Blood and Marrow TRANSPLANTATION

REVIEWS

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IN THIS ISSUE

Cytomegalovirus Infection: The Day After and the Morning Before

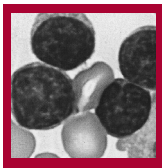
by John R. Wingard, MD, Editor

The deadly consequences of cytomegalovirus (CMV) infection after hematopoietic stem cell transplantation (HSCT) are all too well known to those who trained in the 1980s or before. Those of us remember well the specter of a nonproductive cough and dyspnea suddenly appearing in an allograft recipient two to three months after transplantation. This was a time after transplantation when clinicians and patient alike were beginning to breathe a sigh of relief that the prospects for a successful outcome were bright. Such hopes were quickly dashed by rapid and relentless respiratory failure followed by death.

One of the remarkable success stories in the history of HSCT is the progress made in controlling the danger of CMV infection. Few patients today die from the direct consequences of CMV during the first 3 months after HSCT.

Yet, problems from CMV continue to linger even today. In this transcript of a symposium held at the 2004 Tandem BMT Meetings in Orlando, Florida, several less well studied consequences of CMV infection were discussed. Dr. Michael Boeckh presented data from several recent series indicating that even with the control of CMV infection, indirect effects remain, especially for high-risk patients, that result in a survival disadvantage. Such indirect effects are presumably immunomodulatory, increasing susceptibility to other infections or other sequelae. However, these indirect effects of CMV infection, first noted in solid organ transplant recipients, are still poorly understood. Late onset of CMV infection, once infrequent, now is increasingly common, and only now are strategies being tested to address this.

The fact that these consequences remain "the day after" infection emphasizes the need for prevention of infection altogether when possible. This second topic was addressed by Dr. Garrett Nichols. Dr. Nichols reviewed the current status of methods of screening blood products for transfusion support, the effectiveness of various methods of filtering to reduce the risk of virus transmission, the promise of new testing, and the limitations of these various strategies. Although advances have been made in prevention of viral transmission, it is clear that more work is needed "the morning before."



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Symposium Report

Blood and Marrow TRANSPLANTATION REVIEWS

CMV in Stem Cell Transplantation: New Insights and Options

Adapted from a CME symposium presented at the 2004 Tandem BMT Meeting, February 2004, Orlando, Florida, USA.
This program is funded by an unrestricted educational grant from Roche Laboratories.

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**Prof. Dr. Hermann Einsele gave a presentation entitled "Preemptive Therapy with IV Ganciclovir versus Valganciclovir: PK and Preliminary Safety and Efficacy Data." At Dr. Einsele's request, his data, which are submitted elsewhere for publication, are not presented here.*

Accreditation Statement

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Faculty Disclosure

As an accredited sponsor, the Medical College of Wisconsin must ensure balance, independence, objectivity, and scientific rigor in all its individual or jointly sponsored educational activities. The authors who contributed to this publication have disclosed the following relationships:

Michael Boeckh, MD, indicated that he has a relationship with Bayer AG, GlaxoSmithKline, Roche Laboratories, Vical, and ViroPharm.

Prof. Dr. Hermann Einsele indicated that he has received research grant support from Roche Laboratories.

W. Garrett Nichols, MD, MS, indicated that he is on the speaker's bureau for Pfizer, Roche Laboratories, and Wyeth.

Continuing Medical Education Credit

The Medical College of Wisconsin designates this educational activity for a maximum of 1.0 category 1 credit toward the AMA Physician's Recognition Award. Each physician should claim only those credits that he/she actually spent in the educational activity.

Needs Assessment

Cytomegalovirus (CMV) continues to be a significant cause of morbidity and mortality after hematopoietic stem cell transplantation. As we move toward eliminating the impact of CMV infection and disease on the successful clinical outcomes of these patients, it is important to review accomplishments and identify the remaining challenges.

The content of this publication has been developed to provide insight into the current understanding of the clinical management of CMV infection and disease and to identify future directions and research for advances in therapy and prevention strategies.

Educational Objectives

The information in this publication, should enable the reader to:

- Describe current treatment and prevention strategies for CMV.
- Dicuss emerging CMV treatment options for hematopoietic stem cell transplant recipients.
- Describe remaining challenges and directions for advances in therapy and future management of CMV.

Target Audience

This CME activity will be valuable to physicians, data managers, nurses, and pharmacists who are involved in the care of blood and marrow transplantation patients.

Toward Eliminating the Impact of CMV/CMV Seropositivity on Mortality in Stem Cell Transplant Recipients: Accomplishments and Remaining Challenges

Michael Boeckh, MD

In the current era of prophylactic and preemptive therapy, cytomegalovirus (CMV) is now a rare cause of early mortality after hematopoietic stem cell transplantation (HCT). However, the ultimate goal of completely eliminating the impact of CMV on survival remains elusive. Although the direct effects of CMV (ie, CMV pneumonia, gastrointestinal disease) have been largely eliminated, several recent cohort studies show that CMV-seropositive patients undergoing myeloablative transplantation and CMV-seronegative recipients of a CMV-seropositive graft appear to have a persistent mortality disadvantage compared to CMV-negative recipients with a CMV-seronegative donor. Recipients of T-cell–depleted allografts and/or transplants from unrelated or HLA-mismatched donors seem to be predominantly affected. Reasons for the poor outcome in high-risk patients likely include both incomplete prevention of direct and indirect (or immunomodulatory) effects of CMV as well as consequences of drug toxicities (eg, infections following ganciclovir-associated neutropenia).

CMV Prevention Strategies

The original goal of prevention strategies for CMV was to prevent the devastating impact of CMV pneumonia. During the late 1980s and early 1990s, approximately 30% of CMV-seropositive allogeneic transplant recipients developed CMV pneumonia, and mortality was initially 80% to 90%; even now with combination therapy of intravenous immunoglobulin and ganciclovir or foscarnet, mortality from CMV pneumonia remains at more than 50%. While prevention of this disease remains a primary goal, strategies are also aimed at preventing gastrointestinal disease.

Two strategies for CMV prevention have been developed. One is a prophylactic approach, whereby an antiviral agent is given based on the CMV serostatus; treatment typically starts at engraftment if a ganciclovir product is used or even before transplantation if an acyclovir product is used. Theoretically, such an approach covers direct effects of CMV infection, such as pneumonia and gastrointestinal disease, as well as indirect effects, such as the immunosuppressive effects of CMV, which make individuals susceptible to bacterial and fungal infections, and perhaps, in some settings, acute graft-versus-host disease. Based on the toxicity associated with this approach and the necessity of treating entire populations for a prolonged period of time, the concept of preemptive therapy was developed (Figure 1), in which the patient is monitored weekly with a diagnostic test. As soon as positive test results are obtained, an antiviral drug is given for several weeks. If the diagnostic test becomes positive again, this treatment course is repeated. These preemptive strategies are now widely used. Most centers use these strategies with antigenemia or polymerase chain reaction (PCR)-based diagnostic tests, and they are highly effective in preventing CMV disease.

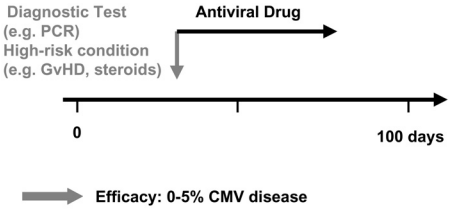

Figure 1. Preemptive therapy of CMV. PCR indicates polymerase chain reaction; GVHD, graft-versus-host disease.

