Kinetics of the Antibody Response against Human Cytomegalovirus–Specific Proteins in Allogeneic Bone Marrow Transplant Recipients

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The humoral immune response against human cytomegalovirus (CMV) was retrospectively investigated in >800 serum samples from 31 recipients of allogeneic bone marrow transplants. To this end, an ELISA was performed that allowed the individual analysis of IgG antibodies against known antigenic domains of CMV-derived phospho- and glycoproteins and nonstructural polypeptides. Twenty-nine patients developed active CMV infection after transplantation, as determined by repeatedly positive samples in polymerase chain reaction (PCR) assays. Seventeen patients responded serologically to viral replication by producing CMV-specific antibodies against a variety of antigens. The response was detectable concomitantly with PCR positivity and was seen as early as 20 days after transplantation. High titers of glycoprotein-specific neutralizing antibodies were correlated with the absence of viral DNA in blood (P < .002). Nineteen patients developed CMV disease. Survival was associated with the production of high titers of CMV glycoprotein–specific antibodies in response to viral replication.

Primary infections or reactivations of human cytomegalovirus (CMV) in immunosuppressed patients frequently result in life-threatening disease. Of special concern are recipients of bone marrow transplants (BMTs) because the most serious manifestation, CMV pneumonitis, is associated with high mortality [1, 2]. Prophylactic ganciclovir therapy has decreased the subsequent incidence of CMV disease in this group of patients [3, 4]. However, prophylactic treatment is complicated by the development of CMV disease after discontinuation of antiviral therapy, and long-term survival of BMT patients may not be improved [5–7]. Sensitive laboratory tests for CMV antigen or DNA have been successfully used to manage preemptive therapy in several different clinical settings [8]. However, the predictive value of positive diagnostic tests for the development of CMV disease is unsatisfactory because a substantial percentage of patients who are positive in these tests never develop CMV disease [9–11]. CMV-specific immunologic effector functions are important in limiting the clinical consequences of CMV infection in the posttransplant period. CD8+ cytotoxic T cells have been suggested to play an important role in the prevention of CMV disease after BMT [12–15]. However, during the first 100 days after allogeneic BMT, some 50% of the recipients are persistently deficient in CD8+ cytotoxic T lymphocytes [12]. Adoptive transfer of CMV-specific CD8+ cytotoxic T cells has been shown to restore transiently cellular immunity against CMV after BMT and prevent CMV disease [15–18]. The role of CD4+ helper T cells in BMT patients is not clearly defined. Their presence, however, is needed for persistence of transferred CD8+ T cells [18].

The importance of the humoral immune response against CMV after BMT has received only little investigation, and the results from clinical studies are controversial [2, 19]. The administration of immunoglobulins to recipients of allogeneic marrow transplants has been found to decrease the incidence of acute graft-versus-host disease and interstitial pneumonia among CMV-seropositive patients [20, 21]. However, this approach has not significantly decreased the incidence of CMV infection, nor has it affected overall survival [3, 4, 21]. An important problem in defining the role of antibodies during the course of CMV infection is the lack of defined assay systems. CMV is a highly complex virus, potentially encoding close to 200 proteins [22]. The humoral immune response against individual antigens has only been partially characterized but appears to involve a considerable number of structural and nonstructural proteins [23]. In recent years, a number of studies have used CMV-specific recombinant antigens to overcome these limitations [23–25]. The major conclusion from these studies was that phosphoproteins and, in particular, the basic phosphoprotein of 150 kDa (pp150, ppUL32) represent the most...
immunogenic antigens for the humoral immune response against CMV [26–28]. Antibodies against these polypeptides are nonneutralizing and therefore can be expected to contribute little to limitation of viral dissemination [29]. Comparably detailed studies involving glyco- and phosphoprotein antigens are lacking. Because glycoproteins can induce neutralizing antibodies, the kinetics of antibody formation against these antigens could be important in the clinical course of infection. In the closely related murine CMV system, it is well documented that neutralizing antibodies can limit viral spread and confer protection from lethal challenge [30–33].

In this study, we used an ELISA involving recombinant CMV-specific peptides in order to determine antibody titers in sera from individual BMT recipients. In total, >800 sera were analyzed from 31 patients. Antigens included antibody-binding domains from structural and nonstructural phosphoproteins and the major envelope glycoproteins. Titters of human CMV-specific antibodies were correlated with detection of viral DNA in blood and clinical outcome of the infection.

