Circulating Cytomegalovirus (CMV) Neutralizing Activity in Bone Marrow Transplant Recipients: Comparison of Passive Immunity in a Randomized Study of Four Intravenous IgG Products Administered to CMV-Seronegative Patients


Forty-two cytomegalovirus (CMV)-seronegative bone marrow transplant (BMT) recipients were randomized in a double-blind fashion to receive one of four commercially available intravenous Ig (IVIgG) products (Gamimmune N, Immune Globulin Intravenous, Gammagard, or Sandoglobulin) at a dose of 500 mg/kg every other week. The four treatment groups were similar in distribution of patient ages, weights, autologous versus allogeneic donor type, and underlying diseases. Every other week administration of IVIgG provided total serum IgG levels within the physiologic range for age.

CMV titers by latex agglutination were stable (average geometric mean titer of 18.4 after the second IVIgG dose), with no statistically significant differences among the four groups. CMV neutralizing activity (CMVNA) and CMV enzyme-linked immunosorbent assay (ELISA) titers were determined on a subset of sera from 27 study patients representing the four product groups. Patient serum samples obtained before IVIgG infusions and 2 weeks after the second IVIgG dose (ie, 3 weeks post-BMT) were assayed for CMVNA and CMV ELISA titers. Geometric mean titers of CMVNA and CMV ELISA varied among the product groups. The highest mean 50% CMVNA was 1:43 for product B, whereas the lowest mean 50% CMVNA was 1:14 for product A; two of the IVIgG product groups showed intermediate 50% mean titers of 1:27 (product C) and 1:28 (product D) for an overall P = .02. CMV ELISA titers (expressed as Paul Ehrlich International units [PEI U]) also showed the highest mean of 2.95 PEI U/mL for product B and the lowest mean of 1.34 PEI U/mL for product A. Intermediate mean values of 2.27 PEI U/mL and 2.03 PEI U/mL were obtained with products C and D, respectively (overall P = .003). The CMV ELISA titers show a minimal correlation (r = .566) to the observed CMVNA titers.

We conclude that commercially available IVIgG products provide passive CMVNA, and that the level of circulating CMVNA is affected by the IVIgG product used.

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cially available IVIG products commonly used in the United States. In the first phase of the study, patients were treated on a weekly basis with IVIG, resulting in the accumulation of IgG with all product groups.

The current report describes antibody studies performed on sera from CMV-seronegative patients receiving 500 mg/kg IVIG every other week. This dosing schedule maintained stable IgG levels within the physiologic range for age and stable CMV titers by latex agglutination after the second dose. Trough serum levels obtained after the second IVIG dose during the third posttransplant week were analyzed for CMVNA and CMV enzyme-linked immunosorbent assay (ELISA) titers, providing the first published comparison of circulating, passively transferred functional CMV antibody levels observed with four different commercial IVIG products.

**MATERIALS AND METHODS**

**Patients and IVIG administration.** Forty-two consecutive CMV-seronegative marrow transplant recipients were entered into this study between July 1988 and August 1989. Patients were randomized to receive one of four commercially available IVIG products (product A, Gamimmune N [Miles, Cutter, Elkhart, IN]; product B, Gammagard [Baxter Hyland, Glendale, CA]; product C, San- doglobulin [Sandoz, East Hanover, NJ]; product D, Immune Globin Intravenous [ARC-Hyland, Glendale, CA]) at a dose of 500 mg/kg every other week for three doses beginning 1 week before BMT. All patients in a given product group received the same lot of IVIG throughout the study had a titer of 5.47 ± 0.2 plaque forming units (PFU)/mL, as measured by plaque assay on HF cells (n = 25).

All serum samples were filtered through 1.2- and 0.22-μm syringe filters (Millipore, Bedford, MA) before use to remove lipid (frequently found in BMT patients receiving IV alimentation) and ensure sterility. The serum samples were heated at 56°C for 30 minutes to inactivate complement. Patient samples from each of four treatment groups were included each time an assay was performed to reduce the potential for bias due to assay to assay variability.

The neutralization plaque assay was performed in HF cells incubated in MEM without FBS with a target range of 10% to 90% viral reduction. Serum samples were prepared in six to eight twofold serial dilutions. Complement was not used in the assay after initial studies showed that the presence of guinea pig complement resulted in variable CMVNA in this bioassay.