Effects of CMV Seropositivity

Most studies over the last decade show that CMV disease incidence has been reduced to 0% to 5% during the first 3 months. However, in the early years of this decade, papers came out that showed that despite the high success rate of preemptive therapy, the overall outcome of CMV-seropositive recipients was still significantly worse than that of CMV-seronegative recipients with CMV-negative donors.

A series of studies has looked at these outcomes. Figure 2 summarizes the studies to date. In the first, Broers et al [1], CMV-seropositive recipients had much worse outcomes. Interestingly, there was a very low incidence of CMV disease, and patients died of bacterial sepsis and other infections. The cohort of this study was relatively small, and others have also looked at the effect of CMV seropositivity. In one analysis, Nichols et al [2] found that in a much larger cohort of

CMV Recipient Serostatus

Mortality Remains High in the Era of Preemptive Therapy

Study	N	% TCD	% MUD	Disease	↑ Mortality
Broers (2000)	115	95	0	Mixed	Yes
McGlave (2000)	1423	23	100	CML	Yes
Craddock (2001)	106	100	100	CML	Yes
Cornelissen (2001)	127	26	100	Mixed	Yes
Kollman (2001)	6978	26	100	Mixed	Yes
Castro (2002)	510	24	100	MDS	Yes
Meijer (2002)					
MR	205	100	0	Mixed	No
MUD	48	100	100	Mixed	Yes
Nichols (2002)	1750	0	57	Mixed	Yes
MR	753	0	0	Mixed	No
MUD/MMR	997	0	100	Mixed	Yes

Figure 2. Effect of CMV serostatus on mortality of transplant recipients: a summary of studies to date. TCD indicates T-cell depleted; MUD, matched unrelated donor; MR, matched related donor; MMR, mismatched related donor.

approximately 1750 patients CMV seropositivity remained a poor prognostic factor in terms of mortality, but the effect seemed to be restricted to the high-risk patients, namely, those with unrelated or HLA-mismatched donors. In the matched-related cohort there was, after adjusting for other factors, no impact on survival of CMV seropositivity prior to transplantation. Additional studies looked at large cohorts of patients. The biggest study perhaps is the National Marrow Donor Program cohort [3], in which more than 6000 patients were analyzed. Virtually all studies showed that CMV seropositivity is still associated with an increase in mortality. Of note, the two studies that analyzed matched-related transplants separately [2,4] found that the effect seemed to be restricted to the highest risk patients. The other studies included a fair number of patients who were T-cell depleted or matched-unrelated transplant recipients, and overall the study outcomes indicate that there are still problems with these high-risk patients. CMV seropositive recipients in a high-risk setting still do not seem to do as well as do negative recipients with negative donors.

The deleterious effects of CMV seropositivity can be divided into 3 categories [5]. One is the direct effect of CMVs, in which the virus causes lytic infection with end organ manifestation, the leading form of which is CMV pneumonia. With the current prevention strategies, CMV disease still occurs in 3 situations: one is breakthrough disease. Preemptive therapy is effective, but in 0% to 5% of cases, 1 in 20 patients, breakthrough disease will occur. Another situation is late CMV disease, which is now, at many centers, the CMV disease manifestation that is most common. A third situation is resistant CMV disease, which typically occurs late after transplantation after extensive exposure to antiviral agents.

Indirect effects of CMV were first identified in the solid organ transplantation setting, where CMV-seropositive and donor +/– recipients in particular were found to have a higher risk of fungal infections. Indirect effects have now been observed in stem cell transplant recipients as well, and they are explained by an immunomodulatory effect of CMV, which may increase the level of immunosuppression, thereby increasing the risk of other infections [6].

Finally, there is the effect of drug toxicity with ganciclovir, a drug that is used in perhaps 80% to 90% of patients in our setting. The leading complication is neutropenia, and especially the consequences of neutropenia, such as invasive bacterial or fungal infection. Foscarnet and cidofovir also have organ toxicities, such as renal failure, which may effect poor outcome.

Author		Depletion	Unrelated Donors	Effect on Mortality	
				Positive Recipient	Negative Recipient
Kollman et al.	6878	Mixed	100%	None	
Nichols et al.	1001*	Mixed	100%	None	Increased
Ljungman et al.	1108	Mixed	100%		

Figure 3. Impact of donor CMV status: summary of study results.

CMV Prevention in High-Risk Patients

How can we improve prevention in high-risk patients? The data in T-cell–depleted patients clearly indicate that the CMV-specific T-cell immunodeficiency seems to be the major problem. Significant advances have been made in adoptive immunotherapy. Although this option is not available everywhere, it can potentially be used in these high-risk patients. There are still some practical issues associated with this strategy, however, such as donor availability. The presence of high-dose steroids may also be an obstacle for the persistence and efficacy of the cells, and finally, the center must have the technical capability to perform the procedures.

Another option is valacyclovir; given in high doses, this drug reduces the incidence of CMV viremia, as shown in a large European study of more than 700 patients [7]. The advantage of this treatment strategy is a very low toxic approach, but the pill burden, 8 grams per day, is a significant obstacle for long-term prophylaxis. Unfortunately, in the high-risk target population that we hope would benefit, the study showed that the approach did not work very well. Finally, with such an approach, monitoring for CMV and perhaps for drug toxicity would still be necessary.

A final approach is to modify current strategies and try to prevent CMV reactivation more effectively by giving more antiviral drug and pushing the drug with more supportive care. Clearly, the limiting factor of ganciclovir is neutropenia and its consequences. A strategy could be designed that includes use of ganciclovir with early use of growth factors to prevent the severe neutropenia that is responsible for the bacterial and fungal infections, combined with a switch to alternative drugs such as foscarnet, which has been shown in randomized trials to be equivalent in preemptive therapy strategies. Together with improved antifungal strategies, this strategy might allow us to more effectively prevent CMV reactivation in high-risk patients. There are interesting data from 2 randomized trials indicating that with increased use of granulocyte colony-stimulating factor, ganciclovir-related bacterial and fungal infections are less common [8,9]. Before such strategies are adopted they should be tested in prospective randomized trials.

Because the available solutions are all unsatisfactory, new drugs would certainly be very welcome, and there are active drug development programs ongoing at several companies. One drug, maribavir, which targets the CMV UL97 gene, will go into clinical trials in the stem cell transplantation setting this summer. There is also a development program for nonnucleoside drugs, and finally, the drug cidofovir has been modified to be less toxic and to be taken orally.

Conclusions

Current CMV prevention strategies appear to have eliminated the survival disadvantage associated with CMV seropositivity in the HLA-matched related sibling setting; however, CMV seropositivity in the recipient continues to be associated with poor outcome in the matched unrelated donor and T-cell–depleted settings. Whether CMV seropositivity of the donor is beneficial for seropositive transplant recipients remains controversial (Figure 3). Additional analyses must be performed to define the role of CMV serostatus with improved HLA-matching strategies. Additional analyses are also needed in the nonmyeloablative transplantation setting. To improve outcomes in high-risk patients, prevention strategies will require targeting both the direct and the indirect effects of CMV infection. This targeting could be accomplished by adoptive

- **Current CMV prevention strategies appear to have eliminated the survival disadvantage associated with CMV seropositivity in the HLA-matched-related transplant setting.**
- **However, CMV seropositivity of the recipient continues to be associated with a higher mortality in the unrelated donor and T-cell depleted myeloablative transplant setting.**
- **Additional analyses are needed define the role of CMV serostatus with improved HLA matching strategies, non-myeloablative conditioning, and improved antifungal prevention strategies.**
- **Randomized trials of intensified CMV prevention strategies are needed in these settings.**
- **Whether CMV seropositivity of the donor is beneficial for seropositive transplant recipients remains controversial.**

Figure 4. Conclusions.

transfer of donor-derived CMV-specific T-cells in T-cell–depleted transplantation or by intensified drug prevention strategies. Randomized trials are needed to evaluate these strategies (Figure 4). In conclusion, large multicenter cohort studies are needed to better define the subgroups of CMV-seropositive patients who may benefit from intensified prevention strategies and to define the impact of CMV donor serostatus in the era of high-resolution HLA matching.

