

RECONSTITUTION OF CELLULAR IMMUNITY AGAINST CYTOMEGALOVIRUS IN RECIPIENTS OF ALLOGENEIC BONE MARROW BY TRANSFER OF T-CELL CLONES FROM THE DONOR

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Abstract Background. Cytomegalovirus (CMV) disease in immunocompromised patients correlates with a deficiency of CD8+ cytotoxic T lymphocytes specific for CMV. We evaluated the safety and immunologic effects of immunotherapy with clones of these lymphocytes in recipients of allogeneic bone marrow transplants.

Methods. Clones of CD8+ cytotoxic T cells specific for CMV proteins were isolated from the blood of bone marrow donors. Fourteen patients each received four intravenous infusions of these clones from their donors beginning 30 to 40 days after marrow transplantation. The reconstitution of cellular immunity against CMV was monitored before and during the period of infusions and for up to 12 weeks after the final infusion. The rearranged genes encoding the T-cell receptor served as markers in evaluating the persistence of the transferred T cells.

Results. No toxic effects related to the infusions were observed. Cytotoxic T cells specific for CMV were

reconstituted in all patients. In vitro measurements showed that cytotoxic activity against CMV was significantly increased ($P < 0.001$) after the infusions in 11 patients who were deficient in such activity before therapy. The level of activity achieved after the infusions was similar to that measured in the donors. Analysis of rearranged T-cell-receptor genes in T cells obtained from two recipients indicated that the transferred clones persisted for at least 12 weeks. Cytotoxic-T-cell activity declined in patients deficient in CD4+ T-helper cells specific for CMV, suggesting that helper-T-cell function is needed for the persistence of transferred CD8+ T cells. Neither CMV viremia nor CMV disease developed in any of the 14 patients.

Conclusions. The transfer of CMV-specific clones of CD8+ T cells derived from the bone marrow donor is a safe and effective way to reconstitute cellular immunity against CMV after allogeneic marrow transplantation. (N Engl J Med 1995;333:1038-44.)

REACTIVATION of latent cytomegalovirus (CMV) infection in immunocompromised patients causes considerable morbidity and mortality.¹⁻⁶ CMV is excreted in the urine after allogeneic bone marrow transplantation by approximately 70 percent of CMV-seropositive recipients and 30 percent of CMV-seronegative recipients whose donors are seropositive.⁷⁻¹⁰ Without ganciclovir prophylaxis, CMV disease develops in half the patients with CMV reactivation.^{7,11,12} CMV pneumonia, the most common form of CMV disease, has a mortality rate of 30 to 60 percent.^{13,14} Although ganciclovir prophylaxis reduces the incidence of CMV disease early after transplantation, it is complicated by the occurrence of severe neutropenia in 30 percent of patients and by an increased incidence of CMV disease of late onset (more than 100 days after transplantation).¹⁵⁻¹⁷ Thus, the development of alternative strategies of prophylaxis is warranted.

Deficiencies in the response of class I HLA-restricted CD8+ cytotoxic T lymphocytes specific for CMV are important in the pathogenesis of CMV disease in immunocompromised recipients of allogeneic marrow transplants.¹⁷⁻¹⁹ These patients receive a conditioning treatment that destroys their T cells, and they depend

on the recovery of virus-specific T-cell immunity by in vivo proliferation of T cells derived from the donor marrow. During the first 100 days after allogeneic marrow transplantation, half the recipients are persistently deficient in CD8+ cytotoxic T lymphocytes specific for CMV. It is in this subgroup of patients that CMV disease occurs.¹⁹

The transfer of syngeneic (i.e., involving genetically identical donors and recipients), polyclonal CD8+ T cells from immune mice to immunosuppressed mice provided protection from a viral challenge. CD4+ T cells were not protective.^{20,21} Similarly, in humans polyclonal populations of lymphocytes obtained from the peripheral blood of the donor have been used successfully to treat the Epstein-Barr virus (EBV) lymphoproliferative syndrome that can develop after allogeneic marrow transplantation. However, the transfer of these unselected lymphocytes also caused graft-versus-host disease (GVHD).²² Enrichment of the lymphocytes in cytotoxic T cells specific for EBV by in vitro culture before transfer appears to reduce the risk of GVHD.²³ A more definitive strategy to reduce the risk of GVHD from allogeneic lymphocytes would be to use T-cell clones with specificity for the antigens of the pathogen being treated. Clones of cytotoxic T lymphocytes specific for CMV can be isolated from normal CMV-seropositive subjects. The predominant specificity of these clones is directed against CMV structural proteins, such as the matrix proteins pp65 and pp150, which are presented for recognition by cytotoxic T lymphocytes before new virions are formed in infected cells.^{24,25}

A preliminary study of three patients established the feasibility of transferring clones of CD8+ cytotoxic

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T lymphocytes specific for CMV from the marrow donor to the marrow-transplant recipient.²⁶ We report the results of a phase I trial in which the safety and efficacy of adoptive T-cell therapy were examined.

