Published in final edited form as:
Biol Blood Marrow Transplant. 2012 January ; 18(1): 66–75. doi:10.1016/j.bbmt.2011.05.010.

Cytomegalovirus Viral Load and Virus-specific Immune Reconstitution after Peripheral Blood Stem Cell versus Bone Marrow Transplantation

A. Guerrero[1,2], **S.R. Riddell**[1,2], **J. Storek**[1,*], **T. Stevens-Ayers**[1], **B. Storer**[1,2], **J. A. Zaia**[4], **S. Forman**[4], **R.S. Negrin**[5], **T. Chauncey**[1,2,3], **W. Bensinger**[1,2], and **M. Boeckh**[1,2]

[1]Fred Hutchinson Cancer Research Center, Seattle, WA

[2]University of Washington, Seattle, WA

[3]Veterans Administration Medical Center, Seattle, WA

[4]City of Hope National Medical Center, Duarte, CA

[5]Stanford Blood and Marrow Transplant Program

Abstract

Peripheral blood stem cell (PBSC) products contain more T cells and monocytes when compared to bone marrow (BM), leading to fewer bacterial and fungal infections. CMV viral load and disease as well as CMV-specific immune reconstitution were compared in patients enrolled in a randomized trial comparing PSBC and BM transplantation. There was a higher rate of CMV infection and disease during the first 100 days after transplantation among PBSC recipients (any antigenemia/DNAemia: PBSC, 63% vs. BM, 42%, P=0.04; CMV disease: PBSC, 17% vs. BM, 4%, P=0.03). By two years, CMV disease rates were similar. The early increase in CMV events correlated temporarily with lower CMV-specific $CD4^+$ T helper and $CD8^+$ cytotoxic T lymphocyte function at 30 days after transplantation in PBSC recipients. By 3 months after transplantation and thereafter, CMV-specific immune responses were similar between BM and PBSC recipients. In conclusion, higher CMV infection and disease rates occurred in PBSC transplant recipients early after transplantation. These differences may be due to a transient delay in CMV specific immune reconstitution following PBSC transplantation.

Introduction

The use of peripheral blood stem cells (PBSC) for transplantation improves survival in patients with high risk hematologic malignancies compared with the use of bone marrow (BM) as a stem cell source (1-3). PBSC products from donors who receive granulocyte colony stimulating factor (G-CSF) contain at least one $\log_{10}$ more T-cells and monocytes than BM, and absolute $CD4^+$ and $CD8^+$ lymphocyte numbers are higher early after PBSC

Corresponding author: Michael Boeckh, M.D., Fred Hutchinson Cancer Research Center, Vaccine and Infectious Disease Division, Clinical Research Division, 1100 Fairview Ave. N, Seattle WA 98109, Phone (206) 667-6706, FAX (206) 667-4411, mboeckh@fhcrc.org.
*Present affiliation: University of Calgary, Calgary, Canada

Conflict-of-interest disclosure: The authors declare no competing financial interests.

transplant (4-6). Overall, recipients of PBSC grafts have less frequent severe infections, especially those of bacterial and fungal etiology (4).

The impact of PBSC transplantation on CMV-specific immune reconstitution, clinical infection and disease is difficult to predict because of unique properties of CMV. Active CMV infection after transplantation is controlled by CMV-specific $CD4^+$ T helper (Th) and $CD8^+$ cytotoxic T lymphocyte (CTL) responses (7, 8). There is evidence for the transfer of donor CMV-specific immunity with the graft, since CMV seropositive recipients of seropositive BM reconstitute CMV-specific T cell immunity earlier than recipients of seronegative BM (9, 10). Although greater numbers of T cells are transferred with PBSC grafts, CMV infects early and committed hematopoietic progenitors (11, 12), which may result in transmission of a greater virus load with PBSC products. Moreover, the bone marrow and blood contain different ratios of naïve, memory and regulatory T cell subsets that could influence the recovery of functional CMV-specific T cells after transplant (13-15). Thus, differences in the cellular composition of BM and PBSC products may lead to different rates of CMV infection after transplantation both by influencing immune recovery and virus reactivation.

$CD8^+$ and $CD4^+$ effector T cells have traditionally been characterized by functional assays that measure production of a single cytokine such as IFNγ, or by cytotoxicity or proliferation assays. Recently, multiparameter flow cytometry has made it possible to simultaneously evaluate multiple qualitative properties of T cells and enabled additional characterization of T cell function based on the production of combinations of cytokines (16, 17). In a prior study, polyfunctional $CD8^+$ CMV-specific T cells have been demonstrated to reconstitute differently in CMV-positive recipients of allogeneic hematopoietic cell transplantation (HCT) depending on the serostatus of the donor, and patients with lower number of polyfunctional $CD8^+$ CMV specific T cells were on antiviral drugs longer than patients with a higher number of polyfunctional $CD8^+$ CMV specific T cells (18). The purpose of this study was to compare quantitative virologic outcomes and to undertake exploratory analyses of CMV-specific T cell immune reconstitution in transplant recipients who participated in a randomized clinical trial of allogeneic BM versus PBSC as a stem cell source for HCT.

Patients and Methods

Study Subjects

Patients participating in a multicenter randomized trial comparing PBSC or BM as a stem cell source for allogeneic transplant were analyzed for CMV infection and CMV-specific immune reconstitution (2). Virologic analysis was done in all subjects who participated in the randomized trial while CMV immunity was assessed in a subset of subjects at FHCRC who signed an additional consent form (Supplemental Figure S1). Conditioning regimens and GvHD prophylaxis were administered as described (19). CMV immune reconstitution studies were performed at one site (Fred Hutchinson Cancer Research Center) in a subset of patients who agreed to provide additional blood samples. Some of the data on qualitative immune reconstitution have been previously published (4). All participants had an HLA-matched related donor and were > 12 years of age. The study was approved by the Institutional Review Board at the FHCRC and all patients gave informed consent.

Virologic testing

Pre-transplant CMV serostatus was determined by an ELISA at FHCRC and Veterans Administration Medical Center (Premier CMV IgG, Meridian Diagnostic, Cincinnati, OH), indirect immunofluorescence assay at City of Hope National Medical Center, Duarte, CA (Virgo; Hemagen Diagnostics Inc., Columbia, MD), and passive agglutination test (Becton

Dickinson, Meylan, France) and microparticle enzyme immunoassay (IMX Systems, Abbot Laboratories, Abbot Park, IL) at Stanford University Medical Center, Stanford, CA. All patients were prospectively monitored for CMV reactivation by pp65 antigenemia and blood cultures (Fred Hutchinson Cancer Research Center, Veteran's Administration Hospital Seattle), plasma PCR for CMV DNA and shell vial cultures (City of Hope National Medical Center), or plasma PCR for CMV DNA (Stanford Medical Center). CMV pp65 antigenemia was quantified as the number of positive cells per slide; CMV DNA was quantified as copy number per mL (4, 20).

