Enzyme-Linked Immunosorbent Assay for Measurement of Cytomegalovirus Glycoprotein B Antibody in Serum

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Received 19 October 2009/Returned for modification 12 January 2010/Accepted 3 March 2010

Measurement of antibody to cytomegalovirus (CMV) glycoprotein B (gB) is valuable in the assessment of the antibody response to infection and to gB-containing vaccines. For this purpose, an enzyme-linked immunosorbent assay (ELISA) with a recombinant CMV gB molecule as the antigen was evaluated. Sera from 168 anti-CMV IgG-positive and 100 seronegative subjects were used to evaluate the anti-gB antibody assay. A cutoff optical density (OD) that would distinguish gB antibody-positive from -negative sera was established. Titers of antibody to gB determined by endpoint dilution were compared with those calculated using regression analysis. The run-to-run and interoperator reproducibilities of results were measured. The mean OD ± 5 standard deviations from 50 anti-CMV IgG antibody-negative sera (0.2472) was used as the cutoff between anti-gB antibody-positive and -negative results. All sera from 100 anti-CMV IgG-seronegative subjects were negative for antibody to gB. All but 1 of 168 sera from seropositive subjects were positive for antibody to gB. Observed antibody levels based on titration to the endpoint were very similar to results calculated using linear regression. The run-to-run consistency of endpoints was excellent, with 38 runs from one operator and 48 runs from another all giving results within 1 dilution of the mean value for each of three anti-CMV IgG antibody-positive serum pools. The geometric mean titer of antibody to gB for 99 sera from seropositive blood donors was 1/10,937. This ELISA gives accurate and reproducible results for the relative quantity of anti-CMV gB IgG in serum over a wide range of antibody levels.

Materials and Methods

Study sera. A total of 268 sera (1 sample from each of 268 subjects) that had been tested for IgG antibody to CMV were used to characterize the CMV gB ELISA. In addition, 66 sera positive for anti-CMV IgG antibody were used to create pools of serum controls with low, intermediate, and high levels of antibody to CMV. Study sera included deidentified remnant diagnostic laboratory samples, deidentified sera from healthy adult blood donors kindly provided by the American Red Cross, and sera from healthy young women who volunteered for vaccine clinical trials. One hundred sera were negative for antibody to CMV, and 168 were positive for IgG antibody to CMV. All sera were from subjects participating in studies approved by the University of Alabama at Birmingham (UAB) Institutional Review Board for Human Use.

Serologic methods. Sera were screened for IgG antibody to CMV by using a commercial microparticle immunoassay (AxSYM System CMV IgG; Abbott Laboratories, Abbott Park, IL) according to the manufacturer’s instructions. The results were recorded as antibody units (AU) per milliliter. Values greater than 15 AU/ml were considered positive for anti-CMV IgG; the highest value achievable is reported by this system as >250 AU/ml. Values less than 10 AU/ml were considered negative. Results from 10 to 15 AU/ml were considered to be in an indeterminate range.

The gB ELISA was performed in 96-well microtiter plates. The procedure used is a modification of a procedure provided to us by Chiron that was used in studies of a CMV gB vaccine sponsored by Chiron (9) and a procedure kindly shared with us by Richard Ward, Director of the Laboratory for Specialized Clinical Studies at Cincinnati Children’s Hospital Medical Center. Recombinant CMV gB (Sanofi Pasteur, Marcy L’Etoile, France) provided for laboratory use by the manufacturer was used as the antigen for the ELISA. The recombinant gB is a mutagenized form of CMV Towne gB in which the transmembrane portion and fusion domains have been deleted and the proteolytic cleavage site has been blocked by site-specific mutagenesis (5, 12). The molecule retains the major antigenic domains, AD-1 and AD-2, and is produced in Chinese hamster ovary cell culture. This CMV gB molecule was originally produced by Chiron as a vaccine antigen and was evaluated in a number of clinical trials (8, 12). The vaccine was acquired by Sanofi Pasteur in 2000, and a recent phase 2 clinical trial showed that the CMV gB vaccine has efficacy for the prevention of maternal CMV infection (10).
TABLE 1. ELISA results for IgG antibody to CMV gB for sera from 50 donors who were negative for anti-CMV antibody by a commercial assay

| Serum dilution | OD | Proportion of sera with OD greater than: |
|---------------|----|----------------------------------------|
|               | Mean | SD | Mean + 3 SD | Mean + 4 SD | Mean + 3 SD | Mean + 4 SD |
| 1/200         | 0.0525 | 0.0389 | 0.1694 | 0.2083 | 2/50 | 0/50 |
| 1/400         | 0.0300 | 0.0222 | 0.0967 | 0.1190 | 1/50 | 0/50 |
| 1/800         | 0.0185 | 0.0125 | 0.0560 | 0.0685 | 1/50 | 0/50 |

RESULTS

Determination of the cutoff between results negative and positive for antibody to gB. Fifty sera from seronegative subjects were used to define the expected OD for sera from uninfected subjects with the CMV gB ELISA. With the Assym assay, these 50 sera had a median anti-CMV IgG level of 1.6 AU/ml, with a range from 0.0 to 8.8 AU/ml, all clearly less than the Assym cutoff of <10.0 AU/ml for negative results. Three dilutions (starting at 1/200) of each anti-CMV IgG-negative serum sample were tested by the CMV gB ELISA. The mean OD ± 1 standard deviation (SD) for the 50 samples at a 1/200 dilution was 0.0525 ± 0.0389. The numbers of sera with OD greater than specified multiples of this SD are shown in Table 1. All 50 samples had OD that were less than 4 standard deviations above the mean OD for the group at a 1/200 dilution. Considering the relatively modest sample size for this determination and the desire to have an assay with a high predictive value for a positive result, it was decided that sera with OD at a 1/200 dilution less than 0.2472, 5 standard deviations above the mean OD for negative sera, would be consid-
data differed from the observed endpoint by more than a log2 of the endpoints calculated from the 1/12,800 starting-dilution. None better agreement when the data used came from serum dilutions that covered the range of the observed endpoints. The log2 median calculated endpoint was 18.30 (range, 16.62 to 19.64). Endpoint between the calculated and observed endpoints was not statistically different from the median calculated endpoint (P = 0.78, less than 1 dilution. With the 1/800 starting-dilution data, 4/18 results were more than 1 dilution (log2 of difference, >1.00) greater than the observed endpoint.

