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Cytomegalovirus-Specific Cytotoxic T Lymphocytes Can Be Efficiently Expanded from Granulocyte Colony-Stimulating Factor–Mobilized Hemopoietic Progenitor Cell Products Ex Vivo and Safely Transferred to Stem Cell Transplantation Recipients to Facilitate Immune Reconstitution

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Uncontrolled cytomegalovirus (CMV) reactivation after allogeneic hematopoietic stem cell transplantation causes significant morbidity and mortality. Adoptive transfer of CMV-specific cytotoxic T lymphocytes (CTLs) is a promising therapy to treat reactivation and prevent viral disease. In this article, we describe the generation of clinical-grade CMV-specific CTLs directly from granulocyte colony-stimulating factor–mobilized hemopoietic progenitor cell (G-HPC) products collected for transplantation. This method requires less than 2.5% of a typical G-HPC product to reproducibly expand CMV-specific CTLs ex vivo. Comparison of 11 CMV CTL lines generated from G-HPC products with 52 CMV CTL lines generated from nonmobilized peripheral blood revealed similar expansion kinetics and phenotype. G-HPC–derived CTLs produced IFN-γ after reexposure to CMVpp65 antigen and exhibited CMV-directed cytotoxicity but no alloreactivity against transplantation recipient–derived cells. Seven patients received CMV-specific CTL lines expanded from G-HPC products in a prophylactic adoptive immunotherapy phase I/II clinical trial. Use of G-HPC products will facilitate integration of CTL generation into established quality systems of transplantation centers and more rapid inclusion of T cell therapies into routine clinical care.

INTRODUCTION

Human cytomegalovirus (CMV) is a herpesvirus that infects more than 50% of the population [1]. Primary infection is generally self-limiting, but results in the establishment of a life-long latent infection subsequently controlled by a robust innate and adaptive cell-mediated immune response [2-6]. CMV reactivation occurs frequently after hematopoietic stem cell transplantation (HSCT), and if uncontrolled, can cause significant morbidity and mortality. Clinical complications include myelosuppression, encephalitis, retinitis, colitis, and pneumonia [5,7-9]. To prevent CMV-related disease, transplantation centers continually monitor viral load to guide the use of preemptive antiviral therapy [10]. Ganciclovir and foscarnet are effective but can cause side effects, including myelosuppression, renal toxicity, and increased risk of bacterial and fungal infections [11,12]. Additional concerns are the emergence of antiviral-resistant strains and onset of late CMV disease [13,14].

The inability of HSCT recipients to adequately control viral replication is related to deficits in cell-mediated immunity, particularly the delayed reconstitution of CMV-specific $CD8^+$ and $CD4^+$ T cells after transplantation [15,16]. Several previous investigators have conducted clinical trials with the aim of rapidly restoring CMV immunity in allogeneic HSCT recipients through the adoptive transfer of CMV-specific cytotoxic T lymphocytes (CTLs) isolated and expanded ex vivo from the blood of healthy donors [17-27]. Initial studies established the feasibility of this approach by infusing CMV-specific T cell clones into patients after HSCT [20]. CMV immunity improved after infusion in a dose-dependent manner, and TCR rearrangements consistent with the infused CTLs could be detected for up to 12 weeks by PCR [20]. Manufacturing the CTL products proved technically difficult and labor-intensive, however, requiring cloning by limiting dilution. Subsequent studies consistently demonstrated the safety of infusing allogeneic CMV-specific T cells with promising clinical outcomes, although direct comparison of these studies is difficult, considering the significant variability in the composition of T cell products, CMV epitopes targeted, method of manufacture, time of infusion, cell dose, and risk of CMV disease. Ultimately, the inclusion of adoptive immunotherapy into routine clinical care will require efficient methods for CTL generation and

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integration of patient and donor management into existing transplantation programs.

To date, clinical trials using ex vivo-expanded virus-specific T cells, including trials conducted at our own center, have used blood collected from donors before starting granulocyte colony-stimulating factor (G-CSF) to mobilize hemopoietic progenitor cells (HPCs). Our institutional experience has been that obtaining blood products from donors before transplantation is often difficult for a range of regulatory, logistic, and geographic reasons. Furthermore, patients with unrelated donors collected overseas have been ineligible for immunotherapy owing to the complexities of obtaining and transporting the separate blood donation before mobilization. A solution to this problem is to expand virus-specific T cells directly from G-CSF–mobilized HPC (G-HPC) apheresis products using cells in excess of the amount required for transplantation. This would have the additional benefit of using well-established transplantation center procedures for donor assessment, infectious disease testing, product collection, labeling, and transport to the processing facility.

Despite the higher T cell content of G-HPC products compared with bone marrow, initial clinical studies revealed no increased risk of acute graft-versus-host disease (GVHD) after allogeneic HSCT [28-32]. More recently, transplantation with G-HPC products has been associated with a greater incidence of chronic GVHD but also improved engraftment kinetics and leukemia-free survival [33-35]. The mechanisms underlying the lack of acute GVHD exacerbation are unclear but may be related to the immunomodulatory effects of G-CSF on antigen-presenting cells or T cells [36-38]. In vitro, T cells exposed to G-CSF have decreased proliferative and Th1 cytokine-producing capacity in response to mitogenic stimulus [39-41]. Whether this alteration of global T cell responses also affects virus-specific memory T cells is unknown.