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Prevention of Transfusion-Transmitted CMV: Current and Future Strategies

W. Garrett Nichols, MD, MSc

Transfusion-transmitted cytomegalovirus (CMV) infection (TT-CMV) is associated with considerable morbidity and mortality in at-risk populations, which include CMV-seronegative neonates, patients with AIDS, and hematopoietic cell transplant (HCT) recipients. The provision of CMV-seronegative blood product support to these individuals became the standard of care in the late 1980s after studies showed this strategy significantly reduced the rate of TT-CMV. The maintenance of CMV-seropositive and CMV-seronegative dual inventories is expensive, however, and some communities with high CMV seroprevalence have found it difficult to maintain adequate supplies of CMV-seronegative products. Thus, alternate methods for the provision of CMV-safe blood products have been pursued, including the use of leukoreduced platelet and red blood cell components.

CMV presents a unique challenge after stem cell transplantation, given that CMV infection, unlike many other transfusion-transmitted infections, is truly ubiquitous. In addition, it is associated with latent, asymptomatic infection in the normal host, which makes clinical detection impossible. The good news is that there is a serologic test that is widely available and inexpensive; a positive serology result essentially means that the donor is infected with CMV. The bad news is that a positive serology result does not equal infectious risk with regard to individual blood components, and indeed most seropositive units do not transmit infection even in the most highly immunosuppressed stem cell transplant recipients. This reality obviously raises some questions. First, given that CMV-seropositive donors rarely transmit infection, what are the features that characterize the infectious blood component? Does CMV reactivation in the normal blood donor that results in plasma viremia account for most transmissions, or do latently infected cells in the blood product transmit infection? Components from CMV-antibody–negative donors can also (albeit rarely) transmit CMV infection; this transmission is usually thought to occur in the setting of donor primary infection prior to seroconversion. One could thus question whether window-period viremia occurs in those individuals, and how common is that particular event. Obviously, if latently infected cells in the blood products account for the majority of cases of viral transmission, then leukoreduction should be highly effective in the prevention of transfusion-transmitted CMV infection. However, if donor reactivation with plasma viremia or window-period viremia in the seroconverting donor are frequent players in transmission events, then nucleic acid testing of individual blood components should make it possible to prevent transfusion-transmitted CMV infection.

Use of Seronegative Blood Components

Two landmark studies, the first by Bowden and colleagues [1] (published in 1986), subsequently confirmed by Miller and colleagues [2] (published in 1991), have looked at the issue of providing all seronegative components to seronegative stem cell transplant recipients. These two studies came out with remarkably equal estimates of the risks of primary CMV infection in the CMV-seronegative host with a seronegative stem cell donor, documenting rates of CMV infection on the order of 3% to 4% with the use of seronegative blood components; this compared favorably to the 30% to 35% incidence rate seen when unscreened blood components were used (Figure 1). These results clearly established seronegative components as the standard of care for preventing primary CMV infection in the high-risk stem cell transplantation setting. Difficulties may arise, however. Seronegative donors can sometimes be hard to find, depending on the location and the prevalence of CMV seropositivity within a particular community. In addition, there is a high cost associated with maintaining dual inventories of blood products. Recent evidence has clearly indicated that CMV is latent in cells of the monocyte and macrophage lineages, however, which suggests that removing these cells from the blood components via leukoreduction techniques can reduce the risk of transfusion-transmitted CMV infection. This theory led to the development and evaluation of leukoreduction methods for this particular indication, such as filtration and more recently process leukoreduction via the use of apheresis machines.

Use of Filtered Blood Products

Studies from 1987 up to the current era that have investigated the use of filtered products for prevention of transfusion-transmitted CMV infection have shown the incidence of primary CMV infection to be on the order of 0% to 3%. The numbers of patients who were evaluated in many individual studies were small, however (eg, 20 to 45). Because of the small sample size, for rare outcomes it cannot be said with confidence that there is not a significant risk for infection using these products. Also, most of these studies were retrospective and did not use prospective monitoring for primary CMV infection to determine whether it was indeed prevented.

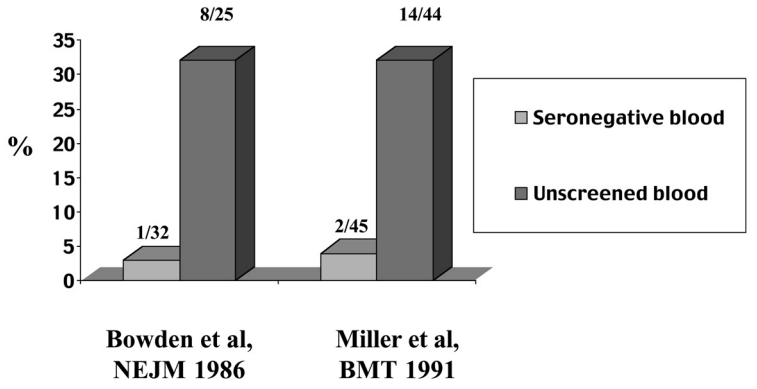


Figure 1. CMV-negative products for seronegative patients: incidence of primary infection after hematopoietic stem cell transplantation.

The largest study, with 500 patients, was a randomized trial by Bowden et al, published in 1995 [3]; importantly, this study also included a control arm. The Bowden study compared the use of CMV-seronegative blood components with filtered blood components from unscreened donors, with filtration performed at the bedside. Bone marrow transplant recipients were the subjects of the study, including 300 seronegative recipients of stem cell transplants from seronegative donors and 196 seronegative recipients of autologous transplants. Virologic assessment was performed weekly, using cultures of blood, throat, and urine specimens to detect the incidence of primary CMV infection, given that this study was carried out prior to the availability of either antigenemia or polymerase chain reaction (PCR)-based assays. The primary analysis was based upon the occurrence of CMV infection between days 21 and 100 after transplantation; this analysis was specified a priori to eliminate the early infections that theoretically could have occurred due to prestudy exposure rather than to the blood products after transplantation per se. The secondary or intent-to-treat analysis included all infections that occurred after randomization. The authors predicted that there would be a baseline CMV infection rate of 1% in the seronegative arm, and powered their study to be able to detect an absolute difference of 5% in the occurrence of primary CMV infection. The primary analysis (which excluded early infections) indicated that the incidence of CMV infection, CMV disease, and CMV-related deaths was no different in patients who received filtered blood products or seronegative blood products. However, the intent-to-treat analysis came to somewhat different conclusions (Figure 2). Although the incidence of primary CMV infection did not differ between the filtered blood arm and the seronegative blood arm (2.4% versus 1.4%), the incidence of CMV disease was statistically different between these 2 arms, 2.4% versus 0%. Three cases of pneumonia occurred during the early transplantation period, in addition to 2 cases of pneumonia and 1 case of gastrointestinal disease occurring approximately 50 days after transplantation; all occurred in the filtered blood arm. All of the cases of pneumonia resulted in death, so the incidence of CMV-related death was 2% in the filtered-blood arm versus 0% in the seronegative arm.