Briefly, the neutralization plaque assay was performed by preparing six to eight twofold serial dilutions of each serum sample using MEM without FBS (MEM-SF) to target a range of 10% to 90% virus neutralization. The CMV stock was diluted to obtain approximately 1,500 to 3,500 PFU/mL. Equal volumes of the serum predilutions (0.2 mL) were combined with the diluted CMV (0.2 mL) and incubated for 1 hour at 37°C. After this incubation, the HF cell monolayers were washed with 1 mL MEM containing 3% FBS and 0.4% agarose (FMC Bioproducts, Rockland, ME), and incubated at 37°C for 7 days. After plaque development was observed, the agarose layers were removed and the monolayers fixed for 30 minutes at room temperature with 1.3% crystal violet stain (Sigma) containing 50% ethanol and 4% formaldehyde in phosphate-buffered saline (PBS). Monolayers were air dried and plaques counted at 13 to 17× magnification. A percent neutralization value was derived for each serum dilution using the formula:

\[
\text{Mean Virus Control Plaque Count} \times \frac{100}{\text{Mean IGIV Dilution Plaque Count}} - \text{Mean Virus Control Plaque Count} = \text{Percent Neutralization}
\]
Assay reproducibility was ensured by using two lots of commercially available IVlgG as controls for each assay. Each control sample had been reassayed at least 20 times to obtain an assigned 50% neutralization titer. A set of assays in this study was considered acceptable if the 50% neutralization titer for the control IVlgG, which were assayed simultaneously with the serum samples, was within an assigned range (mean ± 25%) and the virus control had a demonstrated human CMV (HCMV) titer of 5.27 to 5.67 log10 PFU/mL.

**CMV ELISA assay.** Serum antibody titers to CMV were determined using the Cytomegalovirus II Test kit (Whittaker Bioproducts, Inc, Walkersville, MO) in microtiter plates. No significant differences between ELISA values were obtained in test runs using either heated (56°C for 30 minutes) or unheated clinical serum samples. All calibrators were assayed in at least triplicate determinations and all control and test sera were assayed in at least duplicate. When using dilutions in addition to those specified in the kit, no values needed to be extrapolated beyond the standard curve.

**Statistical analysis.** The mean total IgG concentration at each study time point was generated and compared among IVlgG product groups using the Kruskal-Wallis one-way analysis of variance statistic. Similarly, CMV latex agglutination titers expressed as the average geometric mean titers were compared at each time point among IVlgG product groups.

Statistical analyses involving CMV neutralization bioassays and comparison with ELISA titers were performed using the RS/Explore statistical software program (BBN Software Products Corp, Cambridge, MA). A neutralization curve was generated from each serum sample by plotting serum dilutions versus the plaque count, and a 50% neutralization titer, expressed in dilution values, was predicted from this curve using curve fitting least squares regression analysis. Results of regression analysis were subsequently analyzed by one-way analysis of variance comparing multiple categorical variables (different IVlgG products) against a single continuous response variable (50% neutralization titer).

**RESULTS**

A summary of patient demographics of each IVlgG product group is presented in Table 1. There were no significant differences among groups in terms of patient age, weight, or pretransplant diagnoses. Eight of the 23 allogeneic recipients had CMV-seropositive donors: product A, 1; product B, 2; product C, 0; and product D, 5. All of the patients were monitored weekly while hospitalized using rapid detection with fluorescent monoclonal antibody

<table>
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<tr>
<th>Table 1. Characteristics of CMV-Seronegative BMT Patients</th>
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<tr>
<td>Product Group</td>
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<td>N: All (CMVNA)*</td>
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<td>Median age (yr)</td>
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<td>CML</td>
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<td>Other malignancy</td>
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<td>Nonmalignant disease</td>
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Abbreviation: CML, chronic myelogenous leukemia.

*Subset of patients whose sera was tested for CMVNA.
COMPARATIVE CMV TITRES IN BMT RECIPIENTS

Fig 2. There are no statistically significant differences among mean serum CMV titres (determined by latex agglutination) obtained after administration of any of four IVlgG products post-BMT.

on paired preinfusion and postinfusion sera showed that the median preinfusion CMVNA for all products was less than 1:4, with the following exception: for products A and B, one of the eight pairs of sera from each product tested showed CMVNA in the preinfusion specimen (1:18 and 1:36, respectively). These titers were similar to week +3 CMVNA titers of 1:12, and 1:23, respectively, obtained from the same patients. In addition, two other preinfusion samples tested during the preliminary phase of the study (but for whom paired postinfusion samples were not available) also showed positive 50% CMVNA of 1:37 and 1:18, thereby showing that 4 of 24 preinfusion samples tested had quantifiable CMVNA. Otherwise, all paired samples showed preinfusion antibody activity below the level of detection and positive CMVNA titers at week +3. Mean 50% CMVNA titers were highest for product B at 1:43 (with a predicted range at 95% confidence level of 1:26 to 1:61), whereas the lowest mean 50% CMVNA was 1:14 for product A (95% confidence level, 1:6 to 1:22); two of the IVlgG product groups showed intermediate 50% mean titers of 1:27 (product C) (95% confidence level, 1:14 to 1:39) and 1:26 (95% confidence level, 1:14 to 1:38) for an overall P = .02. Fifty percent CMVNA titers in sera from normal CMV seropositive controls ranged from 1:17 to 1:595.