Use of acyclovir, ganciclovir, intravenous immunoglobulin, and CMV seronegative blood products

Acyclovir was given to herpes simplex virus (HSV) seropositive individuals at a dose of 250 mg/m^2 IV twice daily from the start of conditioning until day 30 after transplantation. Documented HSV infections were treated with acyclovir at a dose of 250 mg/m^2 IV three times daily or 400 mg PO five times daily for one week.

Ganciclovir was administered for any pp65 antigenemia, shell vial cultures or PCR positivity and continued until day 100 as described (21). Intravenous immunogloblin was administered to maintain IgG level above 400 μg/ml (22). CMV seronegative recipients of a seropositive BM or PBSC product received seronegative or filtered blood products throughout the pre- and post-transplant period (23).

Definitions

CMV disease was defined as the identification of virus from one or more visceral sites (lung, gastrointestinal tissue, liver) by culture or histology, or in the BAL by shell vial centrifugation culture, conventional culture, or staining with direct fluorescent antibodies combined with new or changing pulmonary infiltrates on radiologic exam (24). CMV infection was defined as any pp65 antigenemia or plasma PCR positivity. CMV viremia was defined as detection of CMV by rapid or conventional culture. Acute and chronic graft versus host disease (GvHD) were defined as described (25).

CMV-specific immune reconstitution

CMV immune reconstitution studies were done on fresh (LDA, and CTL assay) or cryopreserved peripheral blood mononuclear cells (PBMC) (intracellular cytokine analysis); experiments using cryopreserved cells were performed only in the CMV D+/R+ subset. Denominators for each test differ due to availability of cells (Figure S1).

Cytotoxic T-Lymphocyte assay (CTL)—Short-term CMV-specific CTL lines were expanded in vitro according to previously described methods (10). Briefly, fibroblast lines were established from skin biopsies obtained from the recipient or the HLA identical donor. Recipient PBMC were stimulated twice seven days apart with fibroblasts infected with CMV AD169, and cytolytic activity against HLA-identical and HLA class I-mismatched CMV-infected and mock-infected fibroblasts was then measured using a 5-hour chromium release assay. Specific lysis was calculated according to the standard formula as described (10). A positive CTL response was defined as lysis of HLA-identical CMV-infected targets at a level at least 10% greater than lysis of HLA-identical mock-infected and HLA class I mismatched CMV- and mock-infected targets.

Limiting dilution assay (LDA)—The frequency of CMV-specific T cells was determined by limiting dilution assay (LDA) using methods and calculations previously described (26). Briefly, fresh PBMC were plated in eight serial 2-fold dilutions starting from 10^5 cells per well in the presence of CMV antigen (24 replicates) or mock antigen (12 replicates). Each

well also received 10^4 gamma irradiated (3300 rad) autologous PBMC to serve as antigen presenting cells. After 5 days, the wells were pulsed with 0.6mCi ^{3}H thymidine and harvested after 18-24 hours. Wells were scored positive if the mean ^{3}H thymidine incorporation was >3× that in the corresponding control wells. Precursor frequencies were calculated by the ×2 minimization method (27) with a computer program (28) written by L. Sirinek (provided by C. Orosz, both Ohio State University, Columbus, OH). To compensate for differences between $CD4^+$ and total T cells between PBSC and BM recipients, results were normalized either with absolute lymphocyte count and to calculate the number of CMV-specific $CD4^+$ T cells per L or by the number of CMV-specific $CD4^+$ T cells per 1×10^6 PBMC (4).

Intracellular cytokine staining—Cryopreserved PBMC were thawed and rested overnight. The following day, PBMC were incubated with anti-CD107a PECy5 Ab for 10 minutes, followed by the addition of the co-stimulatory antibodies anti-CD28 (1μg/ml; Becton Dickinson, San Jose, CA, USA) and anti-CD49d (1μg/ml). Cells were then stimulated with CMV pp65 protein (2μg/ml), *Staphylococcus aureus* enterotoxin B (0.05 μg/ml) or medium for 6 hours at 37°C and 5% CO2. Brefeldin A (10 μg/ml, Sigma)/Golgi stop (Becton Dickinson, San Jose, CA, USA) was added during the last 4 hours of incubation. Samples were held overnight at 4 °C and then stained for flow cytometric analysis the following day.

After incubation with EDTA (20 mM), samples were incubated successively with FACSlyse and FACSperm (BD Biosciences), washed, and then incubated for 30 min in the dark at room temperature with a cocktail of antibodies consisting of CD3 ECD (Beckman Coulter, Brea, CA), CD8 PerCypCy 5.5, IFNγ APC, MIP-1β PE and TNFα A700 (BD Bioscience & Pharmingen, San Jose, CA). Lastly, samples were fixed with 1% paraformaldehyde and cell acquisition (range 100,000 to 400,000) was done on an LSRII flow cytometer. Appropriate single color compensation and fluorescence minus one controls were run. Data were initially analyzed with FlowJo Version 8.8.6, and then further with PESTLE version 1.6.2 and SPICE version 5.0 (Simplified Presentation of Incredibly Complex Evaluations) software provided by M. Roederer of the National Institute of Allergy and Infectious Diseases.

Statistical analysis

Time to CMV events was assessed by cumulative incidence curves and the log rank test. Student's t test, Fisher's exact test and the Wilcoxon matched pairs test were done as appropriate on Prism software v5.0.

Results

Study Subjects

A summary of relevant patient characteristics is shown in Table 1, and additional characteristics have been published elsewhere (4) (Figure S1). The two study arms (BM and PBSC) were balanced for CMV serostatus and age. The subset of patients who were studied for CMV-specific T cell immune reconstitution was also balanced between the study arms.

CMV infection and disease

Seropositive recipients—The incidence of pp65 antigenemia or CMV DNA at any level before day 100 was higher in PBSC than in BM recipients (P=.04) (Figure 1A). There was no statistically significant difference between the two groups in the incidence of high (>100 cells/slide or >100,000 copies/μl) viral load by PCR or antigenemia (Figure 1B). CMV disease before day 100 was significantly higher in CMV seropositive recipients of PBSC

(P=0.03) compared to BM (Figure 1C). There was no difference in virologic parameters relative to donor CMV serostatus (Table 2).

Seronegative recipients—There was a trend towards more primary infection in patients that received PBSC than those who received BM (Table 2). In seronegative recipients of seronegative PBSC or BM, infection rates were low and not statistically different (Table 2). There was no CMV disease among seronegative recipients.

Time to cessation of antigenemia after ganciclovir early treatment—There was no statistically significant difference based on transplant type in the time to cessation of pp65 antigenemia or PCR positivity after the start of preemptive therapy in the subset of Fred Hutchinson Cancer Research Center patients (data not shown).