The interpretation of this study was debated quite extensively. Despite the fact that all 6 cases of CMV disease occurred in the filtered arm (and all 5 cases of pneumonia were fatal despite treatment with intravenous ganciclovir and immunoglobulin), the outcome was most commonly ascribed to chance given the low incidence of CMV infection in both populations. Supporting this theory was the fact that 4 of the 5 patients who had early primary CMV infection had equivocal serological results going into transplantation, so it is possible that they were seroconverting at the time of transplantation and therefore should not have been included in the study in the first place. The conclusion by Bowden et al was that the study may have overestimated the risk of CMV infection and/or disease with leukoreduced blood product support, and therefore these 2 approaches were deemed to

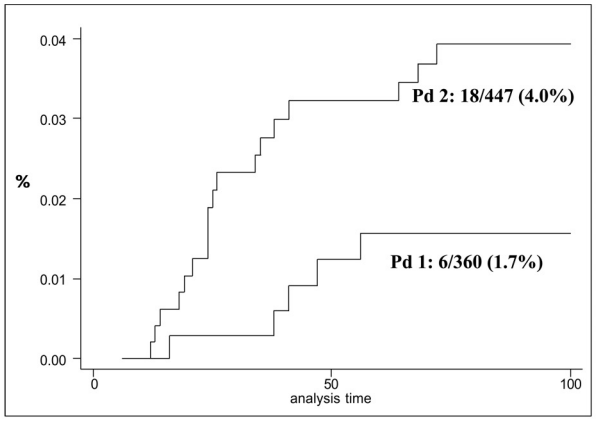


Figure 2. Transfusion strategy and transfusion-transmitted CMV: Fred Hutchinson Cancer Research Center experience. From: Nichols WG, Price TH, Gooley T, Corey L, Boeckh M. Transfusion-transmitted cytomegalovirus infection after receipt of leukoreduced blood products. *Blood*. 2003;101:4195-200. Copyright American Society of Hematology, used with permission.

be equivalent. The results were widely accepted by the medical community, and filtered products have been widely used in stem cell transplantation centers and in other centers for the prevention of primary CMV infection ever since. But even this large 500-patient study was still underpowered for the detection of clinically significant differences, if one considers that it was powered to show equivalence if less than a 500% difference in incidence was seen. The observed difference of 71% in the incidence of primary CMV infection is still excessive according to current accepted standards for equivalence trials. If nothing else, this study highlights the difficulties in achieving clinical and statistical significance for trials that are based on rare outcomes.

Several issues remain to be investigated more thoroughly; the first involves the filtration process. In the randomized trial, filtration was performed at the bedside, whereas current leukoreduction via filtration is most commonly performed at the blood bank. There are fewer filter failures with the latter procedure, and there is also process control, so that filtration at the blood bank should be associated with higher efficacy. The second issue to consider is whether CMV-related death could be fully preventable. Given that transfusion-transmitted CMV-related death occurs in approximately 1 in 30 to 1 in 50 seronegative patients undergoing HCT, one could consider whether prospective surveillance with the antigenemia assay and/or PCR combined with preemptive ganciclovir therapy could improve survival in these patients. Finally, there is the question of the efficacy of new techniques such as process leukoreduction via platelet apheresis machines; these products are now used despite the fact that they were not evaluated in controlled trials. At the Fred Hutchinson Cancer Research Center, we noted an increase in the incidence of primary CMV infection after HCT after these products were initiated; we thus determined that this required further evaluation.

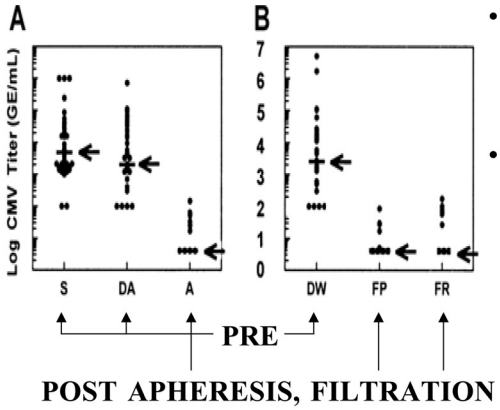
Table 1 summarizes our experience [4]. We analyzed a large cohort of more than 800 seronegative patients who were treated during two distinct time periods, defined by blood bank strategy. All patients during both periods were prospectively screened using the antigenemia assay and treated preemptively with ganciclovir once primary CMV infection was documented. During period 1 (1994-1996, shortly after the randomized control trial was stopped but before analysis was complete), only seronegative products or blood products that were filtered at the blood bank were used. During period 2 (1997-2000), identical screening and treatment protocols were utilized but our blood bank added single-donor apheresis products that were obtained from unscreened donors; these products were administered to patients without additional filtration. The incidence of primary CMV infection differed significantly between these 2 periods, 1.7% during the early period and 4% in the later period, and we wondered whether the apheresis products could account for this difference. Looking at the individual blood products that the patients received, however, we noted that the second period was distinguished by both the increased use of apheresis platelets and the use of filtered products from CMV-positive donors, as physicians became more comfortable with their use. The results of multivariable analysis that examined the receipt of each blood product administered suggested that it was the filtered red blood cells (but not the apheresis products) that were associated with the risk of transfusion-transmitted CMV infection. Thus, it is possible that either apheresis products and/or the filtered products could have accounted for the increase in the risk of primary CMV infection. The most important finding of this study, however, was that screening and preemptive therapy eliminated all cases of early CMV-related death.

Our data are probably not unique. Other studies that have examined the incidence of primary CMV infection with prospective surveillance and more sensitive techniques have found comparable or higher incidences of primary CMV infection. Butt and Clark [5] reported the use of a whole blood PCR assay and documented an astounding 55% incidence of primary CMV infection. These patients were treated preemptively, and only 1 case of CMV disease occurred. This study, however, was very small, and one can question whether the PCR results were falsely positive in a significant portion of these patients. Two subsequent studies [6,7] used either plasma PCR, antigenemia, or peripheral blood

Table 1. Transfusion Strategy and Transfusion-Transmitted CMV (TT-CMV)*

• Although the primary difference between the 2 time periods was the secondary use of apheresis platelets, filtered products from CMV-positive donors also increased during period 2 (P = .035).
• Multivariate analysis results suggested that filtered red blood cells (P = .006) but not apheresis products (P = .42) were associated with a risk of TT-CMV.
• Most importantly, preemptive therapy eliminated all cases of early CMV-related death.

*Based on data from the Fred Hutchinson Cancer Research Center for a large cohort trial looking at more than 800 patients treated during 2 distinct time periods that differed according to blood bank strategy [4]. During period 1 (1994-1996, shortly after the randomized control trial was stopped but before analysis was complete), only seronegative products or blood products that were filtered at the blood bank were used. During period 2 (1997-2000), identical screening and treatment protocols were used but in addition the blood bank added single-donor apheresis products that were obtained from unscreened donors; these products were administered to patients without additional filtration.



- Data confirmed by Visconti et al (prepublished Blood 2003) using spiking experiment
- Reasons for failure?
  - True failures (>5 x 10^6 WBC)
  - Failure to reduce monocyte subset
  - Plasma viremia???