In the present study, we examined the feasibility of generating CMV CTL lines directly from G-CSF–mobilized donors and validated a method requiring only 2 to 4 mL of a typical G-HPC collection (on average, 1%-3% of the total collection) to reproducibly enrich CMV-specific T cells in culture. We compared the expansion kinetics and functionality of these cells and of CTL lines generated from nonmobilized peripheral blood (NMPB). Seven patients received CMV-specific CTL lines expanded from G-HPC products in a prophylactic adoptive immunotherapy phase I/II clinical trial.

METHODS

Participant Details and Eligibility Criteria

Patients who underwent allogeneic HSCT for any hematologic malignancy at Westmead Hospital between October 2006 and July 2011, as well as their HLA-matched (-A, -B, and -DR loci) or 1 antigen-mismatched related or unrelated CMV-seropositive donors, were eligible for inclusion in this study. Participants were recruited from the pool of eligible donor–recipient pairs based on the availability of donors to provide peripheral blood before initiation of G-CSF mobilization and/or the laboratory's capacity for CTL line generation. CTL lines were generated from donors enrolled in a phase I/II clinical trial of prophylactic adoptive transfer of donor-derived CMV-specific T cells. This study was approved by the Human Research Ethics Committees of Westmead Hospital, the University of Sydney, and the Australian Bone Marrow Donor Registry. Informed consent was obtained from all donors and recipients before enrollment, in accordance with the Declaration of Helsinki. The study has been registered on the Australian Clinical Trial Registry (ACTRN12605000213640 and ACTRN12607000224426).

CMV-Specific T Cell Generation

T cell products were generated from approximately 100 mL of peripheral blood collected by venesection from donors before G-CSF mobilization or a proportion of the G-HPC apheresis product. For G-HPC products, absolute $CD34^+$ cells were enumerated using an in-house single-platform viable CD34 flow cytometric assay, as described previously [42,43]. If the $CD34^+$ HPC content exceeded 2.5×10^6 $CD34^+$cells/kg, a proportion of the collection was removed for CTL generation (Table 1). The same method, except for the starting volume of donor product, was used to generate CMV CTLs from NMPB or G-HPC products. In brief, samples were diluted in PBS supplemented with 0.2% human albumin (Albumex 20; CSL, Parkville, Australia) and peripheral blood mononuclear cells (PBMCs) isolated by density-gradient centrifugation using Ficoll-Paque Premium (GE Healthcare, Waukesha, WI). Monocytes were enriched by adherence and differentiated into dendritic cells (DCs) in CellGro DC medium (CellGenix, Freiburg im Breisgau, Germany) supplemented with 1,000 U/mL GM-CSF and 1,000 U/mL IL-4 for 5 to 6 days. DCs were supplemented with 200 U/mL TNF-α (CellGenix) to promote maturation and then transfected at a multiplicity of infection of 20:1 with a clinical-grade adenovirus vector (Ad5F35pp65) encoding the entire CMVpp65 protein (provided by C. Rooney, H. Heslop, and M. Brenner, Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX). Transfected and irradiated DCs were cocultured with autologous PBMCs in AIM V medium CTS (Life Technologies, Carlsbad, CA) with 10% heat-inactivated AB serum (from the Australian Red Cross Blood Service) or autologous plasma to initiate CMV-specific T cell expansion. After 7 days, cultures were restimulated with Ad5F35pp65-transfected DCs and supplemented with 20 U/mL IL-2 (Millipore, Billerica, MA, or Miltenyi Biotec, Bergisch Gladbach, Germany) for 7 days. IL-2 was increased to 50 U/mL for the final week of culture. At the completion of culture, cells were enumerated, washed once, and cryopreserved until use for infusion or quality assurance testing. Release criteria were >50% postthaw viability, <2% $CD14^+$ and <2% $CD19^+$ cells, <10% killing of recipient-derived phytohemagglutinin

Table 1
HPC collection details used for CTL generation

Patient*	Collection Volume, mL	$CD34^+$ Cells, $\times 10^6$/kg	Volume Removed, mL	% of Harvest Removed	TNCs Removed, $\times 10^9$	Reduction in $CD34^+$ Cells, $\times 10^6$/kg
1	245	2.7	4	1.63	1.0	0.04
2†	204	0.9	4	1.96	0.7	0.02
3	378	3.8	4	1.06	1.0	0.04
4	362	9.2	3	1.10	0.8	0.10
5	311	7.5	4	1.29	1.0	0.10
6	249	6.4	4	1.61	0.9	0.10
7	152	7.7	4	2.63	0.9	0.20
8	289	4.1	3	1.38	0.6	0.06
9	349	8.9	3	1.15	0.8	0.10
Donor 1	191	4.7	1.7	2.09	0.4	0.10
Donor 2	206	6.9	1.5	1.94	0.5	0.13
Mean	266.9	5.7	3.3	1.62	0.8	0.09
SD	76.2	2.7	0.9	0.49	0.2	0.05

TNC indicates total nucleated cells.

* CTLs from G-HPCs from donors 1 and 2 were generated for direct comparison with CTLs derived from NMPB.

† Details provided for removal of cells from day 2 collection; a total of 3.1×10^6 $CD34^+$ cells/kg were infused for transplantation.