Analysis of CMV-specific T cells in donor PBMC before and in the PBSC product after G-CSF treatment

We measured the number of CMV-specific T cells by LDA in the peripheral blood of PBSC donors prior to G-CSF treatment and in the stem product after G-CSF treatment. Interestingly, there were significantly fewer (p=.04) CMV-specific T helper cells after G-CSF treatment (Figure 2). In addition, phytohemagglutin (PHA) stimulation also resulted in significantly less lymphocyte proliferation (p=0.005, Wilcoxon matched pairs test) in the post G-CSF stem cell product compared with pre-GCSF PBMC (data not shown). These results suggest that G-CSF mobilization either resulted in a decline in the number of CMV specific CD4+ T helper cells in the stem cell product or altered their ability to respond to antigen stimulation, either of which could contribute to delayed recovery of CMV-specific immune responses observed in PBSC recipients early after transplantation.

Analysis of CMV-specific immune reconstitution in BM and PBSC recipients

$CD8^+$ and $CD4^+$ CMV-specific T cell reconstitution was evaluated at days 30, 80, 180, and 365 post transplantation by cytotoxicity assay (CRA) and limiting dilution assay (LDA). In addition, based on immunocompetent donors (N=41) LDA results, ≥ 33 (25^{th} percentile) CMV-specific $CD4^+$ T cells per 1 $\times 10^6$ PBMC was established a threshold for LDA (Range 13.91 to 6422.61, Median 76.92, 75^{th} percentile 258.61 per 1 $\times 10^6$ PBMC).

The proportion of BM recipients that exhibited CMV-specific lysis greater than 10% at day 30 after transplantation was higher than in PBSC recipients (Figure 3A). This difference did not reach statistical significance, however (P=0.17, Fisher's exact test), and the numerical difference was no longer present by day 80 after transplant. A trend toward higher a proportion (P=0.24, Fisher's exact test) of patient's with ≥ 33 CMV specific $CD4^+$ T cells was documented by LDA at day 30 and day 80 in BM compared to PBSC recipients (Figure 3B). Collectively, these results suggest that despite the higher T cell dose administered to PBSC recipients, CMV-specific T cell responses as measured by T cell proliferation and cytolytic function assays may have been enhanced in BM recipients.

Cytokine production and degranulation by CMV-specific CD8+ T cells after PBSC and BM transplant

We used multiparameter flow cytometry combined with cytokine staining to directly evaluate the presence and function of CMV-specific $CD8^+$ T cells in blood samples that were obtained at days 30 and 80 post transplantation in a subset of PBSC and BM recipients (D+/R+) with available PBMCs. Cryopreserved PBMC were thawed, stimulated with CMVpp65 antigen, and then evaluated for IFNγ, MIP-1β, TNFα and CD107a expression by intracellular staining. No statistical differences were documented in the absolute number of

CMV-specific $CD8^+$ T cells between BM and PBSC recipients at either day 30 or 80 post transplantation (Figure 4 A & B).

Comparison of polyfunctional CMV-specific $CD8^+$ T cells in BM and PBSC recipients

Analyses of intracellular cytokine data using PESTLE and SPICE software allows for the further characterization of T cells as polyfunctional based on concurrent expression of different combinations of cytokines. Sixteen combinations were possible for $CD8^+$ T cells based on the evaluation of four parameters -- IFNγ, MIP-1β, TNFα and CD107a in this study. We grouped polyfunctional T cells as T cells positive for four, three, two or only one of these markers.

At day 30 after transplantation, the overall proportion of the absolute number of polyfunctional CMV-specific $CD8^+$ T cells per liter- positive for 2, 3 and 4 markers in BM was 18%, 20%, 27% compared to 6%, 8%, and 8%, respectively, for PBSC recipients. Single cytokine-producing T cells encompassed the largest proportion of T cells in PBSC recipients at day 30- post transplantation, and this fraction was significantly greater than the amount of single cytokine-producing $CD8^+$ T cells per liter in BM recipients (PBSC, 77% (6.1×10^7 cells per L) versus BM, 36% (6.8×10^6 cells per L) (P=0.006, Student's t-test, Figure 4C). However, by day 80, the proportion of polyfunctional CMV-specific $CD8^+$ T cells was similar between BM and PBSC recipients (Figure 4D).

Beyond single cytokine producing T cells there was no difference in the absolute number of CMV specific $CD8^+$ T cells per liter- positive for 2, 3 and 4 markers between BM and PBSC recipients (data not shown). This lack of quantitative difference prompted us to evaluate of the quality of T cell response rather than the quantity alone.

We analyzed the median fluorescence intensities (MFI) of each marker in quadruple-positive CMV-specific $CD8^+$ T cells to look for any differences in the capacity to produce cytokines or degranulate (29, 30). At day 30, we documented a trend towards higher MFI values for IFNγ, MIP-1β and CD107a, but not TNFα, in BM compared to PBSC recipients. These differences were not significant, however, and were lost by day 80 (data not shown). We further analyzed the effect of polyfunctionality on the MFI values of IFNγ based on the degree of decreasing polyfunctionality of CMV-specific $CD8^+$ T cells by the loss of one marker (TNFα, MIP-1β and CD107a). At day 30, the MFI values of IFNγ in CMV specific $CD8^+$ T cells from BM recipients decreased along with the loss of polyfunctionality compared to CMV specific $CD8^+$ T cells from PBSC recipients, which increased along with the loss of polyfunctionality (Figure 4E). At day 80, MFI values of IFNγ in CMV specific $CD8^+$ T cells from both BM and PBSC recipients decreased with loss of polyfunctionality (Figure 4F). Though not statistically significant these differences in MFI values hint at the complexity of polyfunctional profiles in CMV-specific $CD8^+$ T cells isolated from BM and PBSC recipients. Overall, these observations support the notion that a delay in CMV immune reconstitution early post transplantation in PBSC recipients is transient and associated not just with T cell quantity, but rather with the quality of T response.

Comparison of polyfunctional T cells between patients with no antigenemia and those with antigenemia

To determine the potential functional relevance of mono versus polyfunctional CMV-specific T cells we further compared the absolute number of polyfunctional CMV specific $CD8^+$ T cells at day 30 in patients with and without subsequent antigenemia, regardless of transplant type. In this analysis, we defined polyfunctional T cells as any T cell positive for two or more markers. In patients with no antigenemia by day 80, the average number of polyfunctional T cells at day 30 was 2.14×10^7 per L compared to 4.6×10^6 per L in patients

with any antigenemia (P=0.09, Student's t-test) (Figure 5A). In addition, a similar trend was documented between the average numbers of CMV specific $CD4^+$ T cells in patients with no antigenemia (1.8×10^5 cells per L) compared to those with any antigenemia (5.7×10^3 cells per L) by LDA analysis (P=0.3, Student's t-test)(Figure 4B). These results suggest that regardless of stem cell source, polyfunctional T cells are associated with protection from CMV reactivation.