Figure 3. Filter failures: residual CMV after leukoreduction. From: Dumont LJ, Luka J, VandenBroeke T, Whitley P, Ambruso DR, Elfath MD. The effect of leukocyte-reduction method on the amount of human cytomegalovirus in blood products: a comparison of apheresis and filtration methods. Blood. 2001;97:3640-3647. Copyright American Society of Hematology, used with permission.

mononuclear cell PCRs and demonstrated an incidence of primary CMV infection that ranged from 6% up to 14%. Because preemptive therapy was utilized, however, the incidence of CMV disease was very low. Some intriguing laboratory-based studies have also looked at this issue; their results suggest some possible explanations for these findings. A study by Dumont and colleagues [8] looked at filter failures and/or apheresis failures, both of which can result in residual CMV in the blood product after leukoreduction. Figure 3 shows data for individual blood components with the log CMV titer for preapheresis and postapheresis specimens. There were still a significant number of products after apheresis that contained the CMV genome, and once the filtration process was complete there were still products with detectable CMV. These data have been confirmed by Visconti et al [9], using a spiking experiment rather than products from actual blood donors. So what accounts for leukoreduction failures in these studies? Potential reasons include true filter failures, in which residual white blood cells in the product exceed 5×10^6 cells (the threshold that is theorized to significantly decrease the risk of transfusion-transmitted infection). There could be subset issues, such that significant reductions in the monocyte subset of cells (which ostensibly carry the CMV genome) do not occur despite significant reductions in the total residual white blood cell count in the product. Finally, plasma viremia may be present in these individuals, in which case leukoreduction would not be effective because CMV would be present in the plasma fraction of that blood component.

Table 2. Nucleic Acid Testing for CMV: Little Supporting Evidence

- CMV DNA is rarely detected in seroconverting donors.*
  3/384 (0.8%) Samples from 3/192 donors (1.6%).
  Only 1 sample was CMV-DNA positive prior to seroconversion.
- CMV DNA is rarely detected in seropositive donors.
  0/488 Samples from 60 donors.*
  2/416 (0.5%) Samples from seropositive donors.†

*Drew et al [10].
†Roback et al [11].

Nucleic Acid Testing for CMV in Blood Products

Because plasma viremia may account for some TT-CMV infections, one could question whether there is a role for nucleic acid testing of individual blood products as a strategy for preventing TT-CMV infection. The rationale is that CMV reactivation in the seropositive donor is probably a frequent event, as evidenced by the fact that a significant proportion of circulating cytotoxic T-lymphocytes (CTLs) in healthy blood donors are CMV specific; this finding suggests constant antigenic stimulation of CTLs due to frequent subclinical reactivation. Reactivation in patients with severe illnesses such as sepsis has been documented by PCR and antigenemia assays. More to the point, Dumont and colleagues [8] found CMV DNA in 75% of healthy seropositive donors during the allergy season, a time of ostensible cytokine activation in these individuals. Shortly after the pollen count increased significantly, donors showed a high rate of CMV DNA in the blood. Even if the specificity of their PCR assay is discounted, 4 of 41 donors in this particular study had positive cultures, a result that is hard to dismiss. For the seronegative donor, window viremia could occur prior to serologic conversion, such that nucleic acid testing could be clinically useful. Unfortunately, there is little supportive evidence that nucleic acid testing is either sensitive or specific enough for this indication. Two recent studies [10,11] found that CMV DNA is rarely detected in seroconverting donors or seropositive donors when samples are

- Chemicals cross-link DNA/RNA
  - Selective (no DNA/RNA in platelet/RBC products)
- Amotosalen HCL (S-59)
  - Approved in Canada/Europe for platelet products
  - Inactivates > 10^6 pfu CMV
  - Treatment protected immunocompromised mice from lethal TT-CMV from platelet products[1]

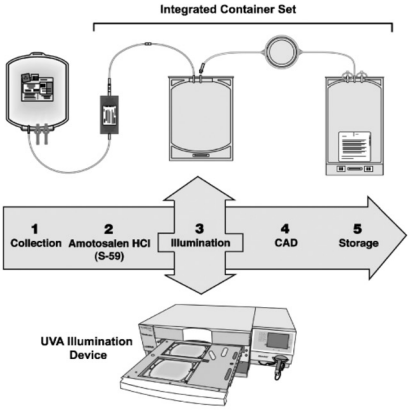


Figure 4. Pathogen inactivation technologies for reducing transfusion-transmitted CMV (TT-CMV) in at-risk populations. Reprinted from [9] with permission from Elsevier.

blinded (Table 2), even when the most sensitive assays are utilized. Historic reports of serosilent infections (seronegative patients who are PCR positive) or the frequent detection of CMV DNA in healthy CMV-positive donors may have been due to unreliable assays. Suffice it to say that nucleic acid testing of blood products to prevent TT-CMV is not yet ready for prime time.

Pathogen Inactivation Technologies

Other prospects on the horizon include the use of pathogen inactivation technologies. These products are being developed not primarily for CMV but for bacterial contamination of individual units of blood. However, these chemicals cross-link DNA and RNA and thus also cross-link CMV; studies have shown that they are also highly effective for the prevention of TT-CMV in animal models. The most extensively studied agent is amotosalen (Figure 4), formerly known as S-59, which is now approved in Canada and Europe for platelet products. In vitro, it inactivates more than 10^6 pfus of CMV, and in animal models treatment of platelet products protected immunosuppressed mice from lethal transfusion-transmitted CMV challenge [12].

Conclusion

Transfusion-transmitted CMV is nearly always found when populations of stem cell transplantation patients are screened; however, this is a low-frequency event that occurs in only 1 in 25 to 1 in 100 patients at risk. Therefore it may take years for individual transplantation centers to see a single CMV-related death, and even these deaths may go undetected if autopsies or other means of detecting CMV disease are not performed. Current research is looking at the use of filtered versus apheresis versus seronegative products, but the most important message to take from studies performed to date is that deaths are preventable with the same surveillance and preemptive therapy approaches used for seropositive HCT recipients. Indeed, reduction of CMV mortality rates occurs one patient at a time.

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This research summary is presented as a brief guide to an important study that appeared in a recent issue of *Biology of Blood and Marrow Transplantation.* The complete paper, including figures, tables and references, can be found in Volume 10, Issue 1, Pages 58-64.

Prolonged Outbreak of Human Parainfluenza Virus 3 Infection in a Stem Cell Transplant Outpatient Department: Insights from Molecular Epidemiologic Analysis

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INTRODUCTION

Community-acquired respiratory virus infections such as human parainfluenza virus type 3 (hPIV3) are significant causes of morbidity and mortality after stem cell transplantation (SCT). We recently experienced a prolonged outbreak of hPIV3 infection in our outpatient department (OPD) over an 11-month period despite intensive infection-control practices. Molecular typing of viral isolates from the outbreak and surrounding community was used to determine whether this outbreak was due to ongoing transmission of a single strain within our outpatient clinic or multiple introductions of virus from the circulating community pool.

PATIENTS AND METHODS

Patient Population and Setting

Stem cell transplant recipients were generally treated as inpatients from the start of transplant-conditioning therapy until recovery from neutropenia. Thereafter, they were followed up at least twice weekly in the OPD until approximately 100 days after transplantation, when they were returned to the care of their referring physician. Inpatients were housed in 1 of 3 transplant-dedicated wards (9SW, 10SW, and 11SW) in individual rooms with positive pressure with respect to the hallway and either high-efficiency particulate air-filtered air or 95% air filtration. Outpatients were treated in a dedicated clinic across the street from the hospital.

Virology Procedures and Definition of hPIV3 Infection

A nasopharyngeal-throat wash or swab and throat swab for viral testing was performed for all patients with upper respiratory tract symptoms throughout the study period; testing consisted of direct fluorescent antibody (DFA; Bartels VRK; Intracel, Issaquah, WA) staining for the community-acquired respiratory viruses (respiratory syncytial virus [RSV], influenza A and B, and the parainfluenza viruses), shell vial testing for RSV, and viral culture. Viral DFA and culture were also performed on all bronchoalveolar lavage, lung biopsy, and autopsy specimens.

Hospital ward–acquired hPIV3 infections were defined as DFA or culture positivity occurring 4 days after admission to one of the inpatient wards. Human PIV3 infections detected while the patient was in the OPD or within 4 days of hospitalization were deemed to have been contracted in the OPD.