METHODS

Patients

Eighteen patients undergoing HLA-identical allogeneic marrow transplantation from a CMV-seropositive related donor at the Fred Hutchinson Cancer Research Center were enrolled in a study to evaluate adoptive immunotherapy with clones of CD8+ T cells specific for CMV, beginning 30 to 40 days after marrow transplantation. The study was approved by the institutional review board, and all patients gave informed consent. Four patients did not receive T-cell infusions: two died before day 30, and two became ineligible because of grade III organ toxicity. None of the patients received prophylactic therapy with ganciclovir or immune globulin. Table 1 shows the clinical characteristics of the 14 treated patients.

Generation and Characterization of the Clones

Polyclonal cytotoxic-T-lymphocyte cultures specific for CMV were established as described elsewhere.^{24,27} Skin-biopsy specimens were obtained from each marrow donor to establish a line of fibroblasts for use as stimulator and target cells. Peripheral-blood mononuclear cells (PBMCs) were obtained from the donor and cultured with autologous fibroblasts infected with the AD169 strain of CMV. The culture medium for the T cells was RPMI, supplemented with 25 mmol of HEPES buffer per liter; 11 percent AB-positive, CMV-seronegative serum from normal blood donors; 4 mmol of L-glutamine per liter; 50 U of penicillin per milliliter; 50 µg of streptomycin per milliliter; and 2.5×10^{-5} mol of 2-mercaptoethanol per liter of solution. CD8+ T cells were cloned from the cultures by the limiting-dilution method after 7 to 14 days, depending on whether CMV-specific cytolytic activity was detected in the cultured cells.^{24,27}

Clones of CD8+ cytotoxic T lymphocytes specific for CMV were isolated by depleting the culture of CD4+ T cells with the use of flasks coated with anti-CD4+ monoclonal antibody (Applied Immune Sciences, Santa Clara, Calif.) and plating the CD8+ T cells in 96-well round-bottomed plates (0.3 to 0.8 cell per well) with 50,000 gamma-irradiated (30-Gy) autologous PBMCs (PBMC_{ir}), 10,000 gamma-irradiated (80-Gy) autologous EBV-transformed B lymphoblasts (LCL_{ir}) as feeder cells, and 25 to 50 U of interleukin-2 per milliliter. Anti-CD3 monoclonal antibody (30 ng per milliliter) or autologous CMV-infected fibroblasts (2000 per well) were added to stimulate the T cells.^{24,27} The clones were transferred to larger wells or tissue-culture flasks, and their numbers were increased to more than 1 billion by cyclic stimulation at 10-to-12-day intervals with either autologous CMV-infected fibroblasts or anti-CD3 monoclonal antibody in cultures supplemented with PBMC_{ir} and LCL_{ir}, with interleukin-2 added on days 1, 5, and 8 after stimulation. The expression of CD3, CD4, and CD8 was determined with a fluorescence-activated cell sorter, and clones of CD3+CD8+CD4- cytotoxic T lymphocytes were selected for use in therapy.²⁷ The sterility of the cultures was confirmed before each infusion. T-cell clones from all 18 donors were successfully generated for the therapy.

Assay for Cytotoxicity

The clones were assayed for HLA-restricted cytolytic activity specific for CMV in a five-hour chromium-release assay.^{17,19} The target cells were autologous and HLA class I-mismatched CMV-infected or mock-infected fibroblasts incubated for 48 hours with 100 U of recombinant interferon gamma (Boehringer-Mannheim, Indianapolis) per milliliter to increase the sensitivity of the assay.²⁸ Spontaneous release of chromium, maximal release, and the percentage of CMV-infected cells killed (specific lysis) were calculated as described elsewhere.^{17,19,24} Cytotoxic-T-lymphocyte clones that lysed more than 30 percent of autologous CMV-infected target cells and less than 5 percent of control target cells with an effector:target ratio of 5:1 were used in therapy.

Table 1. Clinical Characteristics of 14 Recipients of Allogeneic Bone Marrow Transplants Who Underwent Adoptive Immunotherapy.