CMV ELISA values were determined on the subset of 25 sera (representing the 4 IVlgG product groups) that was tested for CMVNA: 7 patients who received product A, 8 patients who received product B, 4 patients who received product C, and 6 patients who received product D. Median CMV ELISA for 21 preinfusion samples was 0.13 Paul Ehrlich International Units (PEI U)/mL. The four patient sera that showed CMVNA before IVlgG infusion also showed significant preinfusion CMV ELISA titers: 1:18 (2.76 PEI U/mL), 1:18 (1.78 PEI U/mL), 1:36 (2.87 PEI U/mL), and 1:37 (3.45 PEI U/mL). All patient samples that were negative for CMVNA before IVlgG also had negligible CMV ELISA titers ranging from 0.03 to 0.19 PEI U/mL. Figure 4 illustrates the comparison of postinfusion CMV ELISA titers versus the IVlgG product. Product B again showed the highest CMV titer with a mean of 2.95 ± 1.17 PEI U/mL. The lowest mean of 1.34 ± 0.55 PEI U/mL was obtained with product A, with intermediate mean values obtained with product C (mean 2.27 ± 0.87 PEI U/mL) and product D (mean 2.03 ± 0.75 PEI U/mL) for an overall P = .003.

DISCUSSION

This clinical study represents the first quantitative comparison of circulating functional anti-CMV activity after passive administration of Ig products into CMV-seronegative individuals. This analysis was performed as part of a larger study investigating the kinetics of two different IVlgG dosing schedules and the comparative antibody titers against other viral pathogens achieved by IVlgG administration to children and adults undergoing BMT.
IV IgG is now widely used in the management of BMT patients for a variety of indications. At our institution, the cost of IV IgG often exceeds the cost of all other medications used in BMT added together. Yet, little information is available regarding the in vivo kinetics of IV IgG in this patient population, the in vivo biologic activity of the antibodies, nor the in vivo comparability of different preparations.

The major objective of this study was to perform an in vivo quantitative comparison of passively transferred functional anti-CMV antibody (ie, viral neutralizing activity) among commercially available IV IgG products manufactured from pooled human plasma by different techniques.1,13

The study period was purposely limited to the first few weeks posttransplant. During this period, endogenous IgG synthesis is markedly depressed after cytoreductive therapy and the potential contribution of passively transferred CMV immunity for the subset of recipients with CMV-seropositive donors (3 of 27 recipients: 1 with product A and 2 with product B) is still minimal. The assays were performed on sera obtained at the “trough” of the passive IgG infusion schedule and thereby represent the lower end of the range of passively transferred functional antibody concentrations.

The experimental data obtained indicate that the individual IV IgG products can affect the titer of passively transferred functional antibody. The donor pool (eg, prevalence of seropositivity for CMV and mean titer of CMV neutralizing antibodies in the donor population), as well as the manufacturing procedure,14 may affect the concentration of functional antibody in the final IV IgG product. As commercial products continue to be developed, it is possible that differences within products and among products will continue. In our study, two of the products were manufactured in an identical manner, but from varying plasma sources (products B and D). We cannot draw any conclusions regarding the impact of lot to lot variability on circulating antibodies.

Reports of pharmacokinetic studies of IgG levels and CMV-specific IgG antibodies after IV IgG administration for BMT patients have indicated a wide range of half-life estimates for both parameters, which lends credence to the possibility that host factors, such as nutritional and infectious status, and changes in function of the reticuloendothelial system after cytoreductive therapy may further alter the fate of passively infused antibodies. Our findings have established the feasibility of detecting functional anti-CMV antibodies in the circulation of BMT patients who receive passive IgG transfusion and of detecting differences in relatively small subsets of patients. However, no conclusions as to the protective efficacy can be drawn from this relatively small study.

We conclude that all commercially available IV IgG products provide functional anti-CMV activity that can be detected in vivo, but that discernable differences in titers of neutralizing activity can be related to the product administered. Additional studies to determine optimal dosing schedules based on the determination of functional CMV antibody titers in seronegative or seropositive patients receiving either standard polyvalent IV IgG products, hyperimmune sera, or monoclonal anti-CMV antibodies should be performed in vivo and correlated with clinical outcomes.

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Circulating cytomegalovirus (CMV) neutralizing activity in bone marrow transplant recipients: comparison of passive immunity in a randomized study of four intravenous IgG products administered to CMV-seronegative patients

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