Infection-Control Measures

Multimodal infection-control measures were in place throughout the study period. All health-care workers, patients, and visitors were required to sign in stating that they did not have cough, sneezing, or uncontrolled rhinorrhea before gaining access to inpatient wards or the OPD. Staff members with these symptoms were restricted from patient care, and symptomatic visitors were prohibited access. Symptomatic inpatients and outpatients underwent virologic testing of nasopharyngeal wash specimens as discussed previously; both inpatients and outpatients were placed in respiratory isolation in individual rooms until they were both asymptomatic and culture negative for respiratory viruses.

Reverse Transcription-Polymerase Chain Reaction, DNA Sequencing, and Nucleotide Sequence Analysis

Reverse transcription-polymerase chain reaction and DNA sequencing were performed at the Centers for Disease Control and Prevention (CDC) on 46 patient hPIV3 isolates by using previously described methods.

Human PIV3 outbreak: characteristics

From September 1998 to July 1999, 93 cases of hPIV3 infection were documented in recipients of stem cell transplants at the Fred Hutchinson Cancer Research Center. Of these, 66 cases (71%) were acquired in our OPD; these infections occurred among 397 patients who attended the OPD during the 11-month period (attack rate, 17%). The outbreak was first recognized in September and October (hereafter designated the outbreak period), when 30 outpatients and 9 inpatients contracted hPIV3 infection. The temporal relationship of our hPIV3 outbreak to the community-wide prevalence of hPIV3 (as determined from data obtained from the National Respiratory and Enteric Virus Surveillance System, CDC, for the Seattle/King County area) is shown in Figure 1. These data demonstrate that the outbreak occurred without a concomitant increase in hPIV3 infections in the community.

Molecular Analysis

Of the 93 cases of hPIV3 infection that occurred during the study period, 46 isolates (representing 49% of cases) were recovered and submitted for sequencing and molecular analysis.

The vast majority of case isolates sequenced (36 isolates of 46 tested; 78%) fell

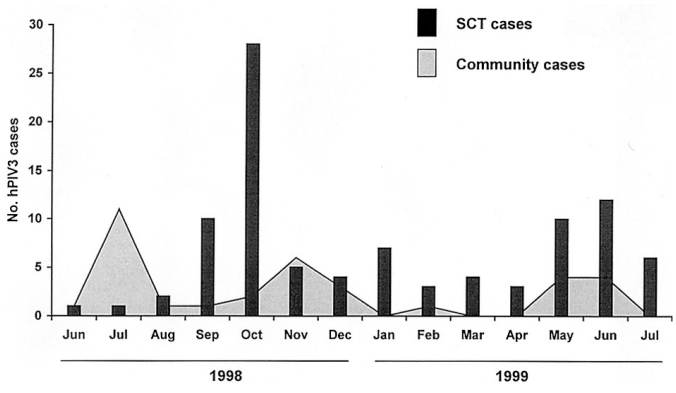

Figure 1. Human PIV3 outbreak at a stem cell transplant (SCT) center and relationship to community-wide prevalence.

into a distinctive cluster (hereafter termed cluster 1), supported by a high bootstrap value of 99%. Within cluster 1, case isolates differed by 3 or fewer nt, and 27 isolates shared identical sequences. Two control isolates from the surrounding community also grouped within this cluster. Three other clusters of case isolates supported by bootstrap values of 80% were also seen.

As expected, most isolates from the outbreak period (23/24; 96%) shared closely related sequences and were grouped within cluster 1; these included 18 (95%) of 19 isolates tested from patients attending the OPD and 5 of 5 isolates from patients on the inpatient wards. In addition, 2 of the 3 community isolates (controls 2 and 6) from the outbreak period fell within cluster 1. Strikingly, 11 (73%) of 15 isolates from the OPD that were obtained from the postoutbreak period still fell within cluster 1, despite the apparent disappearance of isolates from this cluster in the community at large (0 of 5 tested). Isolates from cluster 1 were detected in OPD patients in November (n = 1), December (n = 2), January (n = 4), April (n = 1), May (n = 2), and June (n = 1), suggesting a cycle of ongoing nosocomial transmission; genetically distinct community controls were obtained from December, March, April, and May.

Isolates from cluster 2 did not seem to be tightly linked in time or place.

Epidemiologic Analysis

An intensive prospective investigation of transmission patterns was undertaken during the outbreak period in September and October. Despite patient, family, and staff interviews and extensive chart review, no consistent pattern of exposure was noted (with the exception of OPD attendance for outpatients).

DISCUSSION

Molecular analysis of viral isolates obtained during a prolonged outbreak of hPIV3 infections among stem cell transplant recipients at our institution suggested that this outbreak was associated with a single predominant genotype (cluster 1). Although the finding of related isolates during the outbreak period was expected, we have demonstrated that the persistent, low-level incidence of hPIV3 infections in the post-outbreak period was also primarily due to isolates sharing the same common genotype, suggesting a continuing cycle of nosocomial transmission. That this cycle of transmission occurred in an outpatient setting despite a relatively strict infection control policy is a cause for concern.

In conclusion, molecular sequencing of hPIV3 isolates from SCT recipients suggested that even during the periods of low incidence that followed our easily recognizable outbreak, nosocomial transmission of the virus continued to occur. Further studies to delineate the mechanism of transmission in this setting are clearly needed and should probably focus on issues of environmental contamination or asymptomatic carriage among caregivers or health-care workers.

This research summary is presented as a brief guide to an important study that appeared in a recent issue of *Biology of Blood and Marrow Transplantation.* The complete paper, including figures, tables and references, can be found in Volume 10, Issue 1, Pages 40-48.

Allogeneic versus Syngeneic Killer Splenocytes as Effector Cells for the Induction of Graft-versus-Tumor Effect

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INTRODUCTION

Immunotherapy strategies that aim to amplify an immune response and break tolerance to the tumor cells are mostly based on the use of tumor-syngeneic/autologous effector cells in both experimental models and cancer patients. During the last decade, an increasing number of protocols based on an allogeneic reaction between immunocompetent effector cells and tumor target cells were developed.

Encouraged by the efficient antitumor effects of alloCT in previous studies, yet aware of the associated complications, we compared the efficacy of allogeneic versus syngeneic cell therapy (synCT) by using various sources of lymphokine-activated T and natural killer (NK) cells in an attempt to induce graft-versus-host (GVT) effects in a murine model of mammary carcinoma.

RESULTS

LAK Cells of MiHC-Mismatched or MHC-Mismatched Splenocytes

Antitumor activity across minor histocompatibility complex (MiHC) or major histocompatibility complex (MHC) antigen barriers was tested in irradiated F_1 mice inoculated with 4T1 tumor cells. TBI was given to allow temporal or permanent engraftment of the allogeneic therapeutic cells. MiHC-mismatched lymphokine-activated killer (LAK) cells derived from DBA/2 mice resulted in a delay in tumor-related death, compared with syngeneic BALB-LAK cells (the median survival was 41 and 33 days, respectively. The Kaplan-Meier probability of tumor-free survival was significantly higher after treatment with DBA-LAK cells than with BALB-LAK cells ($P = .0014$).

Treatment with MHC-mismatched parental C57 LAK cells caused severe graft-versus-host disease (GVHD) in F_1 hosts, which resulted in the death of 10 of 10 mice that died free of tumors earlier than mice treated with syngeneic F_1 LAK cells (median survival was 18 versus 38 days, respectively. These results show that cell therapy with MiHC- or MHC-mismatched activated splenocytes improves the probability of tumor-free survival; however, because T cells contained in allogeneic LAK cells aggravate GVHD, an alternative cell source needs to be applied for safer induction of GVT effects.