CHARACTERISTIC	
Age (yr)	
Median	39
Range	16–53
	<i>no. of recipients</i>
Sex (M/F)	11/3
CMV serologic status	
Positive recipient/positive donor	5
Negative recipient/positive donor	9
Conditioning regimen*	
Busulfan, cyclophosphamide	6
Busulfan, cyclophosphamide, TBI	3
Cyclophosphamide, TBI	5
GVHD prophylaxis	
Methotrexate	2
Methotrexate, cyclosporine	7
Cyclosporine, prednisone	3
Tacrolimus, methotrexate	1
Cyclosporine	1
Disease	
Chronic myelogenous leukemia	4
Acute myelogenous leukemia	4
Acute lymphocytic leukemia	1
Acute undifferentiated leukemia	1
Multiple myeloma	1
Myelodysplastic syndrome	3

*TBI denotes total-body irradiation.

Clones of CD8+ cytotoxic T lymphocytes specific for structural virion proteins were identified by their ability to lyse CMV-infected target cells in the presence of dactinomycin to prevent the expression of viral genes, and these clones were selected for use in therapy.²⁴

Treatment Regimen

The clones of CMV-specific cytotoxic T lymphocytes were administered to each marrow-transplant recipient intravenously over a 30-minute period through a Hickman catheter in four escalating doses (33 million, 100 million, 330 million, and 1 billion cells per square meter of body-surface area), each given one week apart. Starting the treatment more than 30 days after transplantation made it easier to distinguish toxic effects related to T-cell infusions from earlier toxic effects due to the conditioning chemotherapy.

Monitoring of Patients

The first three patients received each infusion of T cells in the hospital, where their vital signs and oxygen saturation were monitored before the infusion and 15, 30, 60, and 120 minutes after the start of the infusion. The remaining 11 patients were treated in the outpatient department and were monitored in the same way. Complete blood counts were obtained and liver function was evaluated one day after each infusion and three times weekly until day 100. Chest radiographs were obtained one day after each infusion, and physical examinations were performed weekly. GVHD was graded according to published criteria.²⁹

Immunologic Monitoring

PBMCs were collected before the start of the T-cell infusions, 2 days after each infusion, and 2, 4, 6, and 12 weeks after the completion of therapy. Short-term cultures were generated from the PBMCs and assayed for CMV-specific CD8+ cytotoxic-T-lymphocyte activity.¹⁷ The activity of CMV-specific CD4+ T-helper cells in the PBMCs was also assayed by plating 200,000 PBMCs in triplicate in 96-well round-bottomed plates with medium alone, CMV antigen, or phyto-

Table 2. Reconstitution of Cytotoxic-T-Lymphocyte Responses in Recipients of Bone Marrow Transplants with Adoptive Immunotherapy.*

PATIENT No.	PERCENTAGE OF CMV-INFECTED CELLS KILLED					IN DONOR
	BEFORE 1ST INFUSION	AFTER 1ST INFUSION	AFTER 2ND INFUSION	AFTER 3RD INFUSION	AFTER 4TH INFUSION	
1	2	14	40	55	82	55
2	4	23	47	32	38	31
3	5	15	13	48	54	24
4	0	14	22	30	35	32
5	9	28	28	19	35	24
6	2	12	24	30	26	23
7	3	20	21	41	40	35
8	3	11	19	11	21	58
9	8	15	17	18	12	30
10	0	12	24	7	9	35
11	7	12	15	21	37	30
12	25	30	48	52	64	45
13	37	40	54	13	40	25
14	31	46	27	38	49	42

*PBMCs were tested before the T-cell infusions, two days after infusions 1 through 4, and in all bone marrow donors. Short-term cell lines of cytotoxic T lymphocytes were generated and assayed for CMV-specific cytolytic activity at an effector:target ratio of 10:1.¹⁹ All values are expressed as the difference in the percentage of cells killed (specific lysis) between the CMV-infected and the mock-infected autologous target cells. If this difference was greater than 10 percent, the response was defined as positive.¹⁷ The difference in the percentage of cells killed between the CMV-infected and the mock-infected class I HLA-mismatched target cells was less than 5 percent in all experiments (data not shown).

hemagglutinin (5 μ g per milliliter).¹⁷ The wells were pulsed with tritiated thymidine (1 μ Ci per well) for the final 16 hours of a 96-hour incubation; the cells were then collected for β scintillation counting. The data on T-helper responses were transformed into a stimulation index, defined as the mean number of counts per minute (cpm) for cells exposed to CMV antigen divided by the mean number of counts per minute for cells exposed only to tissue-culture medium.