Materials and Methods

Study population. In a retrospective study, we analyzed the humoral immune response against CMV in a group of 31 selected recipients after allogeneic BMT. Patients were selected on the basis of CMV complications and clinical outcome: 29 recipients were polymerase chain reaction (PCR)–positive after transplantation, indicating CMV replication; 19 patients developed CMV disease (diagnosed as described below); and 10 patients died during an episode of CMV replication. Because patients were selected on the basis of clinical outcome, the mortality rate in this study group does not reflect overall mortality rates after BMT, which are considerably lower in our centers [8]. Nine patients received transplants at the Universitätsklinikum Rudolf Virchow, Berlin, and 22 patients at the Eberhard-Karls-Universität, Tübingen. The primary disease for transplantation was acute lymphocytic leukemia in 7 cases, acute myelogenous leukemia in 6 cases, chronic myelogenous leukemia in 13 cases, aplastic anemia in 2 cases, and multiple myeloma in 3 cases (table 1). Before receiving BMTs, patients received a conditioning regimen, generally consisting of total body irradiation (1200 cGy) and cyclophosphamide (120 mg/kg), or busulfan (10 mg/kg) and cyclophosphamide (120 mg/kg). Most patients received no posttransplant immunosuppressive therapy. Acute (stages II–IV) and chronic graft-versus-host disease was treated with steroids (1–6 mg/kg). Nine patients received Cytooglobin (Bayer, Leverkusen, Germany) at 1 mL/kg body weight every 3 weeks, and 22 patients received Purimun (Cention, Liederbach, Germany) at 7.5–12.5 g weekly. Ganciclovir (Hoffman-La Roche, Basel, Switzerland) therapy was initiated after two positive PCR results. CMV disease was treated with either ganciclovir alone (5 mg/kg body weight intravenously twice daily) or in combination with hyperimmunoglobulins (Cytooglobin or Purimmun) at different concentrations (intravenously every other day). The CMV serologic status of the cohort included 6 cases with donor CMV-seropositive/recipient CMV-seronegative (D+/R−), 10 cases with D−/R+, and 15 cases with D−/R−. The first 100 days after treatment of each individual recipient were represented by 5–16 serum samples. The study population is summarized in table 1.

Clinical evaluation. Onset of CMV replication after BMT was determined by DNA PCR as described previously [8, 34]. CMV disease was diagnosed when patients had clinical and radiologic signs of disease or organ dysfunction associated with the detection of CMV antigens, the presence of typical inclusion bodies in bronchoalveolar lavage fluid (interstitial pneumonia), positive CMV culture, or CMV antigen–positive biopsies (hepatitis, esophagitis, enteritis, retinitis, and encephalitis). Nineteen recipients developed CMV-related disease after BMT, and 6 died of complications related to CMV infection.

ELISA. To detect antibodies against specific human CMV antigens, recombinant polypeptides from the following human CMV proteins were used: pp150 (ppUL32), pp65 (ppUL83), IE1 (pUL123), gH (gpUL75), and gB (gpUL55). gH-specific antigens included the antigenic domain 86 (AD86) from both AD169 and Towne strains. gB-specific antibodies were individually detected using two recombinant antigens representing antigenic domains 1 and 2 (AD-1, AD-2, respectively). The characteristics of the antigens and purification of the polypeptides have been described in detail [35].

ELISA with selected CMV antigens. The experimental details of the recombinant ELISA were described previously [35]. To determine the cutoff of the assay, 13 CMV-negative human sera and 2 independent pools (20 CMV-negative sera each) were analyzed with all antigens, and reactivity was measured as $A_{405}$. A reactivity index (RI) was calculated according to the formula $A_{405}$. The RI cutoff for each individual antigen was defined as median ±2 SD. To define a sample positive with an individual antigen, two different criteria had to be met: reactivity with the prokaryotic fusion partner between $A_{405}$ and $A_{405}$, and an RI higher than the antigen-specific RI cutoff. To determine the variability of the assay, individual samples were analyzed at least three times in different experiments. The SD calculated for each antigen was 10% at the maximum. Serum samples from patients were blinded before analysis.

The serologic status of donor and recipient before transplantation and selected samples from the recipient after transplantation were analyzed with a commercially available ELISA (Enzygnost

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Anti-CMV/IgG; Behring, Marburg, Germany). CMV-specific reactivity was calculated according to the manufacturer’s instructions.