Discussion

In this study, we compared the temporal pattern of CMV reactivation, CMV disease and CMV-specific immune reconstitution in patients enrolled in a randomized trial of BM or PBSC as a graft source for transplantation. We observed a higher incidence of CMV infection and CMV disease early after transplantation in PBSC recipients. We hypothesized that the differences in virologic data resulted from differences in the reconstitution of a CMV-specific immune response between BM and PBSC recipients after transplantation.

In contrast to the findings in non-randomized studies, we observed a higher rate of CMV infection and disease in PBSC recipients. Walker et al. showed a delay in the development of CMV antigenemia in PBSC recipients, but no overall difference in the incidence of antigenemia between BM and PBSC recipients (31). Trenschel et al. reported a similar incidence of CMV antigenemia, but less persistent antigenemia and a trend towards less CMV disease in PBSC recipients (32). On the other hand, Manteiga et al. found higher rates of CMV infection and disease in PBSC recipients (33), which is in agreement with our findings. Possible reasons for these differences include different proportions of unrelated donors, use of T cell depletion in some studies, high-dose acyclovir prophylaxis, and differences in the patient populations in terms of age, disease status and prior therapy across these studies.

Our results stem from a randomized study with a well-balanced distribution of pre- and post-transplant risk factors for CMV (Table 1). The increase in early CMV reactivation that we observed in PBSC recipients was not explained by earlier onset of GvHD or an increased use of corticosteroids. Therefore, it is theoretically possible that the increase of CMV reactivation resulted from higher rates of CMV transfer in the donor PBSC product, perhaps within granulocytes or hematopoietic progenitors. Granulocyte transfusions have been associated with an increased risk of CMV infection (34-36). An alternative and not mutually exclusive explanation for the differences in CMV infection and disease is that G-CSF treatment promotes the emergence of CMV from latency, either by direct effects of G-CSF signaling in latently infected cells or by reducing the ability of T cells to limit reactivation (37). Heightened transfer of CMV is partially supported by a trend towards a higher transmission rate in the CMV D+/R- setting, but not among R+ patients (Table 2). However, the number of D+/R- patients in this cohort was too small and the CMV event rate in the D +/R- BM recipients was unusually low (38), making it difficult to test this possibility statistically. No definitive conclusions can be drawn and larger studies are needed to more thoroughly examine this question.

Mielcarek et al. proposed that monocytes in G-CSF-treated PBMCs are capable of inhibiting T cell proliferation, possibly resulting in lower GVHD following HSCT (39, 40). We did not find any association between the number of monocytes and CMV-specific $CD4^+$ T cells after G-CSF treatment (data not shown). We did observe, however, that G-CSF treatment lowered the number of CMV specific $CD4^+$ T cells in the donor product detected by a functional assay that requires T cell proliferation. Our proposal that a deficiency of functional CMV specific $CD4^+$ T cells in PBSC contributes to the delayed recovery of immunity in PBSC recipients is supported by work done by Pourgheysari et al., who

demonstrated that early post-transplant deficiencies in CMV specific $CD4^+$ T cells are associated with a high risk of viral reactivation in patients following allogeneic HSCT (90% of the patients in that study were PBSC recipients) (41). Impairment of both $CD4^+$ and $CD8^+$ T cells prior to 100 days after transplantation was also associated with late viral reactivation in that study (41). A plausible explanation for the virologic differences observed in our study is impairment of the quantity or quality of CMV-specific T cells early after PBSC transplantation. At day 30 post transplantation in PBSC recipients, we documented (a) fewer CMV specific $CD4^+$ T cells, (b) less CMV specific cell lysis, (c) a greater proportion of $CD8^+$ CMV-specific T cells that mainly produced a single cytokine and (d) a lower proportion of polyfunctional T cells together with a lower capacity for cytokine production and cytotoxicity.

The correlation of polyfunctional T-cells with control of viral infection was first studied in HIV infection (42). Betts et al. demonstrated that individuals with nonprogressive HIV infection maintain polyfunctional HIV-specific $CD8^+$ T cells that correlate inversely with viral load. Similarly, Darrah et al. demonstrated that protection against *Lesihmani major* infection in mice is associated with polyfunctional $CD4^+$ T cells with enhanced effector function (30). Polyfunctional T cells are also known to have a higher capacity for cytokine production and cytotoxicity compared to single-cytokine producing T cells (29). When we compared CMV-specific T cell function between patients with no antigenemia versus those with antigenemia, we found a trend towards larger number of polyfunctional T cells in patients with no antigenemia. These observations further support the association of polyfunctional T cells with protective immunity.

It is important to recognize that our polyfunctional studies were done on a small number of patients due to limited sample availability. Because of the resulting low statistical power for many of the comparisons, we have primarily noted the trends we observed. Our findings are consistent, however, with a prior study by Lilleri et al. who also found that polyfunctional CMV-specific T cells producing IFNγ and IL-2 were associated with protection (43). In addition, the increase of MFI values of IFNγ with decreased CMV-specific $CD8^+$ polyfunctionality in PBSC recipients at day 30, aligns with the work of Krol et al., who suggested that production of IFNγ alone may be a sign of T cell exhaustion during viral infection (44). We recently showed, using CMV-specific tetramers, that HCT patients with ≥ 7 positive tetramer cells per mL in at least one blood sample before day 65 post-transplant were statistically protected from CMV infection (45), although the protection was not complete. Polyfunctional T cells may provide an improved functional marker of protection; but larger prospective studies are needed to demonstrate this conclusively.

In conclusion, PBSC recipients early after post transplantation demonstrate more CMV reactivation and disease. These findings appear to be temporally related to a transient delay in CMV-specific immune reconstitution, perhaps as a consequence of G-CSF treatment. Whether, T cell polyfunctionality is a definitive marker of protection from CMV reactivation requires larger prospective studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Kristen White, Patrick Sudour and Jennifer Cullison for laboratory assistance, Chris Davis for database services, and Cara Varley for chart review.

