and 2,2-azino-diethylbenzthiazoline sulfonic acid substrate (Zymed Laboratories, San Francisco, Calif.) was added to each well. After 10 min at room temperature, the reaction was stopped with 0.1 ml of 2 M sodium azide.

The plates were read at 405 nm on a microELISA automated reader (MR 580, Dynatech Laboratories, Alexandria, Va.). The mean absorbance value for the HEL wells was subtracted from that of the CMV wells for each serum sample. A difference of greater than 0.1 was considered positive (see below). All sera found to be negative by single-dilution ELISA testing were also tested by CF and RIA.

RESULTS

Ninety-three serum samples were compared by CF, RIA, and ELISA for detection of IgG antibodies to CMV (Fig. 1 and 2). There was excellent correlation between ELISA and CF (74/77) or RIA (92/93) in determining the presence or absence of CMV antibodies; however, the ELISA titer was usually 10 to 100 times that determined by CF. The one serum sample that was positive by ELISA and negative by CF and RIA gave an absorbance value of 0.113 on initial testing. On repeat testing this became 0.087, a value which would be considered negative. Also, the two specimens that were positive by CF and negative by ELISA and RIA were from a patient with acute CMV disease. IgM antibodies were detected in both sera by RIA, suggesting that this class of antibody was responsible for the positive CF test.

Sixteen serum samples were anticomplementary by CF. Testing by ELISA revealed that 10 were negative (absorbance value, ≤0.100) and six were positive (four with absorbance values of between 0.101 and 0.999 and two with values of ≤1.000).

For the large-scale screening project an absorbance value of less than 0.1 for a serum specimen diluted 1:1,000 was determined to reliably predict the absence of IgG antibodies to CMV (Fig. 3 and 4). Confirmation of this was obtained by testing of the negative serum pool on 61 separate occasions. The mean absorbance value (0.023 plus two standard deviations (0.041 × 2) equaled 0.105, with a range of 0 to 0.203.

There were 479 sera tested from the homosexual health fair, and 35 (7%) were negative by ELISA. Only 15 (2%) of 644 homosexual men attending the sexually transmitted disease clinic were seronegative by ELISA. The prevalence of seropositivity between the populations studied was found to be statistically significant by chi-square analysis (P < 0.001).

The 50 negative sera were also tested by CF and RIA. One sample from the homosexual health fair with an initial absorbance value of 0.095 was found to be positive by CF (1:16) and RIA (1:10,000). Repeat testing by ELISA revealed an absorbance value of 0.268. Another serum from a subject screened at the health fair was found to be negative by ELISA and RIA but found to have a titer of 1:8 by CF.

DISCUSSION

Our results confirm the reliability of ELISA in detecting IgG antibodies to CMV when compared with RIA and CF.
Of an initial 93 serum samples tested, the correlation between ELISA and RIA (98.9%) or CF (96.1%) was excellent. Additionally, ELISA was able to predict the absence of IgG antibodies to CMV in 49 of 50 homosexual men. The single false-negative serum was positive on repeat ELISA testing. Because RIA and CF testing was not done on all samples screened, the false-positive rate could not be determined. Although other investigators have reported similar findings (1, 2, 7), our method of testing is somewhat different. All reagents, including antigens, can be obtained commercially, and we have incorporated a cell control antigen into our determinations. This was found to be essential, as occasional sera had cell control antigen absorbance values equal to or greater than those achieved with viral antigens.

The high prevalence of IgG antibodies to CMV in homosexual men in San Francisco as reported here is in accordance with the findings of Drew et al. (4). It is of interest that we found a slightly but significantly ($P < 0.025$ by chi-square testing) higher frequency of antibodies (98 versus 93.5%) in the patients attending the same sexually transmitted disease clinic as Drew studied. This may be due to the fact that ELISA is more sensitive than anticomplement immunofluorescence used by those investigators (2). Although not tested by us, the prevalence of CMV seropositivity in a group of male heterosexuals attending the same sexually transmitted disease clinic was 54.3% (4). By large-scale screening we were able to demonstrate a statistically significant difference in CMV seroprevalence between homosexual males in San Francisco attending either a sexually transmitted disease clinic or a homosexual health fair. Unfortunately, aside from sexual orientation, no additional information was available to allow further analysis. Another recent study from New York City with CF testing has confirmed the high prevalence of CMV antibodies (90%) in male homosexuals (6).

We have shown the utility of ELISA for large-scale screening for CMV IgG antibodies. Recently developed automated equipment might be utilized in large-scale screening when the knowledge of CMV serology (e.g., in blood banks) is considered important.

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LITERATURE CITED