In Vivo Persistence of Adoptively Transferred CD8+ Clones

A reverse-transcriptase polymerase chain reaction (PCR) was used to identify the variable genes (V_{α} and V_{β}) of the T-cell receptor expressed by the infused cytotoxic-T-lymphocyte clones and by clones isolated from the patients after the infusions. RNA was isolated from the clones with a total-RNA separator kit (Clontech, Palo Alto, Calif.), and 1 to 2 μ g of total RNA was reverse-transcribed to first-strand complementary DNA (cDNA).³⁰ The segments of the V_{α} and V_{β} genes expressed in the cDNA samples prepared from each clone were determined with 25 5' V_{β} primers for 24 known major V_{β} families (Clontech) and 31 5' V_{α} primers, with 3' primers derived from the respective constant (C_{β} and C_{α}) sequences.³¹

Viologic Monitoring

Shell-vial and conventional cultures of buffy coat, urine, and throat for CMV were monitored weekly until day 100.

RESULTS

Safety of Adoptive Transfer of CMV-Specific Clones

No patient had significant changes in blood pressure, heart rate, temperature, or oxygen saturation during the T-cell infusions. One patient had a transient fever after the fourth infusion; another patient with a history of chills after transfusions had chills during infusions 2, 3, and 4. Blood-chemistry values, chest radiographs, and blood counts were unchanged in all the patients.

Before receiving the infusions, two patients had

grade I GVHD, and five patients had grade II or III GVHD. The dose of the immunosuppressive agents was not increased in the first two patients, but prednisone (2 to 3 mg per kilogram of body weight per day) was given to all five with more severe GVHD and then tapered during or after the infusions, with no flare of GVHD. In three patients GVHD developed during or after the completion of T-cell therapy. GVHD of grade I or II appeared five days after the first infusion and six days after the third infusion in two of these patients. They received 1 to 1.5 mg of prednisone per kilogram per day, which was tapered over a period of two to three weeks; they tolerated subsequent infusions of higher doses of cells without recurrent GVHD. Grade II GVHD developed two weeks after the final infusion in the third patient. Four patients had no evidence of GVHD during the study.

Reconstitution of CMV-Specific Immunity

PBMCs were collected before and after the T-cell infusions in order to evaluate CMV-specific reactivity of cytotoxic T lymphocytes. Eleven of the 14 patients lacked CMV-specific cytotoxic T lymphocytes immediately before the first infusion of CMV-specific clones. In all 11 patients, such lymphocytes were detected two

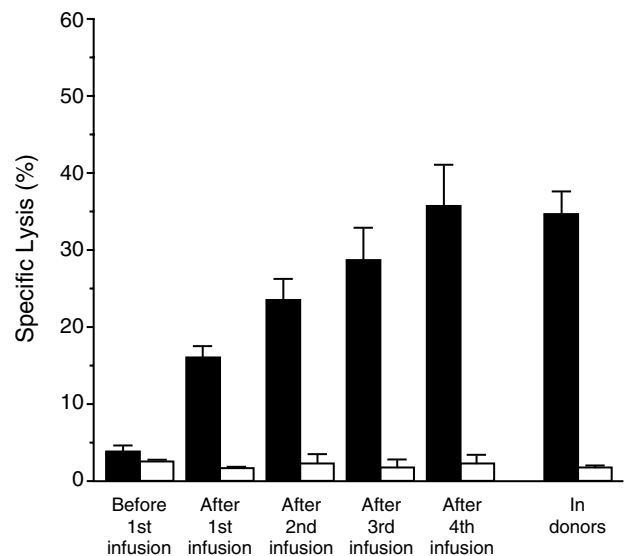


Figure 1. Reconstitution of Responses of CMV-Specific Cytotoxic T Lymphocytes with Adoptive Immunotherapy in 11 Patients. PBMCs from the recipients were tested before the first infusion of T cells and two days after each infusion; PBMCs from the donors were also tested. Solid bars indicate CD8+ cytotoxic-T-cell activity specific for CMV, shown as the mean (\pm SD) difference in the percentage of infected cells killed (specific lysis) between autologous CMV-infected fibroblasts and mock-infected fibroblasts. Open bars indicate nonspecific lytic activity, shown as the mean difference in the percentage of specific lysis between class I MHC-mismatched allogeneic CMV-infected fibroblasts and mock-infected fibroblasts. The responses measured after the fourth infusion were significantly higher than those measured before the first infusion ($P < 0.001$ by the paired t-test) and did not differ significantly from the responses in the healthy donors ($P = 0.86$ by the paired t-test).