Grant support: The work was supported by the following NIH grants: CA18029, CA15704, HL 93294, AI053193; CA049605, CA030206, AI058148, CA980416

References

1. Haspel RL, Miller KB. Hematopoietic stem cells: source matters. Curr Stem Cell Res Ther. 2008; 3:229–236. [PubMed: 19075753]
2. Bensinger WI, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med. 2001; 344:175–181. [PubMed: 11172139]
3. Bensinger WI, Clift R, Martin P, et al. Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: a retrospective comparison with marrow transplantation. Blood. 1996; 88:2794–2800. [PubMed: 8839878]
4. Storek J, Dawson MA, Storer B, et al. Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. Blood. 2001; 97:3380–3389. [PubMed: 11369627]
5. Ottinger HD, Beelen DW, Scheulen B, Schaefer UW, Grosse-Wilde H. Improved immune reconstitution after allotransplantation of peripheral blood stem cells instead of bone marrow. Blood. 1996; 88:2775–2779. [PubMed: 8839875]
6. Storek J, Witherspoon RP, Maloney DG, Chauncey TR, Storb R. Improved reconstitution of CD4 T cells and B cells but worsened reconstitution of serum IgG levels after allogeneic transplantation of blood stem cells instead of marrow. Blood. 1997; 89:3891–3893. [PubMed: 9160701]
7. Lacey SF, Diamond DJ, Zaia JA. Assessment of cellular immunity to human cytomegalovirus in recipients of allogeneic stem cell transplants. Biol Blood Marrow Transplant. 2004; 10:433–447. [PubMed: 15205665]
8. Widmann T, Sester U, Gartner BC, et al. Levels of CMV specific CD4 T cells are dynamic and correlate with CMV viremia after allogeneic stem cell transplantation. PLoS One. 2008; 3:e3634. [PubMed: 18982061]
9. Boland GJ, Vlieger AM, Ververs C, De Gast GC. Evidence for transfer of cellular and humoral immunity to cytomegalovirus from donor to recipient in allogeneic bone marrow transplantation. Clin Exp Immunol. 1992; 88:506–511. [PubMed: 1318805]
10. Li CR, Greenberg PD, Gilbert MJ, Goodrich JM, Riddell SR. Recovery of HLA-restricted cytomegalovirus (CMV)-specific T-cell responses after allogeneic bone marrow transplant: correlation with CMV disease and effect of ganciclovir prophylaxis. Blood. 1994; 83:1971–1979. [PubMed: 8142663]
11. Kondo K, Xu J, Mocarski ES. Human cytomegalovirus latent gene expression in granulocyte-macrophage progenitors in culture and in seropositive individuals. Proc Natl Acad Sci U S A. 1996; 93:11137–11142. [PubMed: 8855322]
12. Kondo K, Kaneshima H, Mocarski ES. Human cytomegalovirus latent infection of granulocyte-macrophage progenitors. Proc Natl Acad Sci U S A. 1994; 91:11879–11883. [PubMed: 7991550]
13. Di Rosa F, Pabst R. The bone marrow: a nest for migratory memory T cells. Trends Immunol. 2005; 26:360–366. [PubMed: 15978522]
14. Blache C, Chauvin JM, Marie-Cardine A, et al. Reduced frequency of regulatory T cells in peripheral blood stem cell compared to bone marrow transplantations. Biol Blood Marrow Transplant. 16:430–434. [PubMed: 19883775]
15. Tayebi H, Tiberghien P, Ferrand C, et al. Allogeneic peripheral blood stem cell transplantation results in less alteration of early T cell compartment homeostasis than bone marrow transplantation. Bone Marrow Transplant. 2001; 27:167–175. [PubMed: 11281386]
16. Chattopadhyay PK, Hogerkorp CM, Roederer M. A chromatic explosion: the development and future of multiparameter flow cytometry. Immunology. 2008; 125:441–449. [PubMed: 19137647]
17. Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. Nat Rev Immunol. 2008; 8:247–258. [PubMed: 18323851]

18. Zhou W, Longmate J, Lacey SF, et al. Impact of donor CMV status on viral infection and reconstitution of multifunction CMV-specific T cells in CMV-positive transplant recipients. Blood. 2009; 113:6465–6476. [PubMed: 19369230]
19. Nash RA, Pepe MS, Storb R, et al. Acute graft-versus-host disease: analysis of risk factors after allogeneic marrow transplantation and prophylaxis with cyclosporine and methotrexate. Blood. 1992; 80:1838–1845. [PubMed: 1391947]
20. Landry ML, Ferguson D, Stevens-Ayers T, de Jonge MW, Boeckh M. Evaluation of CMV Brite kit for detection of cytomegalovirus pp65 antigenemia in peripheral blood leukocytes by immunofluorescence. J Clin Microbiol. 1996; 34:1337–1339. [PubMed: 8727936]
21. Boeckh M, Bowden RA, Gooley T, Myerson D, Corey L. Successful modification of a pp65 antigenemia-based early treatment strategy for prevention of cytomegalovirus disease in allogeneic marrow transplant recipients. Blood. 1999; 93:1781–1782. [PubMed: 10084817]
22. Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood. 1996; 88:4063–4071. [PubMed: 8916975]
23. Nichols WG, Price TH, Gooley T, Corey L, Boeckh M. Transfusion-transmitted cytomegalovirus infection after receipt of leukoreduced blood products. Blood. 2003; 101:4195–4200. [PubMed: 12531791]
24. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis. 2002; 34:1094–1097. [PubMed: 11914998]
25. Flowers ME, Parker PM, Johnston LJ, et al. Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: long-term follow-up of a randomized trial. Blood. 2002; 100:415–419. [PubMed: 12091330]
26. Koelle DM, Schomogyi M, Corey L. Antigen-specific T cells localize to the uterine cervix in women with genital herpes simplex virus type 2 infection. J Infect Dis. 2000; 182:662–670. [PubMed: 10950757]
27. Taswell C. Limiting dilution assays for the determination of immunocompetent cell frequencies. I. Data analysis. J Immunol. 1981; 126:1614–1619. [PubMed: 7009746]
28. Clouse KA, Adams PW, Orosz CG. Enumeration of viral antigen-reactive helper T lymphocytes in human peripheral blood by limiting dilution for analysis of viral antigen-reactive T-cell pools in virus-seropositive and virus-seronegative individuals. J Clin Microbiol. 1989; 27:2316–2323. [PubMed: 2555391]
29. Kannanganat S, Ibegbu C, Chennareddi L, Robinson HL, Amara RR. Multiple-cytokine-producing antiviral CD4 T cells are functionally superior to single-cytokine-producing cells. J Virol. 2007; 81:8468–8476. [PubMed: 17553885]
30. Darrah PA, Patel DT, De Luca PM, et al. Multifunctional TH1 cells define a correlate of vaccine-mediated protection against Leishmania major. Nat Med. 2007; 13:843–850. [PubMed: 17558415]
31. Walker CM, van Burik JA, De For TE, Weisdorf DJ. Cytomegalovirus infection after allogeneic transplantation: comparison of cord blood with peripheral blood and marrow graft sources. Biol Blood Marrow Transplant. 2007; 13:1106–1115. [PubMed: 17697973]
32. Trenschel R, Ross S, Husing J, et al. Reduced risk of persisting cytomegalovirus pp65 antigenemia and cytomegalovirus interstitial pneumonia following allogeneic PBSCT. Bone Marrow Transplant. 2000; 25:665–672. [PubMed: 10734302]
33. Manteiga R, Martino R, Sureda A, et al. Cytomegalovirus pp65 antigenemia-guided pre-emptive treatment with ganciclovir after allogeneic stem transplantation: a single-center experience. Bone Marrow Transplant. 1998; 22:899–904. [PubMed: 9827819]
34. Meyer-Koenig U, Hufert FT, Duffner U, Neumann-Haefelin D, Henschen M. G-CSF-mobilised granulocyte transfusion to an ALL patient complicated by cytomegalovirus transmission. Bone Marrow Transplant. 2004; 34:1095–1096. [PubMed: 15502855]
35. Nguyen Q, Estey E, Raad I, et al. Cytomegalovirus pneumonia in adults with leukemia: an emerging problem. Clin Infect Dis. 2001; 32:539–545. [PubMed: 11181115]