The Effect of Gamma Irradiation on AlloCT

Naive or LAK C57 cells were irradiated in vitro (7.5 Gy) before injection into F_1 mice that had been inoculated with 4T1 tumor cells 24 hours earlier. Both naive and LAK allogeneic effector cells given without in vitro irradiation induced a severe allogeneic reaction against the F_1 hosts that finally led to GVHD-related death of 17 of 17 mice in each experimental group after a median of 13 and 26 days, respectively. However, in vitro irradiation of effector cells before inoculation into the mice totally abrogated the GVHD-related death of all hosts. Irradiation of naive or LAK cells significantly prolonged overall survival as compared with nonirradiated effector cells ($P = .0000$ and $P = .0004$ for naive and LAK cells, respectively). These results show that although irradiation of allogeneic effector cells used for immunotherapy prevented GVHD and did not abrogate antitumor effects in vitro, allogeneic cell therapy (alloCT) induced by the administration of a single dose of irradiated donor lymphocytes was not sufficient to provide a long-lasting GVT effect in vivo.

Phenotypic Characterization of Effector Cells Applied to Cell Therapy

Phenotypic analysis of naive splenocytes revealed 32% $CD3^+$, 3% $DX5^+$, and 4% $CD3^+$ $DX5^+$ cells. After recombinant interleukin-2 (rIL-2) activation in vitro, all 3 cell subsets increased to levels of up to 45%, 20%, and 10%, respectively. In an attempt to achieve an antitumor effect by an enriched $DX5^+$ cell subset without the $CD3^+$ cells responsible for GVHD, we performed a phenotypic analysis of spleen and bone marrow (BM) cells derived from 2 strains of severe combined immunodeficiency disease (SCID) mice lacking the T-cell compartment of the immune system. BM cells of C.B-17 and C57 SCID mice contain a small proportion of $DX5^+$ cells, whereas splenocytes derived from both strains contain 47% and 52% $DX5^+$ cells, respectively. These results show that SCID spleen or BM-derived cells can be propagated in vitro and can provide sufficient numbers of $DX5^+$ cells, without contamination of T cells, for application in a therapeutic experimental model.

Antitumor Reactivity as Measured by Tumor Clonogenic Assay In Vitro

Antitumor activity was evaluated by tumor clonogenic assay in vitro in lung cells derived from F_1 mice inoculated with 4T1 tumor cells and treated with LAK cells 13 days before lung harvest. The antitumor activity of allogeneic LAK cells derived from MiHC-mismatched DBA/2 mice or MHC-mismatched normal and SCID C57 mice was compared with that of syngeneic F_1 or normal BALB and SCID C.B-17-derived LAK cells. Cells isolated from the lungs of tumor-bearing untreated control mice contained 435 to 1015 tumor colonies in vitro, as determined in 5 separate experiments. Allogeneic LAK cells derived from MiHC-mismatched or MHC-mismatched normal and SCID mice were more efficient in preventing tumor growth (4%, 4%, and 1% tumor colonies, respectively) than syngeneic LAK cells derived from BALB, F_1, and C.B-17 SCID mice (14%, 21%, and 37% tumor colonies, respectively).

Effect of Allogeneic versus Syngeneic SCID-Derived LAK Cells on the Survival of Tumor-Bearing Mice

F_1 mice inoculated with 4T1 tumor cells were treated 24 hours later with LAK cells derived from spleen or BM cells of C57 SCID and C.B-17 SCID mice. There was no apparent difference in the median survival of experimental groups treated with the various SCID effector cells. However, compared with alloCT with effector cells derived from normal mice, C57 SCID-derived LAK cells did not cause GVHD; 0 of 18 and only 2 of 11 mice treated with allogeneic C57 SCID-LAK spleen and C57 SCID-LAK BM cells, respectively, died of GVHD, whereas 15 of 15 and 10 of 10 mice treated with naive splenocytes or LAK cells derived from normal C57, respectively, died of GVHD. These results show that allogeneic SCID-derived LAK cells constitute a safe source of allogeneic effector cells for GVT induction without causing GVHD.

Effect of AlloCT versus synCT on Antitumor Activity In Vivo by Adoptive Transfer Experiments

F_1 mice inoculated with 4T1 tumor cells were treated 24 hours later with naive or LAK cells derived from either normal allogeneic C57 and SCID mice or syngeneic F_1 and C.B-17 SCID mice. Fourteen days after tumor inoculation, lung cells were adoptively transferred intradermally into naive BALB mice. Serial weekly measurements of tumor size show that lung cells derived from F_1 mice treated with naive allogeneic C57 and LAK cells or C57 SCID-LAK cells did not give rise to any tumor colony growth in the adoptive recipients, whereas syngeneic F_1 or C.B-17 SCID-LAK cells resulted in exponential tumor growth similar to that observed in untreated tumor-bearing control mice. Follow-up of survival showed that most secondary hosts inoculated with lung cells derived from F_1 mice treated with naive allogeneic C57 and LAK cells or C57 SCID-LAK cells remained tumor-free for >200 days. Kaplan-Meier probability of tumor-free survival emphasized the advantage of alloCT over synCT in exerting antitumor effects ($P = .0003$ for C57 LAK cells versus F_1 LAK cells and $P = .0001$ for C57 SCID-LAK cells versus C.B-17 SCID-LAK cells).

DISCUSSION

Because allogeneic naive lymphocytes and LAK cells contain both T and NK cells, we directed our efforts toward investigating the GVT effects induced by irradiated LAK cells, in which radiosensitive T cells cannot exert GVHD, or we used a source of NK cells alone in an attempt to prevent T cell-mediated GVHD. Indeed, irradiated LAK did not cause GVHD but were unable to mediate a long-lasting GVT effect.

Unfortunately, probably because of a lack of memory NK cells, such GVT effects were short acting and thus not sufficient to exert a long-lasting in vivo effect. Because allogeneic NK cells, as well as irradiated LAK cells, including radiosensitive T cells, did not cause GVHD, the possibility of exploiting their GVT capability over longer periods of time through the use of multiple doses given at short intervals should perhaps be considered.

Taken together, our results support the use of allogeneic cell-mediated immunotherapy based on the use of T cell-depleted NK cells for the induction of safe antitumor activity.

In summary, these experiments, in accord with our previous investigations and supported by indirect data from clinical studies, suggest a clear advantage of alloCT over synCT in exerting GVT effects and emphasize the potential use of intentionally mismatched NK cells, possibly in repeated doses, to achieve safe and effective immunotherapy of minimal residual disease. Clinical studies currently in progress are aiming to further exploit the use of allogeneic cell-mediated immunotherapy, possibly in conjunction with HSCT, for prevention or minimizing relapse, which continues to be the single major barrier to successful HSCT.

Journal Watch

A scan of recent medical literature identified these articles of special importance in the science and clinical application of blood and marrow transplantation.

Le Blanc K, Rasmusson I, Sundberg B, et al: Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet. 2004;2004:1439-1441.

The mesenchymal stem cells found in bone marrow are not immunogenic, and in fact are immunosuppressive: they not only go unrecognized by alloreactive T-cells and natural killer cells, but inhibit T-cell proliferation. Transplanted along with identical sibling hematopoietic stem cells, mesenchymal cells have been reported to reduce graft-versus-host disease (GVHD). Haploidentical stem cells were used for the treatment for severe GVHD in an allogeneic stem cell recipient.

The patient was a 9-year-old boy with acute lymphoblastic leukemia in third remission. He developed grade IV acute GVHD soon after receiving blood stem cells from a matched, unrelated female donor. After the child's progressive, severe GVHD proved unresponsive to all other treatments, haploidentical mesenchymal stem cells were isolated from his mother. After 3 weeks of culture, a volume of 90 x 10^6 mesenchymal stem cells was harvested. The patient received intravenous transplantation with these cells, 2 x 10^6 per kg.