days after the first infusion of T cells (Table 2). These responses persisted and increased in magnitude after successive infusions to such a degree that the reactivity of cytotoxic T lymphocytes after the fourth infusion did not differ significantly from that of the marrow donors ($P=0.86$) (Fig. 1). In two patients (Patients 9 and 10), the reactivity of CMV-specific cytotoxic T lymphocytes appeared to recover to levels below those detected in the donors (Table 2), but this reflected high levels of nonspecific lysis against autologous mock-infected target cells, which obscured the detection of CMV-specific lysis. This nonspecific activity remained after the depletion of natural killer cells (data not shown).

All 14 patients had reconstituted CMV-specific cytotoxic T lymphocytes by days 42 to 49 after marrow transplantation, whereas in previous studies of patients

who did not receive adoptive immunotherapy, more than half were deficient in such responses at day 50.^{17,19} Moreover, the recovery of endogenous cytotoxic T lymphocytes specific for CMV required the presence of CD4+ CMV-specific T-helper cells,^{17,19} whereas Patients 1 through 9 had reconstituted cytotoxic T lymphocytes specific for CMV in the absence of detectable CMV-specific CD4+ helper T cells (mean stimulation index after the fourth infusion, 1.4; range, 0.2 to 1.9). The mean stimulation index after the fourth infusion in the five patients in whom these helper cells recovered was 4.1 (range, 2.6 to 6.2).

High doses of immunosuppressive therapy for severe GVHD may affect the survival, the activity, or both of infused cytotoxic T lymphocytes. Nine patients with GVHD received 1 to 3 mg of prednisone per kilogram