36. Nichols WG, Price T, Boeckh M. Donor serostatus and CMV infection and disease among recipients of prophylactic granulocyte transfusions. Blood. 2003; 101:5091–5092. author reply 5092. [PubMed: 12788795]
37. Smith MS, Goldman DC, Bailey AS, et al. Granulocyte-colony stimulating factor reactivates human cytomegalovirus in a latently infected humanized mouse model. Cell Host Microbe. 8:284–291. [PubMed: 20833379]
38. Nichols WG, Corey L, Gooley T, Davis C, Boeckh M. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. J Infect Dis. 2002; 185:273–282. [PubMed: 11807708]
39. Mielcarek M, Graf L, Johnson G, Torok-Storb B. Production of interleukin-10 by granulocyte colony-stimulating factor-mobilized blood products: a mechanism for monocyte-mediated suppression of T-cell proliferation. Blood. 1998; 92:215–222. [PubMed: 9639519]
40. Mielcarek M, Martin PJ, Torok-Storb B. Suppression of alloantigen-induced T-cell proliferation by CD14+ cells derived from granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells. Blood. 1997; 89:1629–1634. [PubMed: 9057645]
41. Pourgheysari B, Piper KP, McLarnon A, et al. Early reconstitution of effector memory CD4+ CMV-specific T cells protects against CMV reactivation following allogeneic SCT. Bone Marrow Transplant. 2009; 43:853–861. [PubMed: 19104497]
42. Betts MR, Nason MC, West SM, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood. 2006; 107:4781–4789. [PubMed: 16467198]
43. Lilleri D, Fornara C, Chiesa A, Caldera D, Alessandrino EP, Gerna G. Human cytomegalovirus-specific CD4+ and CD8+ T-cell reconstitution in adult allogeneic hematopoietic stem cell transplant recipients and immune control of viral infection. Haematologica. 2008; 93:248–256. [PubMed: 18245650]
44. Krol L, Stuchly J, Hubacek P, et al. Signature profiles of CMV-specific T-cells in patients with CMV reactivation after hematopoietic SCT. Bone Marrow Transplant. 2010 Nov 8. Epub ahead of print.
45. Gratama JW, Boeckh M, Nakamura R, et al. Immune monitoring with iTAg MHC Tetramers for prediction of recurrent or persistent cytomegalovirus infection or disease in allogeneic hematopoietic stem cell transplant recipients: a prospective multicenter study. Blood. 116:1655–1662. [PubMed: 20508161]

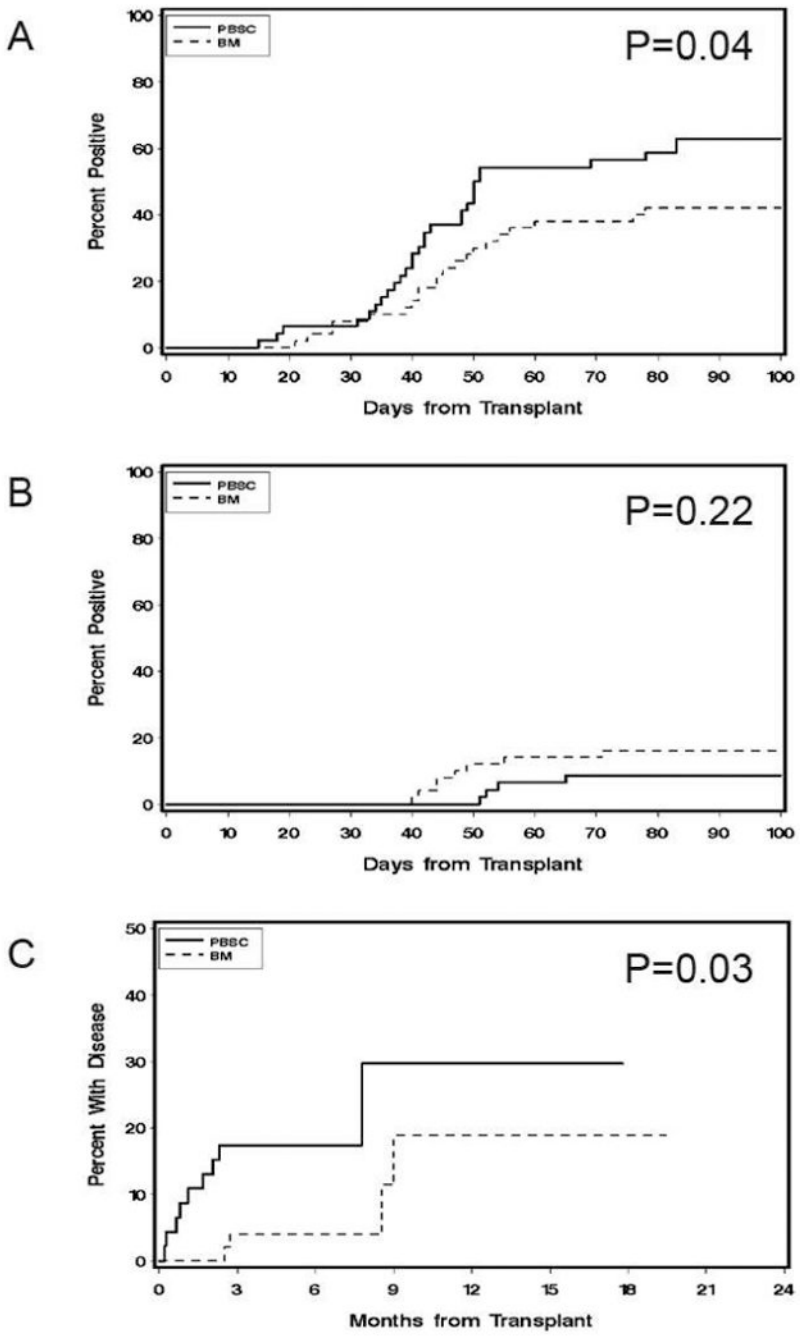

Figure 1. Differences in CMV infection and disease between BM and PBSC recipients

Time to positive antigenemia or PCR positivity at any level (A), antigenemia > 100 positive cells per slide or CMV DNA>100,000 copies per mL plasma (B) and CMV disease (C) among CMV seropositive recipients (BM n= 50, PBSC n=46).