Within days after transplantation, the child's clinical condition improved significantly. Several weeks later, DNA analysis of bone marrow showed minimal residual disease. When the patient developed mild GVHD soon afterward, fluorescence in situ hybridization of a colonic biopsy specimen revealed 4% female epithelium. Another transplant with the mother's mesenchymal stem cells was given, 1 x 10^6/kg. Again, the patient's clinical condition improved. One year after transplantation, he remained well with minimal residual disease in blood and bone marrow. In culture studies, the patient's lymphocytes showed no alloreactivity to the mother's mesenchymal stem cells.

In this case of severe GVHD, transplantation with haploidentical mesenchymal stem cells yielded a dramatic immunosuppressive effect. In particular, the mesenchymal cells appeared to contribute to rapid healing of the damaged gut epithelium. Further study is needed to evaluate the use of mesenchymal stem cells for GVHD prevention and treatment.

Cogle CR, Yachnis AT, Laywell ED, et al: Bone marrow transdifferentiation in brain after transplantation: a retrospective study. Lancet. 2004;363:1432-1437.

There is controversy regarding the potential for transdifferentiation of adult hematopoietic stem cells. Some animal and short-term human studies have suggested that transplanted bone marrow cells can have reparative effects in the brain. Postmortem brain samples from bone marrow transplant recipients were studied to seek evidence that adult hematopoietic cells can contribute to human neuropoiesis.

The study included autopsy specimens from three women who underwent therapeutic transplantation of hematopoietic stem cells from their brothers. All three patients had acute or chronic myelogenous leukemia; the transplants were performed up to 6 years before death. Samples from a woman and a man who died of other causes were studied for comparison. Immunohistochemical studies, fluorescence in situ hybridization, and tissue analyses were performed to assess the presence of long-term, multilineage, donor-derived neurogenesis that was not attributable to cell fusion.

In all three stem cell recipients, the brains showed hippocampal cells containing a Y chromosome. No cells were identified as having a fusion sex chromosome karyotype; all had just one X chromosome. One percent of all neurons were transgender neurons, while one to two percent of all glial cells were transgender astrocytes and microglia. The transgender cells were found mostly in the hilus and subgranular layer of the dentate gyrus.

This retrospective study confirms that transplanted human hematopoietic stem cells can transdifferentiate into brain cells. Transdifferentiation into neurons, astrocytes, and microglia is demonstrated, without evidence of fusion, several years after transplantation. The findings support the concept that bone marrow cells can migrate to the brain and transform into neural cells, thus participating in repair of brain tissue.

CMV in Stem Cell Transplantation: New Insights and Options

CME Assessment Test

1. Which is the following is true of CMV infection?
A. CMV is now a rare cause of early mortality after hematopoietic stem cell transplantation.
B. Poor outcomes in high-risk patients are probably due to direct and indirect (immunomodulatory) effects of CMV and toxicity of drugs used to treat CMV.
C. CMV-seropositive transplant recipients still have significantly worse outcomes than CMV seronegative recipients with CMV-negative donors.
D. All of the above.

2. Which of the following effects of ganciclovir toxicity is the leading complication in transplantation patients?
A. Organ toxicity.
B. Neutropenia.
C. Immunosuppression.
D. All of the above.

3. Which of the following developments may significantly impact the treatment of CMV infection?
A. Improved antifungal prevention.
B. High-resolution HLA matching.
C. Adoptive transfer of donor-derived CMV-specific T-cells in T-cell–depleted transplantation.
D. All of the above.

4. Which of the following is NOT true of preemptive CMV therapy in transplant recipients?
A. It involves regular biologic testing for CMV detection.
B. In 0% to 5% of cases, 1 in 20 patients, breakthrough CMV disease will occur.
C. Treatment may begin even before transplantation if an acyclovir product is used.
D. Preemptive strategies are now widely used, and they are highly effective in preventing CMV disease.

5. Which of the following may compromise the absorption of orally administered ganciclovir for CMV therapy?
A. High-dose chemotherapy.
B. Intestinal GVHD.
C. Total body irradiation.
D. All of the above.

6. With currently available treatment strategies, what is the rate of mortality from CMV pneumonia in CMV-seropositive allogeneic transplant recipients?
A. More than 50%.
B. 80%-90%.
C. Less than 10%.
D. 10%-30%.

7. Which of the following strategies is the standard of care for preventing primary CMV in the high-risk stem cell transplantation setting?
A. Use of seronegative blood components.
B. Use of filtered blood products.
C. Nucleic acid testing for CMV in blood products.
D. All of the above.

8. Which of the following factors has an impact on prevention of transfusion-transmitted CMV infection?
A. CMV infection is ubiquitous.
B. CMV is associated with latent, asymptomatic infection in the normal host, making clinical detection impossible.
C. Most seropositive units do not transmit infection even in the most highly immunosuppressed stem cell transplant recipients.
D. All of the above.

9. According to 2 landmark studies, what are the risks of primary CMV infection in the CMV-seronegative host with a seronegative donor when seronegative versus unscreened blood components are used?
A. 10%-20% versus 80%-90%.
B. 3%-4% versus 30%-35%.
C. 30%-35% versus 3%-4%.
D. No significant difference.

10. Which of the following is true of nucleic acid testing for CMV in blood products?
A. Two recent studies found that CMV DNA is frequently detected in seroconverting donors or seropositive donors when samples are blinded.
B. Nucleic acid testing has proven to be a highly effective new strategy for the prevention of transfusion-transmitted CMV.
C. High rates of CMV DNA have been found in healthy seropositive donors during the allergy season.
D. Only a small proportion of circulating cytotoxic T-lymphocytes in healthy blood donors are CMV specific.

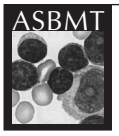
CME Assessment Test Answer Sheet

Release Date: February 15, 2004 **Last Review Date: February 15, 2004** **Expiration Date: February 15, 2006**

Instructions

(1) Read the articles in the supplement carefully. (2) Circle the correct response to each question on the Answer Sheet. (3) Complete the evaluation Form. (4) To receive CME credit, fax the completed Answer Sheet and Evaluation Form to the MCW CME office (414-456-6623) or mail to the Office of Continuing Education, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226. No processing fee is required.

1.	A	B	C	D	**6.**	A	B	C	D
2.	A	B	C	D	**7.**	A	B	C	D
3.	A	B	C	D	**8.**	A	B	C	D
4.	A	B	C	D	**9.**	A	B	C	D
5.	A	B	C	D	**10.**	A	B	C	D



CME Evaluation Form

Please evaluate the effectiveness of this CME activity on a scale of 1 to 5, with 5 being the highest, by circling your choice. Fax with the Answer Sheet to the MCW CME Office, 414-456-6623, or mail to the Office of Continuing Education, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226.

Overall Quality of the CME Activity	1	2	3	4	5
Articles in the supplement were presented in a clear and effective manner.	1	2	3	4	5
The material presented was current and clinically relevant.	1	2	3	4	5
Educational objectives were achieved.	1	2	3	4	5
The CME activity provided a balanced, scientifically rigorous presentation of therapeutic options related to the topic, without commercial bias.	1	2	3	4	5

Please comment on the impact (if any) that this CME activity might have on your management of patients.

Would you benefit from additional CME programs on this topic? Yes No

I have read these articles on diagnosis and management of cytomegalovirus infection following stem cell transplantation, published in *Bone Marrow Transplantation Reviews*, and have answered the CME test questions and completed the Evaluation Form for this activity.

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