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Immunotherapy with Donor T Cells Sensitized with Overlapping Pentadecapeptides for Treatment of Persistent Cytomegalovirus Infection or Viremia

Guenther Koehne [1,2,3,*], Aisha Hasan [2,4], Ekaterina Doubrovina [2,4], Susan Prockop [2,4], Eleanor Tyler [2,3], Gloria Wasilewski [4], Richard J. O'Reilly [2,3,4]

[1] Adult Bone Marrow Transplantation Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York
[2] Transplantation Biology Laboratory, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, New York
[3] Weill Cornell Medical College, New York, New York
[4] Bone Marrow Transplantation Service, Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, New York

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A B S T R A C T

We conducted a phase I trial of allogeneic T cells sensitized in vitro against a pool of pentadecapeptides (15-mer peptides) spanning the sequence of CMVpp65 for adoptive therapy of 17 allogeneic hematopoietic cell transplant recipients with cytomegalovirus (CMV) viremia or clinical infection persisting despite prolonged treatment with antiviral drugs. All but 3 of the patients had received T cell–depleted transplants without graft-versus-host disease (GVHD) prophylaxis with immunosuppressive drugs after transplantation. The CMVpp65-specific T cells (CMVpp65CTLs) generated were oligoclonal and specific for only 1 to 3 epitopes, presented by a limited set of HLA class I or II alleles. T cell infusions were well tolerated without toxicity or GVHD. Of 17 patients treated with transplant donor (n = 16) or third-party (n = 1) CMVpp65CTLs, 15 cleared viremia, including 3 of 5 with overt disease. In responding patients, the CMVpp65CTLs infused consistently proliferated and could be detected by T cell receptor V_β usage in CMVpp65/HLA tetramer + populations for period of 120 days to up to 2 years after infusion. Thus, CMVpp65CTLs generated in response to synthetic 15-mer peptides of CMVpp65 are safe and can clear persistent CMV infections in the post-transplantation period.

INTRODUCTION

Cytomegalovirus (CMV) infections remain a major cause of morbidity and mortality in allogeneic hematopoietic cell transplant (HCT) recipients [1,2]. Although prophylactic or preemptive treatment with ganciclovir or foscarnet has reduced the incidence and mortality of early CMV infections, prolonged antiviral treatment may delay recovery of virus-specific immune responses and predispose patients to late-onset disease [2-5]. Furthermore, treatment with antiviral drugs often cannot be sustained because of complicating myelosuppression or nephrotoxicity [2].

Reconstitution of CMV-specific $CD8^+$ cytotoxic T cells (CMVCTLs) after HCT is correlated with control of CMV infections [2,6-14]. Riddell et al. [15,16] first demonstrated that adoptive transfer of donor-derived $CD8^+$ CMVCTL clones sensitized with autologous CMV-infected fibroblasts could protect allogeneic marrow recipients from infection. Subsequent studies employing CMV-specific, predominantly $CD8^+$, T cell lines sensitized with autologous dendritic cells (DCs) or peripheral blood mononuclear cells (PBMCs) loaded with lysates of CMV-infected cells [17,18] or single peptides of immunodominant antigens, such as CMVpp65 [19] or DCs transduced to express immunogenic CMV proteins [20], have further documented the potential of such cells to prevent or treat CMV disease. However, regulatory concerns persist regarding the use of infected cell lysates or virus-transduced cells. Similarly, sensitization with single peptides presented by specific HLA alleles, however prevalent, may limit their broad application.

We previously reported a method for generating CMVCTL by sensitization with autologous DCs loaded with a pool of 138 synthetic pentadecapeptides (15-mers) with 11 amino

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* Correspondence and reprint requests: Guenther Koehne, MD, PhD, Adult Bone Marrow Transplantation Service, Division of Hematologic Oncology, Department of Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065.
E-mail address: koehneg@mskcc.org (G. Koehne).

