

# Cytotoxic T-Lymphocyte Response to Cytomegalovirus After Human Allogeneic Bone Marrow Transplantation: Pattern of Recovery and Correlation With Cytomegalovirus Infection and Disease

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The high rate of severe cytomegalovirus (CMV) disease after bone marrow transplantation (BMT) is related to the profound immunodeficiency posttransplant. Because cytotoxic T lymphocytes (CTL) have been implicated in resistance to viral infections, we examined the restoration of the CMV-specific CTL response in 20 patients who received bone marrow from HLA-matched, CMV-seropositive donors. Blood specimens were obtained from patients at 1, 2, and 3 months after BMT and from the donors on a single occasion. Peripheral blood mononuclear cells were cocultured with CMV-infected donor-derived fibroblasts for 2 weeks and then tested for cytotoxicity against CMV-infected and uninfected autologous or HLA-mismatched fibroblasts. Cytolytic activity was detected in all 20 donors, with specificity for autologous CMV-infected targets demonstrable in 17 (median CMV-specific lysis at an effector:target ratio of 15:1 was 32%, range 18% to 83%). Specific lysis was mediated by CD8<sup>+</sup>,

class I-restricted T cells, as shown by inhibition with anti-class I monoclonal antibody and by selective depletion of effector cells. By contrast, CMV-specific CTL were detected in only 10 of 20 patients after BMT (median lysis 29% at 3 months post-BMT). None of these 10 patients developed CMV pneumonia, whereas 6 of the 10 patients with an undetectable CMV-specific CTL response after BMT died with CMV pneumonia. These results demonstrate that restoration of CMV-specific CTL responses may require an extended time period after BMT in some patients, and that such patients are at increased risk of developing severe CMV disease. Approaches to reconstitute CMV immunity in BMT patients by adoptive transfer of CMV-specific CD8<sup>+</sup> CTL clones derived from the bone marrow donor are now being pursued.

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**I**NFECTION due to cytomegalovirus (CMV) represents a significant complication of bone marrow transplantation (BMT).<sup>1,2</sup> After allogeneic BMT, CMV infection occurs in approximately 60% to 70% of patients who are CMV seropositive before transplant or who are seronegative but receive bone marrow from a seropositive donor, and is the principal infectious cause of death.<sup>1</sup> A fatal outcome from CMV infection is primarily related to the occurrence of interstitial pneumonia, which develops in one third of infected patients.<sup>1,3</sup> Despite the development of new treatment regimens using antiviral drugs and Ig, CMV pneumonia still has a fatality rate of approximately 50%.<sup>4,6</sup>

The natural history of CMV infection has been extensively studied. Following primary infection, immunocompetent hosts experience a mild or subclinical illness. The virus then enters a latent phase that usually persists for the life of the host, but CMV can reactivate and cause clinical disease if the host is immunosuppressed.<sup>7</sup> Animal models have been developed to elucidate the nature of the immune responses required to control active infection and maintain the virus in a latent state. Studies in murine models with murine CMV have suggested that CD8<sup>+</sup> CMV-specific cytotoxic T lymphocytes (CTL) are responsible for elimination of active infection and protective immunity.<sup>8-10</sup> These results have been supported by inferential studies in humans.<sup>11-15</sup>

The peak incidence of CMV infection is during the first 3 months after BMT, and is thought to be related to the profound immunodeficient state induced by the treatment.<sup>16-21</sup> In light of the global immunologic dysfunction present during this time period, it has been difficult to precisely define the nature of the specific immunologic defects predisposing these hosts to CMV infection. However, BMT recipients demonstrate lymphopenia, with a diminished number of mature cytotoxic effector T cells, and exhibit impaired generation of new CTL effectors from lymphoid precursors in the early posttransplant period. As suggested by murine studies, this deficiency of CD8<sup>+</sup> T cells

may contribute to the high incidence of CMV infection and the development of severe CMV disease.

Earlier investigations among BMT recipients have analyzed cytotoxic responses of lymphocytes obtained directly from the peripheral blood for CMV-infected target cells using long-duration cytotoxicity assays.<sup>14,15</sup> The lytic activity measured with this approach reflects several populations of cytolytic effector cells including both T cells and natural killer (NK) cells.<sup>14,15,22</sup> Thus, it was often not possible in these prior studies to distinguish the contributions to lytic activity of CD8<sup>+</sup> class I major histocompatibility complex (MHC) restricted T cells from non-MHC restricted NK cells.<sup>14,15</sup> Furthermore, the use of more aggressive posttransplant immunosuppression in recent years could potentially alter the tempo of immunologic reconstitution to pathogens such as CMV.<sup>23</sup> Efficient culture systems have been developed that permit in vitro activation and expansion of class I

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MHC antigen-specific T cells, so that it is possible to evaluate the presence of all potentially virus-reactive T cells, not just circulating lymphocytes exhibiting direct lytic activity. These methods have been used to detect and characterize CMV-specific CD8<sup>+</sup> CTL in CMV-seropositive subjects with no evidence of active CMV infection,<sup>11,24-27</sup> and should be applicable to an analysis of the presence of CMV-specific CTL in BMT recipients.