**Observed versus calculated endpoints.** The calculation of endpoints creates the opportunity for additional errors besides those that could affect the accuracy of any ELISA method, such as variation in the quality and consistency of reagents and errors or inconsistency in incubation times, pipetting, blocking, or washing. Calculation errors arise mainly from using OD dilution data that are not on the falling portion of the curve and from a failure of the data points to describe a straight line. Based on the experience gained testing the first 100 anti-CMV IgG-positive sera, it was determined that the criteria for a valid run should include the following. (i) At the highest dilution used in endpoint calculation, the OD must be ≤1.1, or the difference in the OD between the lowest and highest dilutions used in endpoint calculation must be ≥0.9. (ii) The difference in the OD between the lowest dilution used for endpoint calculation and the next-to-lowest dilution must be ≥0.15, and the difference in the OD between the highest and next-to-highest dilutions used must be ≥0.15. (iii) The r2 for the straight line used to determine the endpoint must be ≥0.95.

**Reproducibility.** Three pools of sera were prepared for use as a quality control measure and for assessing the run-to-run and interoperator consistency of the assay results. Twenty sera with Axysym anti-CMV IgG levels from 15 to 89 AU/ml were mixed to form a low-positive control; this pool had an anti-CMV IgG level of 77.6 AU/ml. Twenty-five sera with anti-CMV IgG levels from 90 to 190 AU/ml were mixed to form an intermediate positive control; this pool had an anti-CMV IgG level of 137.4 AU/ml. Twenty-one sera with anti-CMV IgG levels from 200 to >250 AU/ml were mixed to form a high-positive control; this pool had an anti-CMV IgG level of >250 AU/ml.

The run-to-run reproducibility of assay results was assessed for each of two operators and between the two operators. For operator 1, mean log2 endpoints ± 1 SD (for 38 runs) for low-, intermediate, and high-positive controls were, respectively, 12.38 ± 0.28, 12.68 ± 0.43, and 13.89 ± 0.33. For operator 2, the corresponding results (for 48 runs) were 12.23 ± 0.42, 12.65 ± 0.36, and 13.96 ± 0.48. Results within 2 dilution (log2, 1.0) were considered acceptable in terms of reproducibility. For each operator, 100% of the results with positive controls from the runs discussed above were within ±1.0 log2 (one 2-fold dilution) of the operator’s mean. In addition, statistical comparison of results showed that there was no statistically significant difference between the two operators for low (P = 0.12), intermediate (P = 0.96), or high (P = 0.61)-positive-control serum results.

**DISCUSSION**

This ELISA method for measuring IgG antibody to CMV gB allows calculation of the endpoint titer by using regression analysis of the more vertical portion of the OD–dilution curve. The calculated endpoints are very similar over a wide range of anti-gB antibody levels to endpoints observed by diluting sera to the point where the OD is below the cutoff value indicating no detectable antibody to gB. The assay provides excellent run-to-run and interoperator reproducibility. A similar ELISA method was used at Chiron (now Novartis) to measure levels
of antibody to CMV gB in sera from vaccine trial participants and controls (9). The geometric mean titer (GMT) of anti-gB antibody reported for 200 seropositive adults tested at Chiron was 3,186, compared with a GMT of 10,937 reported here for 99 seropositive blood donors.

The anti-gB antibody assay is able to distinguish sera that are positive or negative for anti-CMV antibody nearly as well as a commercial assay that uses whole virus as an antigen, based on results with sera from 168 anti-CMV antibody-positive subjects. However, without further evaluation using a larger sample size, the gB ELISA cannot be recommended for this purpose. The antigen used for this assay is the same molecule used as an antigen in an investigational CMV gB vaccine. The availability of a purified recombinant gB molecule very likely contributed to the sensitivity and specificity of this assay for the detection of persons with antibody to CMV. The fact that all but 1 of 168 anti-CMV antibody-positive subjects tested exhibited antibody to gB provides further evidence that the recombinant gB molecule used as an antigen contains highly conserved immunogenic domains that are recognized by antibodies stimulated by infection in all CMV strains.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants from the Division of Microbiology and Infectious Diseases, National Institutes of Allergy and Infectious Diseases (PO1-AI43681 and U01-AI063565), and by grant 5UL1 RR025777-02 from the NIH National Center for Research Resources. Research support was also provided by Sanofi Pasteur, Marcy L’Etoile, France. D. J. Hackett was the recipient of an Infectious Diseases Society of America Education and Research Resources. Research support was also provided by Sanofi Pasteur, Marcy L’Etoile, France. D. J. Hackett was the recipient of an Infectious Diseases Society of America Education and Research Resources. Research support was also provided by Sanofi Pasteur, Marcy L’Etoile, France. D. J. Hackett was the recipient of an Infectious Diseases Society of America Education and Research Resources. Research support was also provided by Sanofi Pasteur, Marcy L’Etoile, France. D. J. 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