Neutralization analysis. Neutralization assays have been described in detail previously [35].

Statistical analysis. Statistical analyses were performed using the SPSS program (SPSS, Chicago). Differences between groups were calculated by use of the Fisher’s exact test.

Results

Kinetics of antibody response to CMV proteins after BMT. To measure antibody titers against individual CMV antigens, we used an ELISA with recombinant proteins containing previously defined antibody-binding domains. Antigens were derived from two broad categories. The first category was structural and regulatory phosphoproteins, including pp150 (pUL32), pp65 (pUL83), and immediate-early 1 (IE1, pUL123). Available evidence suggests that these antigens do not induce neutralizing antibodies during natural infection [29]. Therefore, we will refer to these antigens collectively as “nonneutralizing antigens” and to the corresponding antibodies as “nonneutralizing antibodies.” The second category from which antigens were derived comprises glycoprotein antigens, including the immunodominant domains AD-1 and AD-2 of glycoprotein B (gB, gpUL55) and AD-86 of glycoprotein H (gH, gpUL75). Since the glycoprotein-specific domains that were used in the ELISA induce neutralizing antibodies during natural infection, we refer to these as “neutralizing antigens” and to the respective antibodies as “neutralizing antibodies” [25, 36, 37].

Twenty-nine patients developed evidence of CMV infection after BMT, as determined by repeatedly positive PCR assay results. In 17 patients, there was a clear serologic response to infection. Patients were classified as serologic responders when, as a consequence of viral replication, an increase in the antibody titer of >2 RI was detected against at least two different antigens (an example is shown in figure 1A). The first positive PCR result was obtained at day 29. Increased antibody titers against a number of antigens were seen at the same time. Similarly, a second patient responded to viral replication with a sharp increase in CMV-specific antibody titers simultaneously with viral replication (figure 1B). The observed response was independent of the CMV serologic status of donor and recipient before transplantation. In both situations, D’/R’ (figure 1B) and D’/R (figure 2B), an increase in CMV-specific antibodies could be detected.

Twelve patients were defined as serologic nonresponders. Figure 1C illustrates the course of a patient in the D’/R category who was repeatedly found to be PCR-positive between days 54 and 98, yet did not mount an antibody response. The slight increase in antibody titer observed at day 98 was most likely the result of the intravenous administration of immunoglobulin (IVIG) (see below). The pattern associated with the absence of a serologic response in a CMV-seropositive recipient is illustrated in figure 1D. Despite several episodes of PCR positivity, this patient’s pretransplant antibody titers gradually declined until day 344, when he became CMV-seronegative.

Serologic responses to CMV antigens in the absence of PCR positivity were also seen. Figure 2A shows a patient who, during the observation period of 300 days after BMT, never tested positive by PCR despite a clear serologic response to CMV antigens. Increasing antibody titers were also observed in patients who were PCR-positive at a separate time point after BMT (figure 2B). This patient had positive PCR results between days 82 and 150 but no serologic response. However, at about day 230 and again between days 315 and 400, a considerable increase in antibody titers was seen in the absence of PCR positivity. Serologic responses in the absence of concurrent PCR positivity were found in 11 patients.

Effect of passively transferred immunoglobulins on titers of CMV-specific antibodies and neutralization capacity of sera. Because all 31 patients had received IVIG preparations, the impact of this treatment on individual CMV-specific antibody titers was evaluated. Six patients were CMV-seronegative before initiation of immunoprophylaxis, and their data were used to establish baseline antibody titers that resulted from IVIG without interference from endogenously synthesized immunoglobulins. Five received Cytoglobin (0.1 mL/kg body weight every 3 weeks), and 1 received Purimun (7.5 g weekly). In general, there was no qualitative difference between immunoglobulin preparations with respect to resulting antibody titers in patients. An example of the antibody titer achieved with IVIG in a single seronegative recipient of marrow from a seronegative donor is shown in figure 3. After prophylactic administration of IVIG, the RIs for antibodies specific for nonneutralizing antigens such as pp150 were <4, and glycoprotein-specific antibodies did not exceed RIs of 3 at the time of transplantation. Increasing the amount of passively transferred antibodies during courses of ganciclovir therapy resulted in antibody titers of RI ≤5 against nonneutralizing antigens and glycoprotein-specific antibodies ≤4 RI (days 44–80 after BMT). The overall profile of individual CMV-specific antibodies that was seen before day 100 was identical to that of the administered immunoglobulin preparation (data not shown). In contrast, the patients’ own immune responses differed drastically with respect to the resulting overall antibody titers and profile (i.e., high antibody titers against gH developed in response to viral replication). Antibodies against gH were present at only very low levels after IVIG infusion (figure 3).