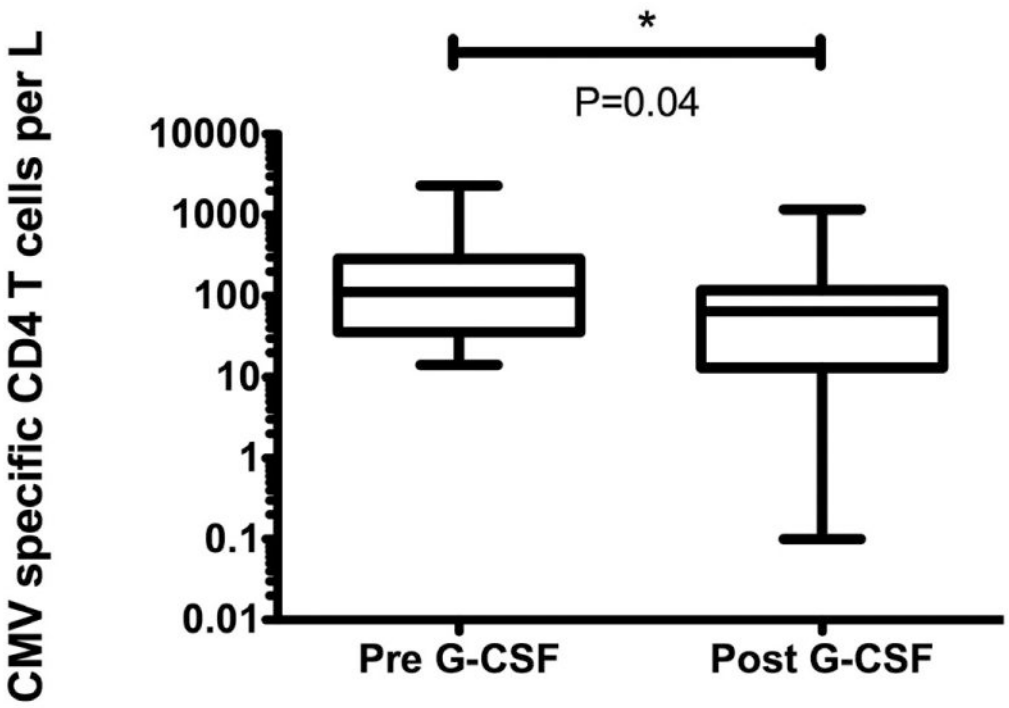

Figure 2. Effect of G-CSF on donor peripheral blood mononuclear cells (PBMC) and stem cell (SC) product

Figure (A) represents the number of CMV-specific $CD4^+$ T helper cells in donor PBMC and the stem cell product, pre and post G-CSF treatment (n=12). P value is from the Wilcoxon matched pairs test.

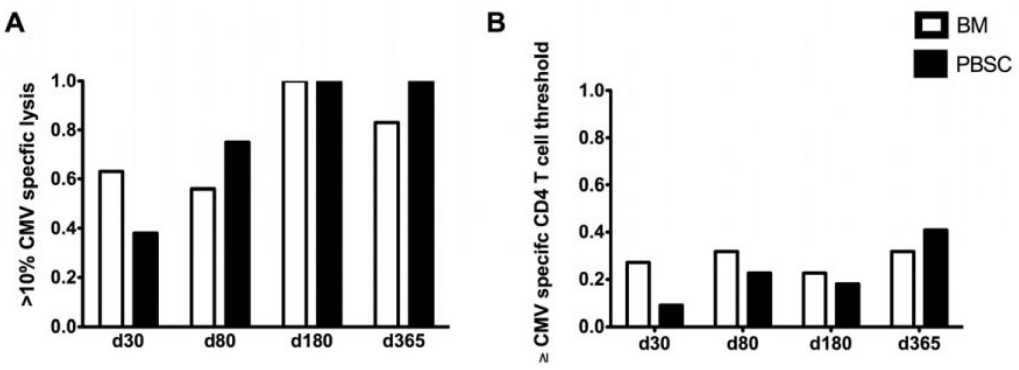

Figure 3. Differences in CMV-specific T cell immune reconstitution between BM and PBSC recipients

Figure (A) represents the proportion of patients with CMV-specific cell lysis greater than 10% between BM and PBSC recipients at day 30 (BM n= 8, PBSC n= 8), 80 (BM n= 9, PBSC n= 8), 180 (BM n= 6, PBSC n= 7) and 365 (BM n= 6, PBSC n= 4) post-transplant. Figure (B) represents the proportion of patients with ≥ 33 CMV-specific $CD4^+$ T cells per 10^6 PBMC as determined by limiting dilution assay at day 30, 80, 180 and 365 (BM n= 22, PBSC n= 22) post-transplant.