In this prospective study of BMT donor-recipient pairs, we have used sequential *in vitro* stimulation of peripheral blood lymphocytes with autologous CMV-infected fibroblasts to analyze seropositive marrow donors for the presence of class I MHC-restricted CMV-specific CTL and their BMT recipients for the development of a CMV-specific CTL response after transplant. The results of these studies were correlated with the occurrence of CMV infection and CMV disease in the BMT recipients during the first 3 months after transplant. The data suggest that generation of class I-restricted CTL responses to CMV may greatly influence the capacity of the host to limit the severity of CMV infection after BMT.

#### MATERIALS AND METHODS

**Patient population.** The investigation was conducted prospectively among 20 allogeneic marrow transplant recipients and their healthy marrow donors. The patients were selected for study if they had a human histocompatibility leukocyte antigen (HLA)-identical, mixed lymphocyte culture-nonreactive sibling marrow donor seropositive for CMV IgG antibody. Informed consent was obtained from both the patients and the marrow donors, and the study protocol was approved by the institutional review board of the Fred Hutchinson Cancer Research Center (Seattle, WA). Characteristics of the study population are summarized in Table 1.

The methods of BMT and posttransplant care have been reported previously.<sup>28</sup> Each patient received cyclosporine plus a short course of methotrexate as prophylaxis for graft-versus-host disease (GVHD), and trough serum cyclosporine levels were

monitored weekly.<sup>29</sup> Due to the seropositive donor status, the patients were transfused with blood products that were not screened for antibody to CMV. Sixteen of the 20 recipients were CMV seropositive before transplant, with 14 of 16 receiving CMV prophylaxis to day 30 after transplant with high-dose intravenous acyclovir,<sup>30</sup> and 2 of 16 receiving prophylactic foscarnet until day 75.

**Expansion of CMV-specific CTL *in vitro*.** CMV-specific CTL reactivity in patients was evaluated at 1, 2, and 3 months after transplant and compared with the response in marrow donors. A modified culture system,<sup>26</sup> based on previously described methods,<sup>11,24</sup> was developed to expand CMV-specific CTL *in vitro*. Briefly, skin biopsies were obtained from each marrow donor to establish fibroblast lines for use as both stimulator and target cells. Fibroblast lines were grown in Waymouth's medium supplemented with 20% fetal calf serum (FCS), and used between passages 4 and 12. The fibroblasts were tested before use for mycoplasma contamination by DNA hybridization (Mycoplasma T.C. II; Gen-Probe, Inc, San Diego, CA). Autologous fibroblasts were plated in 6-well plates at  $0.5 \times 10^6$  cells/well, and infected for 2 hours with the human CMV strain AD 169 (American Type Culture Collection, Rockville, MD) at a multiplicity of infection (MOI) of 5:1 before initiation of lymphocyte culture. CMV strain AD 169 was initially propagated in human foreskin fibroblasts, and the infectivity of the resultant virus stocks used for the study was  $3 \times 10^6$  plaque-forming units/mL. Peripheral blood mononuclear cells (PBMC) were obtained from whole blood containing preservative-free sodium heparin (Lymphomed, Inc, Rosemont, IL) by Histopaque (Sigma, St Louis, MO) gradient centrifugation. The cells were resuspended in RPMI-HEPES supplemented with 10% CMV seronegative human AB serum and  $5 \times 10^{-5}$  mol/L 2-mercaptoethanol (Sigma), and dispensed at  $10^7$  cells/well in the 6-well plates containing autologous CMV-infected fibroblast stimulators. After 1 week of incubation at 37°C in a humidified 5% CO<sub>2</sub> atmosphere, the cultured cells were harvested, washed, and recultured at a ratio of 20:1 with fresh CMV-infected fibroblast stimulators in 6-well plates with autologous irradiated (3.5 Gy) PBMC as filler cells. Forty-eight hours after restimulation, half of the culture supernatant was replaced by fresh medium containing recombinant interleukin-2 (rIL-2; Hoffmann-LaRoche, Inc, Nutley, NJ) to achieve a final concentration of 2 U/mL. This culture system resulted in preferential persistence and expansion of CD8<sup>+</sup> T cells with CMV-specific cytolytic activity.<sup>26,27</sup>