We and others [25, 38] have shown that CMV-specific antiglycoprotein antibody titers correlate with neutralizing capacity, whereas phosphoprotein antibodies do not. This correlation was also seen during this study (figure 3). During IVIG treatment, neutralization titers of 1:100 were never exceeded, whereas these titers increased to >1:2000 after onset of glycoprotein-specific antibody synthesis. It is important to note that the increase in neither glycoprotein-specific antibodies nor neu-
Figure 1. CMV-specific antibody response after allogeneic bone marrow transplantation. CMV-specific serologic status of donor and recipient before transplantation is indicated (D/R). CMV antigen-specific antibody reactivity (reactivity index, RI) against nonneutralizing antigens is shown by solid lines; glycoprotein-specific antibodies are represented by dashed lines. Reactivities against specific antigens are illustrated by symbols. Arrows indicate time points when polymerase chain reaction was positive for CMV. Kinetics are shown as days before and after transplantation. A and B, serologic responders; C and D, serologic nonresponders.
Figure 2. CMV-specific antibody response after bone marrow transplant (BMT) in absence of simultaneous polymerase chain reaction (PCR) positivity. CMV-specific serologic status of donor and recipient before BMT is indicated (D/R). Antigen-specific antibody reactivity (reactivity index, RI) against nonneutralizing antigens is shown by solid lines; glycoprotein-specific antibodies are represented by dashed lines. Reactivities against specific antigens are illustrated by symbols. Arrows indicate time points when PCR was positive for CMV. Kinetics are shown as days before and after BMT. A, Serologic response in absence of PCR positivity over entire observation period. B, Absence of serologic response during PCR positivity and serologic response at later times after BMT without PCR positivity.
Figure 3. Intravenous immunoglobulin and CMV-specific antibody profiles after bone marrow transplant (BMT). Serologic analysis of CMV-seronegative recipient after BMT. CMV-specific serologic status of donor and recipient before BMT is indicated (D/R). Antigen-specific antibody reactivity (reactivity index, RI) against nonneutralizing antigens is shown by solid lines; glycoprotein-specific antibodies are represented by dashed lines. Reactivities against specific antigens are illustrated by symbols. Neutralization capacity of sera at days 38, 120, 248, and 390 are shown. Arrows indicate time points at which polymerase chain reaction was positive for CMV. Kinetics are shown as days after transplantation. NT, neutralizing titer.

Titers of CMV-specific antibodies and viral replication. Next we wanted to determine if there was a correlation between titer of glycoprotein-specific antibodies and levels of viral DNA in blood. We used a glycoprotein-specific RI of 4 as a baseline value because this was the maximal titer that could be achieved by passive transfer of IVIG and represented a value common to all patients. Because antibody titers changed rapidly before, during, and after periods of viral replication, we operationally defined two phases in which the correlation was analyzed.

The “prereplication” phase was defined as the period of time before viral DNA was detectable. Twenty-nine patients were included in this group. At the time of the first positive PCR result (20–153 days after BMT), 24 (83%) of 29 patients had glycoprotein titers of <4 RI. This antibody level was the result of either low titers over the entire prereplication phase (e.g., patient in figure 1C) or loss of CMV-specific antibodies after BMT (e.g., patient in figure 4C). Five patients had glycoprotein titers >4 RI during this phase (table 2). However, these recipients were CMV-seropositive before transplantation, and their antibody levels gradually declined over the posttransplant period, presumably secondary to catabolism of IgG.