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acid overlaps spanning the amino acid sequence of CMVpp65 [21]. With this approach, we were able to generate CMVpp65 peptide–specific T cell lines (CMVpp65CTLs) from each CMV-seropositive donor tested, regardless of HLA type, and to characterize these lines as to their epitope specificities and HLA restrictions [21]. We now report results of a phase I trial reassessing the safety and antiviral activity of escalating doses of transplant donor-derived CMVpp65CTLs generated by this technique in allogeneic HCT recipients with CMV infections or persistent CMV viremia. By defining the epitope specificity, HLA restriction, and TCR V_β usage of the T cells infused, we were also able to sequentially follow their growth and persistence in vivo and correlate their expansion with clearance of infection.

MATERIALS AND METHODS

Design of Clinical Trial

This single-institution phase I trial was designed to assess the toxicity and activity of escalating doses of CMVpp65CTLs derived from T cell lines generated from CMV-seropositive healthy marrow transplant donors by sensitization in vitro with autologous, cytokine-activated monocytes (CAMS) loaded with a pool of synthetic 15-mer peptides spanning the sequence of CMV protein pp65 [21]. The trial was approved by the institutional review/privacy board at Memorial Sloan-Kettering Cancer Center, the National Marrow Donor Program, and the Food and Drug Administration. Eligible patients were allogeneic HCT recipients who either had clinical CMV infection or CMV viremia that was persistent despite at least 2 weeks of treatment with antiviral drugs or those who could not be maintained on antiviral drugs because of associated toxicities.

Four dose levels of transplant donor-derived CMVpp65CTLs were sequentially evaluated: group 1 (n = 3) received 5×10^5 T cells/kg; group 2 (n = 4), 1×10^6 T cells/kg × 1 dose; group 3 (n = 3), 2×10^6 T cells/kg × 1 dose; group 4 (n = 6), 1×10^6 T cells/kg × 3 weekly doses. Endpoints included incidence and severity of toxicities and acute graft-versus-host disease (GVHD) as well as the clinical and virological responses observed and their correlation with alterations in CMV-specific T cells detected after infusion.

Patient and Donor Characteristics

Characteristics of the 16 patients who received transplant donor-derived CMVpp65CTLs, including diagnoses, disease status at time of transplantation, conditioning regimen, and type of transplantation, are summarized in Table 1. All recipients were CMV-seropositive before transplantation.

All patients had been previously treated with antiviral drugs, according to standard of care before administration of CMVpp65CTLs. Antiviral therapy was maintained after CMVpp65CTL infusion in 13 patients but had been discontinued in 4 patients (unique patient numbers [UPN] 4, 5, 8, and 11) because of intolerable toxicities at time of CMVpp65CTL infusion.

UPN 17 was referred from an outside center with reactivation of drug-resistant CMV after a 9/10 HLA-matched (HLA-A mismatch) HCT from a seronegative unrelated donor. This patient was treated with partially matched third-party CMV cytotoxic T cells (CTLs) under an institutional review board and Food and Drug Association–approved single patient use-IND.

Generation of Antigen-Presenting Cells

Autologous transplant donor-derived CAMS and Epstein-Barr virus–transformed B lymphocyte cell lines (EBV-BLCLs) were generated as previously described [21-24]. To identify HLA restrictions of CMVpp65CTL, a panel of EBV-BLCLs of defined HLA types were generated as previously described [21,22].

Generation of Clinical Grade CMVpp65CTLs

Cultures of CMVpp65-specific T cells from seropositive transplant donors were initiated at first detection of CMV viremia or before reactivation for seropositive transplant recipients at risk. $CD3^+$-enriched T cell fractions, isolated from PBMC by depletion of adherent monocytes and immunoadsorption of natural killer cells, were initially stimulated at an effector to stimulator ratio of 20:1 with irradiated (6000 cGy) autologous CAMS loaded with the pool of overlapping pentadecapeptides of CMVpp65 (Invitrogen, Boston, MA) and propagated in vitro with weekly restimulation at an effector to stimulator ratio of 4:1 and supplementation with IL-2 beginning at day 10 to 16, as previously described [21,22]. After 28 days, T cells were harvested, counted, and tested for antigen-specific cytotoxicity and lack of alloreactivity [21-23] as well as for microbiological sterility and endotoxin levels. Aliquots of CMVpp65CTLs meeting release criteria were