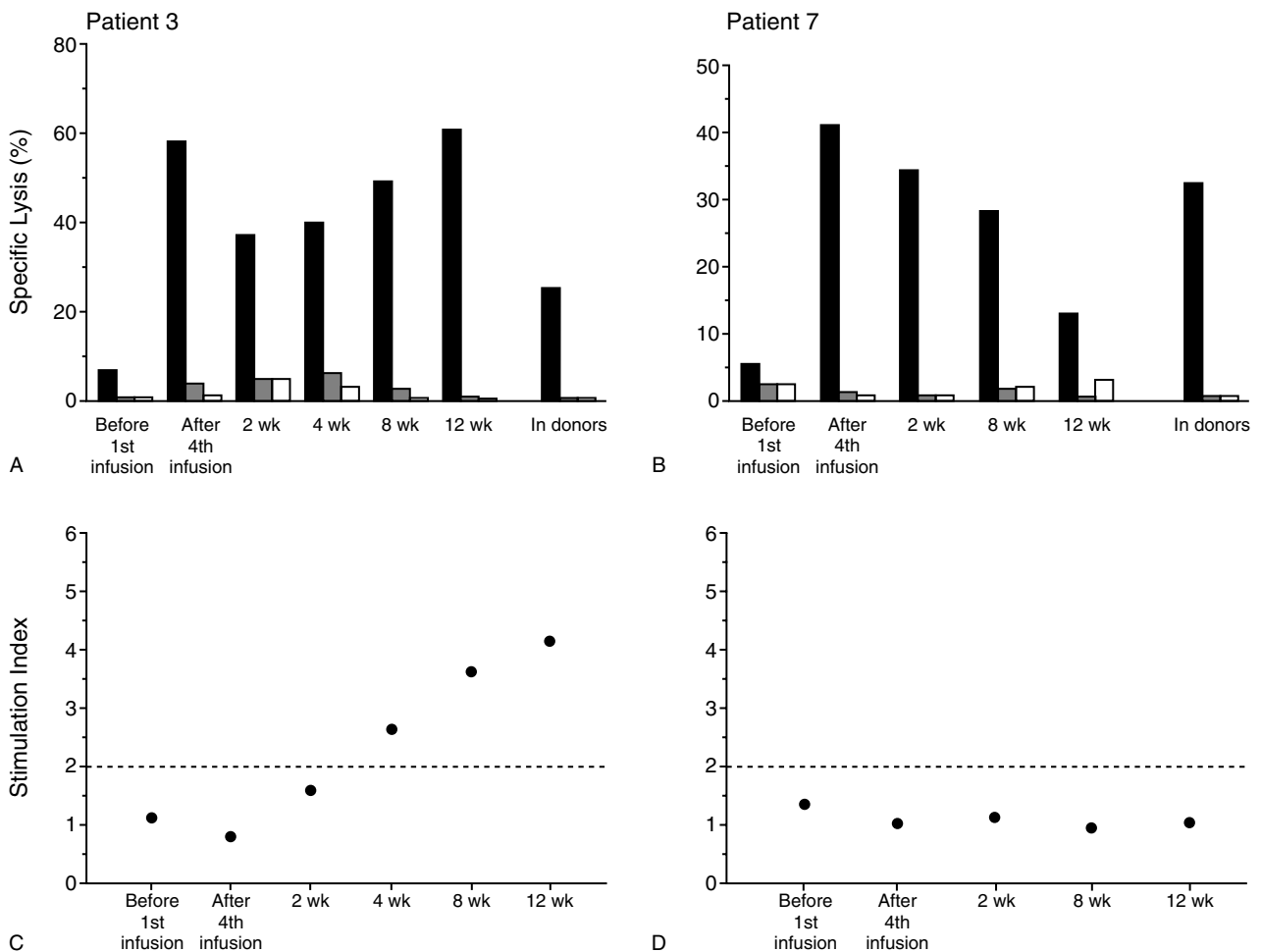


Figure 2. Persistence of CD8+ Cytotoxic-T-Lymphocyte Responses Specific for CMV in Patients 3 and 7, in Whom CD4+ T-Helper Responses Specific for CMV Were and Were Not Recovered, Respectively.

Panels A and B show the results of an assay for CD8+ cytotoxic T lymphocytes for CMV in cell lines derived by stimulation of PBMCs with autologous CMV-infected fibroblasts. The target cells include CMV-infected (solid bars) and uninfected (shaded bars) autologous fibroblasts and CMV-infected class I HLA-mismatched allogeneic fibroblasts (open bars). Data are shown at an effector:target ratio of 10:1. Panels C and D show the responses of CD4+ T-helper cells obtained at intervals of up to 12 weeks after the final infusion, expressed as a stimulation index defined as the mean number of counts per minute for cells exposed to CMV antigen divided by the mean number of counts per minute of cells exposed to medium. A stimulation index greater than 2.0 indicates a positive lymphoproliferative response.¹⁷

per day in addition to cyclosporine or tacrolimus (FK 506). In six of these patients, including five who had deficient responses of helper T cells, the magnitude of the reconstitution of cytotoxic-T-lymphocyte responses was equal to or greater than that of the donors. Nonspecific cytolytic activity obscured the responses of cytotoxic T lymphocytes in two patients (Patients 9 and 10); in one patient (Patient 8), who was receiving 3 mg of prednisone per kilogram per day to treat grade III GVHD, the response of CMV-specific cytotoxic T lymphocytes after therapy was less than that of the marrow donor.

Persistence of Transferred CD8+ Clones

All the patients maintained cytotoxic-T-lymphocyte responses specific for CMV for at least eight weeks after the completion of T-cell therapy (Fig. 2). We used rearranged V_α and V_β genes for the T-cell receptor as

molecular markers to evaluate the survival of the transferred clones in two patients (Patients 3 and 7) who received only one or two clones of cytotoxic T lymphocytes in each infusion (Table 3). The six clones recovered at each point up to 12 weeks after the treatment of Patient 7 expressed V_α and V_β T-cell-receptor genes that were identical to those of the infused clones; similar results were obtained for Patient 3 (Table 3). Sequencing of PCR products from representative clones (sequenced through the VJ_α [variable joining α] and VDJ_β [variable diversity joining β] regions) confirmed that they were identical to the sequences in the infused clones. Two clones of cytotoxic T lymphocytes specific for CMV were isolated from a third patient (Patient 8), who was receiving high-dose prednisone (3 mg per kilogram per day), two weeks after the fourth infusion of T cells. The V_α and V_β genes expressed by these clones were

Table 3. V_α and V_β Genes Expressed by the T-Cell Clones Transferred from the Donors and by Representative Clones Recovered from Patients 3 and 7.*