A “postreplication” phase could be analyzed for 24 patients after an initial period of PCR positivity and antiviral therapy. Two groups of patients could be differentiated (table 2): those who became PCR-positive again after a first episode of viral replication (n = 14) and those who did not (n = 10). In the group of patients with multiple episodes of viral replication, 12 (81%) of 14 had glycoprotein-specific antibody titers of <4 RI, whereas in the group who remained PCR-negative, 8 (80%) of 10 had glycoprotein titers of >4 RI; these 8 recipients belonged...
Figure 4. CMV-specific antibody response (reactivity index, RI) and disease. CMV-specific serologic status of donor and recipient before BMT is indicated (D/R). Antigen-specific antibody reactivity against nonneutralizing antigens is shown by solid lines; glycoprotein-specific antibodies are represented by dashed lines. Reactivities against specific antigens are illustrated by symbols. Kinetics are shown as days before and after bone marrow transplantation. Arrows indicate time points at which polymerase chain reaction was positive for CMV. Shaded boxes illustrate period of CMV disease. Black bars represent periods of ganciclovir therapy. Neutralization capacity of sera at individual time points is indicated. Panels represent patients surviving CMV disease (A, B), dying of CMV disorder (C), and dying of CMV-unrelated sepsis (D). IP, interstitial pneumonia; E, enteritis.
to the group of serologic responders, who continuously maintained high titers of glycoprotein-specific antibodies that were synthesized during the initial phase of PCR positivity.

In line with the above-mentioned results, neutralization titers of \( \geq 1:1000 \) were seen in patients with glycoprotein-specific antibody titers \( \geq 4 \) RI, whereas those with glycoprotein-specific antibody titers \( \leq 4 \) RI had neutralization titers of \( \leq 1:1000 \) (data not shown). These data suggest that high titers of glycoprotein-specific antibodies were associated with the absence of viral DNA in peripheral blood (\( P < .002 \)).

Correlation of glycoprotein-specific antibodies with CMV disease. Finally, the effect of antibodies on the development of CMV disease was analyzed. Nineteen patients developed CMV disease according to the criteria described in Materials and Methods. It should be mentioned that all PCR-positive patients in our study population were treated with ganciclovir for various time periods. Examples of this treatment in connection with CMV disease are indicated in figure 4. Nine patients from our study population survived, 6 died of complications related to CMV infection, and 4 died of complications not related to CMV. Fourteen of 19 patients had glycoprotein-specific antibody titers \( \leq 4 \) RI when CMV disease was diagnosed (mean, 2.8; range, 1.9–4.0). As already mentioned, 5 of 19 CMV-seropositive recipients who gradually lost antibodies still had glycoprotein-specific antibody titers \( \geq 4 \) RI when CMV disease was diagnosed (mean, 5.0; range, 4.3–6.5). Patients who survived CMV infection (\( n = 9 \)) were characterized by the production of high glycoprotein-specific antibody titers (mean, 9.2 RI; range, 5.0–21.0) in response to viral replication (figure 4A, 4B). In contrast, the 6 patients who died of CMV-related disease had glycoprotein-specific antibody titers of \( < 4 \) RI (mean, 2.5; range, 1.9–3.9), and none of these patients had de novo glycoprotein antibody responses in the posttransplant period (figure 4C). The 4 patients who died from complications unrelated to CMV (bacterial and fungal sepsis) also developed high glycoprotein-specific antibody titers (mean, 7.1 RI; range, 4.2–10.2) in response to CMV replication (figure 4D).

In conclusion, among our cohort of 31 patients, 19 BMT recipients developed CMV disease. Survival of CMV disease in all patients (\( n = 9 \)) was correlated with serologic response to viral replication. In contrast, of the recipients who died of CMV disease (\( n = 6 \)), all were serologic nonresponders.

### Discussion

In this study, we analyzed in detail the CMV-specific antibody response after BMT. Patients were selected for analysis on the basis of CMV infection and different clinical outcome. They were treated according to current clinical management practice for BMT recipients: preemptive therapy using ganciclovir plus IVIG administration [8, 39]. Antibody titers against major CMV-specific structural and nonstructural antigens were determined independently and correlated with virologic and clinical findings. Our data showed that a CMV-specific humoral immune response occurred very early after transplantation and that high titers of glycoprotein-specific antibodies were associated with an improved clinical outcome.

The importance of the humoral immune response over the course of a CMV infection after BMT remains ill-defined. One major problem with the analysis of the CMV-specific humoral immune response has been the lack of defined assay systems. The presence of CMV-specific neutralizing antibodies (thought to represent the most relevant antibody type for protective immunity) is difficult to determine, and a detailed study involving hundreds of serum samples is almost impossible. On the other hand, conventional serologic tests, such as complement fixation or ELISA, do not measure virus-neutralizing antibodies because the predominant antibody reactivity that is detected in these assay systems lacks neutralizing capacity [25, 40]. However, the increase in neutralization capacity is correlated with glycoprotein-specific antibody levels. The determination of this antibody specificity can therefore serve as a surrogate marker for determining neutralization capacity of a given serum.