Table 1
Patient and Donor Characteristics

UPN	Age	Diagnosis	Disease Status at HCT	Graft	Donor Type	CMV IgG D/R	Weight at HCT, kg	Conditioning	GVHD Proph	GVHD Status before CMV CTLs	GVHD Proph/Treatment at CMV CTL Administration	GVHD status at CMV CTLs
1	60.8	SAA	Stable disease	BM unmodified	MUD	+/+	56.1	Flu/TBI	CSA/MTX	-	CSA/MMF	-
2	62.6	NHL	Partial remission	PBSC unmodified	MRD (bro)	+/+	67.9	Flu/Cyclo/TBI	CSA/MMF	Skin/gut III	CSA/MMF/Methylpred	Skin/gut III
3	44.5	SAA	Refractory	TCD PBSC (Isolex)/E	MUD	+/+	73.5	Flu/Cyclo/Thio	TCD	-	-	
4	38.5	MM	Second partial remission	TCD PBSC (Isolex)/E	MRD (sis)	+/+	98.4	Bu/Mel/Flu	TCD	-	-	
5	45.8	MDS/RAEB	Refractor cytopenia	TCD PBSC (Isolex)/E	MRD (sis)	+/+	85	Bu/Mel/Flu	TCD	-	-	
6	59.5	AML	First remission	TCD PBSC (Isolex)/E	MRD (sis)	+/+	104.7	Bu/Mel/Flu	TCD	-	-	
7	69.6	MM	Second partial response	TCD PBSC (Isolex)/E	MRD (bro)	+/+	82.7	Bu/Mel/Flu	TCD	-	-	
8	65.1	AML	Third remission	TCD PBSC (Isolex)/E	MRD (sis)	+/+	82	Bu/Mel/Flu	TCD	-	-	
9	7.9	ALL	First remission	TCD PBSC (Isolex)/E	MRD (bro)	+/+	37	TBI/Thio/Cyclo	TCD	-	-	
10	60.7	AML	First remission	TCD PBSC (Isolex)/E	MUD	+/+	921	Bu/Mel/Flu	TCD	-	-	
11	60.9	MPD Myelofibrosis	First chronic phase	TCD PBSC (CliniMACS)	MRD (bro)	+/+	48.9	Bu/Mel/Flu	TCD	-	-	
12	44.2	MDS/RAEB	Refractory anemia	TCD PBSC (CliniMACS)	MRD (sis)	+/+	98.2	Bu/Mel/Flu	TCD	-	-	
13	57	ALL	First remission	TCD PBSC (CliniMACS)	MRD (sis)	+/+	63.6	Bu/Mel/Flu	TCD	-	-	
14	63.5	MM	Second partial remission	TCD PBSC (CliniMACS)	MRD (sis)	+/+	103.1	Bu/Mel/Flu	TCD	-	-	
15	47.1	NHL	First remission	TCD PBSC (CliniMACS)	MRD (bro)	+/+	45.3	TBI/Thio/Cyclo	TCD	-	-	
16	52.5	MM	Second partial response	TCD PBSC (CliniMACS)	MUD	+/+	53.7	Bu/Mel/Flu	TCD	-	-	

D indicates donor; R, recipient; proph, prophylaxis; SAA, severe aplastic anemia; BM, bone marrow; MUD, matched unrelated donor; Flu, fludarabine; TBI, total body irradiation; CSA, cyclosporine A; MTX, methotrexate; MMF, mycophenolate mofetil; NHL, non-Hodgkin lymphoma; PBSC, peripheral blood stem cells; MRD, matched related donor; bro, brother; cyclo, cyclophosphamide; methylpred, methylprednisolone; TCD, T cell depleted; E, E-rosetting; Thio, thiotepa; sis, sister; Bu, busulfan; Mel, melphalan; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess blasts; AML, acute myeloid leukemia; MM, multiple myeloma; ALL, acute lymphoid leukemia.