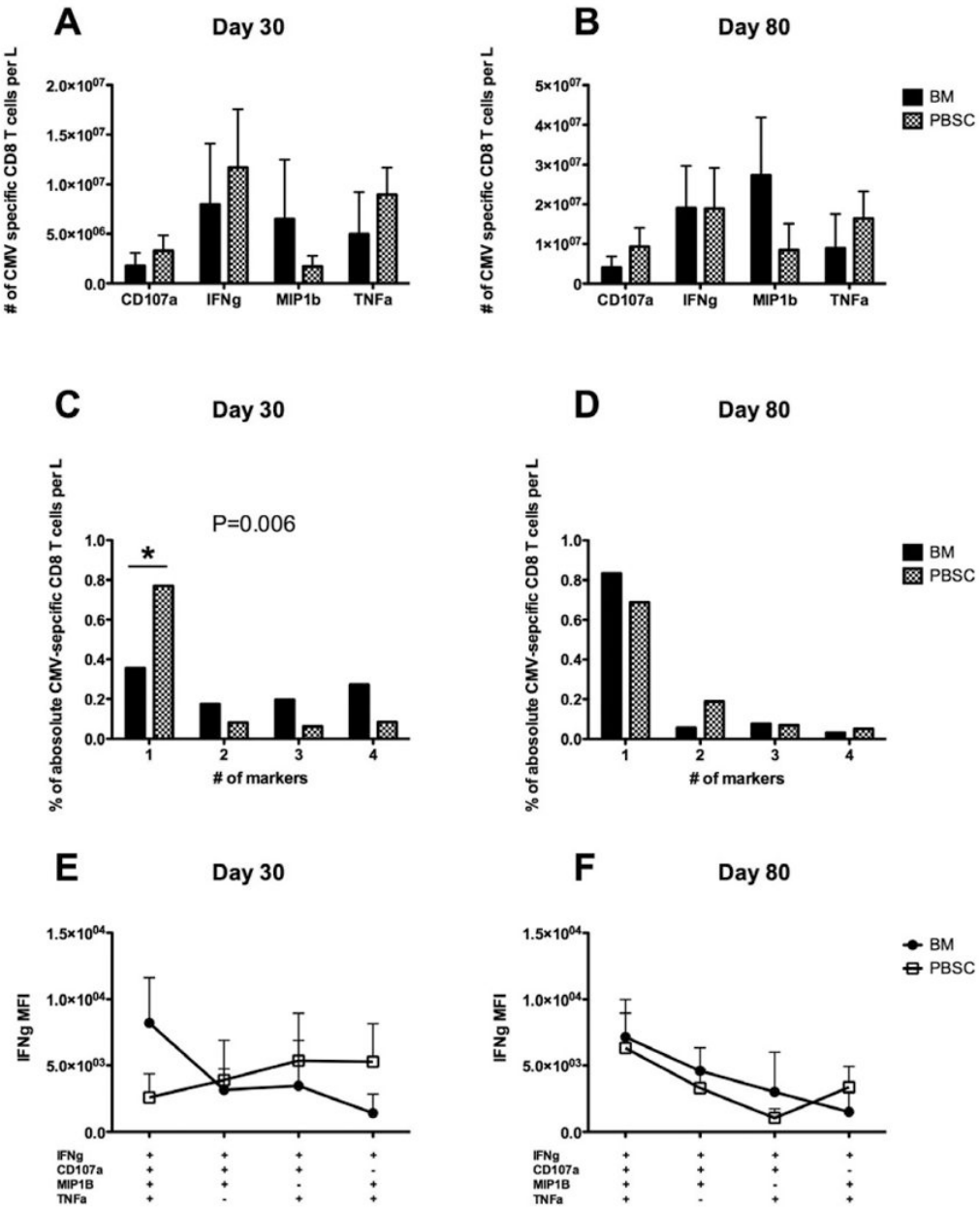

Figure 4. Differences in polyfunctional CMV-specific CD8$^+$ T cells between BM and PBSC recipients

Figures (A & B) represent the absolute number of CD8$^+$ T cells in BM (n=7†) and PBSC (n=6†) recipients positive for CD107a, IFNγ, MIP-1β and TNFα at d30 and d80 post transplantation. Figures (C & D) represent the absolute proportion of polyfunctional CMV-specific CD8$^+$ T cells within BM and PBSC recipients at day 30 and 80. Figures (E & F) represent the MFI of IFNγ based on the degree of decreasing polyfunctionality of CMV-specific CD8$^+$ T cells by the loss of one marker (CD107a, MIP-1β and TNFα). Polyfunctional T cells grouped by T cells positive for 1, 2, 3, or 4 of the markers based on the 16 possible combinations according to SPICE. P value is a result of the student's t test. Bars represent the mean value within each group and error bars are the standard error of the mean.

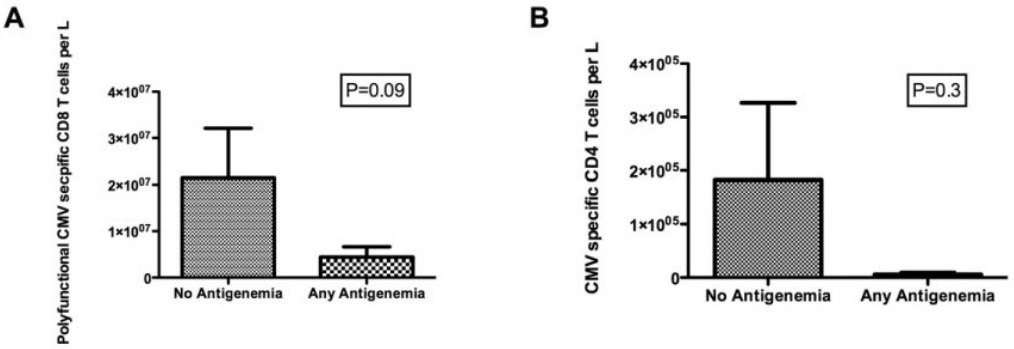

Figure 5. Comparison of CMV-specific polyfunctional CD8$^+$ T cells between patients with no antigenemia and those with antigenemia

Figure (A) represents the number of polyfunctional T cells per L at day 30 in patients with no (n=5†) and any subsequent antigenemia (n=7†) at day 30 regardless of stem cell source. Figure (B) represents the number of CMV-specific CD4 T cells per L (by LDA) at day 30 in patients without (n=20†) and any subsequent antigenemia (n=11†) †Includes patients that were eligible for randomization but did not participate in the randomized trial. Bars represent the mean value within each group and error bars are the standard error of the mean.

Table 1

Patient characteristics

Characteristic	BM	PBSC
	N=91	N=81
Recipient age (median, range)	42 (12-55)	42 (15-55)
Recipient sex (male/female)	62/29	56/25
Underlying disease status		
Less advanced	51 (56%)	41 (51%)
More advanced	40 (44%)	40 (49%)
CMV serostatus		
Recipient positive/donor positive	36 (40%)	29 (36%)
Recipient positive/donor negative	14 (15%)	17 (21%)
Recipient negative/donor positive	14 (15%)	15 (18%)
Recipient negative/donor negative	27 (30%)	20 (25%)
Acute GvHD by day 100		
Grade 2-4	64%	57%
Grade 3-4	12%	15%
Chronic clinical extensive GvHD at 3 years	52%	63%

*Analysis of CMV virologic endpoints was performed in all randomized patients; analysis of CMV immune reconstitution was performed in a subset that agreed to additional blood draws and skin biopsies.

Table 2

Incidence of CMV infection and disease before 2 years by CMV serostatus.

Manifestation of CMV	BM					PBSC				
	R+ N=50	D+/R+ N=36	D-/R+ N=14	D+/R-N=13	D-/R-N=27	R+ N=46	D+/R+ N=29	D-/R+ N=17	D+/R-N=15	D-/R-N=20
pp65 antigenemia or CMV DNA in plasma (day 100)										
Any level	42%*	42%	43%	0**	7%	63%*	59%	71%	19%**	10%
≥ 10 + cells/slides or > 1000 copies per mL	24%	25%	21%	0	0	26%	31%	18%	19%	5%
≥ 50 + cells/slides or > 10,000 copies per mL	16%	17%	14%	0	0	9%	14%	0	0	%%
CMV disease										
• Day 100 after HCT	4%†	3%	7%	0	0	17%†	14%	24%	0	0
• Two years after HCT	8%	6%	14%	0	0	20%	17%	24%	6%	0

R, recipient; D, donor

*P = 0.04, between BM and PBSC recipients that are R+

**P = 0.03, between BM and PBSC recipients that are D+/R-

†P = 0.03, between BM and PBSC recipients that are R+