Approximately 50% of patients in our study population mounted a humoral immune response to CMV, and this response occurred as soon as 20 days after transplantation. This finding indicates that during the first 100 days after BMT, the period of highest risk for developing clinical CMV-related complications, a humoral immune response in principle could modify CMV infections in these patients. Despite the severe immunosuppression in these patients, production of virus-specific antibodies was detected concomitantly with PCR positivity. For several reasons, this response was likely anamnestic.

First, it is well established that a de novo humoral immune response cannot be generated within the first year after BMT (reviewed in [41]). In our study population, we had three D+/
R⁻ and four D/R⁺ combinations with antibody production, indicating that IgG synthesis was mediated by B cells adoptively transferred from the donor or residual B cells in the recipient, a situation also seen by others [42–45].

Second, antibody avidity against all CMV-specific antigens was found to be continuously high, reflecting immunoglobulin synthesis from memory B cells rather than a primary immune response (unpublished data).

Third, we have previously shown that the primary humoral immune response against CMV in immunocompetent and immunosuppressed persons is characterized by a delayed synthesis of glycoprotein-specific antibodies, whereas during a secondary response, antibodies against phospho- and glycoprotein-specific antigens developed simultaneously [35]. Glycoprotein-specific antibody production was not delayed in the BMT patients.

It is well established that CMV-specific antibodies cannot prevent virus reactivation. However, various studies have provided evidence that antiviral antibodies have a modulatory effect on the infection and the subsequent clinical course of CMV disease [4, 46]. In the murine CMV system, neutralizing antibodies effectively limit viral spread during reactivation, an effect that is dependent on antibody titer [31, 32]. A similar situation seems to exist in BMT patients. In the prereplication phase, high glycoprotein-specific antibody titers were associated with absence of PCR positivity, indicating an effective suppression of viral dissemination. At the time of the first PCR-positive sample, glycoprotein titers were in the range of 4 RI, mediating neutralization titers of <1000 (P < .002). A similar correlation was found in the postreplication phase. Patients who were able to react to viral replication with production of glycoprotein-specific antibodies of RI >4 (19/24) remained PCR-negative throughout the remainder of the observation period (n = 11) or as long as titers stayed above an RI of 4 (n = 8), whereas patients who were initially unable to mount a glycoprotein-specific immune response (12/31) experienced additional periods of PCR positivity (n = 6) or died during the course of generalized CMV infection (n = 6).

A correlation between antibody titers and PCR positivity during phases of viral replication and therapy was not attempted because it was not possible to differentiate between effects mediated by antibodies or by the concomitant ganciclovir therapy.

Thus, in all situations in which transplant recipients maintained or developed a high glycoprotein-specific humoral immune response against CMV, no viral replication could be detected in the blood by PCR, and no clinical symptoms related to CMV infection were seen. This is in agreement with our findings in liver transplant recipients [35].

Previous studies that analyzed the influence of antibodies on the outcome of CMV infections in BMT patients were inconclusive [2, 19, 45]. A potential explanation for these discordant results is provided by our data showing that the synthesis and catabolism of CMV-specific antibodies after BMT is a highly dynamic and complex process, and single time point determinations are insufficient to reflect this situation accurately. In addition, as mentioned above, commercially available tests do not allow determination of neutralizing antibodies. In our study group, 19 patients developed CMV disease after BMT. Six patients died of CMV-related complications, and 9 survived. With respect to clinical outcome, we observed a near perfect correlation between the capability to react by the production of glycoprotein-specific antibodies during CMV disease and favorable clinical outcome. Four patients survived CMV-related complications but died of causes unrelated to CMV infection. These patients also developed high glycoprotein-specific antibody titers.

Studies with the murine CMV model have demonstrated that humoral and cellular immunologic effector functions contribute to the defense against CMV [31–33, 47, 48]. After BMT, a correlation between CMV-specific CD8⁺ T cell activity and recovery from active infection has been demonstrated [10]. However, adoptive transfer studies have shown that CD4⁺ T cell–mediated immune effector functions are necessary to maintain CD8⁺ T cell activity [18]. Thus, including the data presented in our study, T cell– and B cell–related activities seem to be linked to the clinical outcome of CMV infection after BMT. For the clinical management of the patient, it would be important to investigate whether cooperation of the respective immunologic effector functions is necessary to successfully combat a CMV infection or whether individual components of the immune system are sufficient.

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