TRANSFERRED CLONES				CLONES RECOVERED AFTER INFUSION												
PATIENT AND INFUSION NO.	CLONES INFUSED	V_α^\dagger	V_β^\dagger	TIME RECOVERED‡	CLONE 1		CLONE 2		CLONE 3		CLONE 4		CLONE 5		CLONE 6	
					V_α	V_β	V_α	V_β	V_α	V_β	V_α	V_β	V_α	V_β	V_α	V_β
Patient 3§																
1	58G9	4, 14	16													
2	16D7	4, 14	16													
3	52D5	4, 14	16	After infusion 3	ND	16	ND	16	ND	16						
4	19C7	8, 7	6													
	18H9	18, 11	2	After infusion 4	18, 11	2	18, 11	2	18, 11	2	18, 11	2	18, 11	2	8, 7	6
				2 wk after infusion 4	18, 11	2	8, 7	6	8, 7	6						
				4 wk after infusion 4	8, 7	6¶										
				12 wk after infusion 4	18, 11	2										
Patient 7																
1	1A3	3, 12	7													
2	10E6	2, 17	14													
	3H6	5	11	After infusion 2	3, 12	7	5	11	5	11	3, 12	7	3, 12	7	3, 12	7
3	10B5	15	19													
4	10E6	2, 17	14	After infusion 4	2, 17	14	2, 17	14	2, 17	14	2, 17	14	15	19	15	19
				4 wk after infusion 4	15	19	2, 17	14	2, 17	14	2, 17	14	2, 17	14	2, 17	14
				8 wk after infusion 4	15	19	15	19	2, 17	14	2, 17	14	2, 17	14	2, 17	14
				12 wk after infusion 4	2, 17	14	2, 17	14	2, 17	14	2, 17	14	2, 17	14	2, 17	14

*The rearranged V_α and V_β genes of the clones recovered after the infusions were used as markers for T cells derived from the infused clones. One to six clones were recovered at intervals after treatment. All the recovered clones were found to express V_α and V_β genes identical to those expressed by the T cells previously transferred to the patient. Eight weeks after the fourth infusion, for example, the V_α and V_β genes in Patient 7 that corresponded to clone 10B5 (given as the third infusion) were found in two isolates, and the V_α and V_β genes corresponding to clone 10E6 (given as the fourth infusion) were found in four isolates. Numbers shown under the headings " V_α " and " V_β " refer to the standard nomenclature for the genes. ND denotes not determined.

†Primers of the V_α and V_β families were used in the reverse-transcriptase PCR of cDNA synthesized from the infused clones. Alternate 5' and 3' C_α and C_β primers or β -actin primers served as positive controls, and distilled water substituted for the cDNA template served as a negative control. Two different VJ_α transcripts were expressed in a fraction of clones, as described elsewhere.³²⁻³⁴

‡PBMCs were obtained 2 days after infusions 2, 3, and 4 and 2, 4, 8, and 12 weeks after the final infusion. CMV-specific clones were isolated from the PBMCs, as described in the Methods section, and were evaluated for expression of the V_α and V_β genes for the T-cell receptor.

§The PCR products resulting from T-cell-receptor amplification reactions for the samples from Patient 3 were gel-purified with GeneClean (Bio 101, La Jolla, Calif.) and cloned into pBSSK⁻ (Stratagene, La Jolla, Calif.) for sequencing through the VJ and VDJ regions. Sanger dideoxy sequencing was performed with Sequenase II (USB, Cleveland) and a primer to either the C_α or the C_β region of the amplified sequence of the T-cell receptor.³⁵ The sequences of the $V_\alpha J_\alpha$ and $V_\beta D_\beta J_\beta$ gene segments from clones 58G9, 16D7, and 52D5 were identical and were designated as $V_{\alpha 42} J_{\alpha 42}$, $V_{\alpha 11} J_{\alpha 49}$, and $V_{\beta 16} D_{\beta 2} J_{\beta 2}$, respectively, by comparison with the data base, suggesting that these clones were derived independently from the same founder cell in the polyclonal culture. The $D_{\beta 2}$ sequence was most consistent with sequences of the $D_{\beta 2}$ family, but additions and deletions to the N region did not permit an unambiguous determination to be made. The T-cell-receptor genes expressed by clones 19C7 and 18H9 were also sequenced and identified as $V_{\alpha 30} J_{\alpha 32}$, $V_{\alpha 7} J_{\alpha 41}$, $V_{\beta 60}$, $D_{\beta 2.1} J_{\beta 2.1}$ and $V_{\alpha 18} J_{\alpha 7}$, $V_{\alpha 11} J_{\alpha 2.1}$, $V_{\beta 2} D_{\beta 2.1} J_{\beta 1.3}$, respectively. The reverse-transcriptase PCR performed of all clones recovered from Patient 3 used primers specific for $V_\alpha J_\alpha$ and $V_\beta D_\beta J_\beta$, synthesized from the sequences identified in the infused clones. The 5'-3' sequences of the J_α primers were TG-GCTGGACAGCAAGCAGAGTG, GGGTTTACATGAGTTTGGTCCAG, and GGTATGACCACCATTGGTCC. The 5'-3' sequences of the $D_\beta J_\beta$ primers were GCTCCCGTAAGGCTGGAATCTTG, TATGGTGTGAGCAACTGTCCCTC, and GAAGTCTCATTTGTTAGTCCC.

¶The recovered clone was sequenced through the VDJ region of the β chain to confirm sequence identity with clone 19C7.

||The recovered clone was sequenced through the VDJ (β chain) and the VJ (α chain) to confirm sequence identity with clone 18H9.

identical to those expressed by two clones given in the fourth infusion (data not shown).

Since CD4+ helper T cells sustain host virus-specific responses of CD8+ cytotoxic T lymphocytes during chronic viral infection,^{19,36} we analyzed whether the recovery of helper T cells specific for CMV influenced the in vivo persistence of adoptively transferred CD8+ cytotoxic T lymphocytes. The magnitude of the CMV-specific responses of cytotoxic T lymphocytes decreased with time in patients in whom there was no recovery of CD4+ T-helper responses specific for CMV (Fig. 2). By contrast, the recovery of a T-helper response after the infusions was associated with sustained or increased responses of cytotoxic T lymphocytes, suggesting that the recovery of adequate T-helper function may facilitate the maintenance of transferred CD8+ cytotoxic T lymphocytes (Fig. 2).

Virologic Monitoring

Patients were tested weekly for CMV in the urine, throat, or blood. Ganciclovir was administered to two patients with urinary CMV, according to the practice standard at the time of the study. CMV may be excreted in the urine in persons without deficient T-cell function.³⁷ In one patient with a CMV-positive throat culture before the T-cell infusions, the virus was cleared after the first infusion. The cultures in the remaining 11 patients were negative, and none of the 14 patients had CMV viremia or CMV disease.

DISCUSSION

This study shows that adoptive immunotherapy with CD8+ T-cell clones can safely restore CMV-specific cytotoxic-T-lymphocyte responses in recipients of allogeneic bone marrow transplants. Fifty-six infusions of CMV-specific cytotoxic-T-lymphocyte clones were administered to 14 patients without any major toxic effects.

In other studies, donor PBMCs were transferred to marrow-transplant recipients to treat relapses of leukemia, but GVHD developed in 9 of 11 recipients (grade I in 6 and grade III in 3).³⁸ In the 14 patients we studied, immunotherapy with clones that were selected for their capacity to recognize CMV antigens presented in association with class I HLA molecules reduced the risk of GVHD. All the T-cell clones administered as therapy recognized structural virion proteins; further study of the clones given to seven patients identified the pp65 and pp150 proteins of CMV as the dominant target antigens (data not shown).^{24,25}

Several results support our conclusion that the reconstitution and persistence of cytotoxic-T-lymphocyte responses specific for CMV were due to the transfer of cytotoxic-T-lymphocyte clones from the donor. First, selective recovery of cytotoxic T lymphocytes specific for CMV in the absence of CMV-specific helper T cells was not observed in any of 56 patients previously evaluated who had not received adoptive immunotherapy,^{17,19} but such recovery was observed in all 11 patients

who lacked CD4+ T-helper responses in this study. Second, the infusion of increasing doses of cells resulted in an increased CMV-specific cytotoxic-T-lymphocyte response in the recipient. Finally, the persistence of the transferred clones was demonstrated in three patients when the rearranged T-cell V_α and V_β genes were used as molecular markers.

The infusion of T-cell clones in patients who required high-dose immunosuppressive therapy for severe GVHD restored cytotoxic-T-lymphocyte responses specific for CMV, but not always to the level present in the immunocompetent donors, suggesting that such patients may benefit from higher doses of T cells. Patients who had a progressive decline in the response of cytotoxic T lymphocytes did not recover CD4+ CMV-specific T-helper responses, suggesting that the concurrent transfer of CD4+ T-helper cells or the administration of interleukin-2 may have application.^{36,39} A potential problem with the administration of interleukin-2 early after allogeneic marrow transplantation is that it may worsen GVHD.

The absence of CMV viremia and CMV disease in the 14 patients who received adoptive immunotherapy suggests that studies of the efficacy of this approach as prophylaxis against CMV infection are warranted.

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