**Cytotoxicity assay.** The panel of target cells used for each cytotoxicity assay included autologous and HLA-mismatched CMV-infected and uninfected fibroblasts. All fibroblast lines used as HLA-mismatched targets were lysable by autologous effector cells. Fibroblast targets were preincubated before use with recombinant interferon- $\gamma$  (rIFN $\gamma$ ; Schering, Inc, Kenilworth, NJ) for 48 hours at 800 U/10<sup>6</sup> cells, because this has been shown to increase the sensitivity and specificity of the assay for CMV-specific CTL by enhancing HLA-class I antigen expression.<sup>25</sup> Fibroblast targets were labeled with <sup>51</sup>Cr (100  $\mu$ Ci/10<sup>6</sup> cells; New England Nuclear, Boston, MA), and an aliquot was infected overnight with CMV AD 169 at an MOI of 5:1. Fibroblasts were then trypsinized, suspended at 10<sup>5</sup> cells/mL in 10% FCS, and 100  $\mu$ L (10<sup>4</sup> cells) dispensed in triplicate into 96-well round-bottom plates together with 100  $\mu$ L of effector cell suspensions at an effector:target (E:T) ratio of 15:1. Higher E:T ratios were also evaluated when enough lymphocytes were available. Although more lysis was detected at higher E:T ratios, it did not improve the ability to detect CMV-specific CTL in donors or patients. To confirm that specific lysis was class I-restricted, cytotoxicity was also assayed against targets preincubated for 20 minutes at 22°C with 25  $\mu$ g/mL of the anti-HLA class I monoclonal antibody (MoAb) W6/32 (a gift of Dr D. Geraghty, Fred Hutchinson Cancer Research Center).<sup>31</sup> After incubation of

**Table 1. Characteristics of the 20 Marrow Donors and Recipients**

	Donors	Recipients
Median age, in years	39	40
(range)	(18-54)	(17-56)
Sex (male/female)	11/9	11/9
Underlying disease:		
Acute nonlymphocytic leukemia		9
Acute lymphocytic leukemia		2
Chronic myelogenous leukemia		5
Hodgkin's lymphoma		1
Non-Hodgkin's lymphoma		2
RAEB in transformation		1
Pretransplant conditioning regimen:		
Cyclophosphamide/FTBI		15
VP-16/BCNU/cyclophosphamide		3
Busulfan/cyclophosphamide		2
Pretransplant CMV serology:		
Positive	20	16
Negative	0	4*
Patients alive at 3 mo after transplant		14

Abbreviations: RAEB, refractory anemia with excess of blasts; FTBI, fractionated total body irradiation.

\*Two of these four patients seroconverted after transplant.



**Table 2. Effects of  $\alpha$  Class I MoAb Blocking or Selective Depletion of T-Cell Subsets on Cytotoxicity of CMV-Specific T-Cell Cultures From Three CMV Seropositive Subjects**

Subject	Percent Specific Lysis of Targets at E:T of 15:1*				
	Untreated Effector and Target	Target Preincubated With $\alpha$ Class I MoAb	Effector Treated With		
			C Alone	$\alpha$ CD4 + C	$\alpha$ CD8 + C
1	28	14	33	26	5
2	47	25	39	38	4
3	36	21	41	38	0

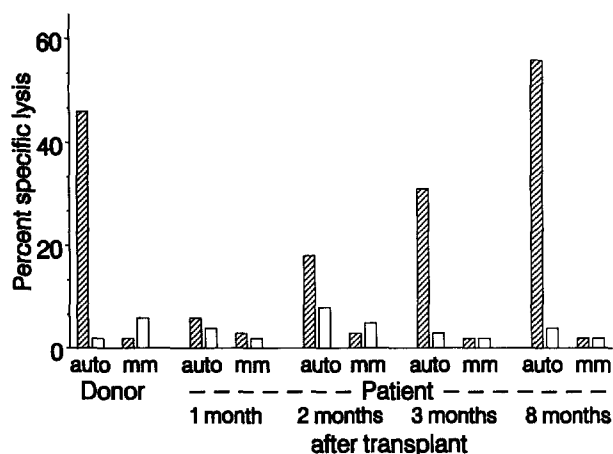
Abbreviations:  $\alpha$ , anti; C, complement.

\*Lysis of autologous CMV-infected fibroblasts targets. Lysis of autologous uninfected and HLA-mismatched CMV-infected and uninfected targets was <5% in all three subjects (data not shown).

months after allogeneic BMT (Fig 2). The preferential lysis of autologous CMV-infected fibroblasts compared with HLA-mismatched CMV-infected fibroblasts in all patients with detectable CMV-specific CTL activity (Fig 2) and the inhibition of lysis of autologous CMV-infected target cells by an average of 49% ( $P = .0002$ ) indicate that CTL activity in these cultures was MHC class I-restricted. The magnitude of the median specific cytolytic response in these recipients at 3 months after transplant was quantitatively similar to that measured among the corresponding marrow donors (Figs 1 and 2).

Of the three recipients who received marrow from a donor with nonspecific lytic activity, one developed detectable CMV-specific CTL activity after transplant and two did not. One of these recipients was only evaluable until day 30 due to early leukemic relapse, and the other was CMV seronegative and remained CMV seronegative during the 3-month study period despite receiving bone marrow from a CMV seropositive donor.

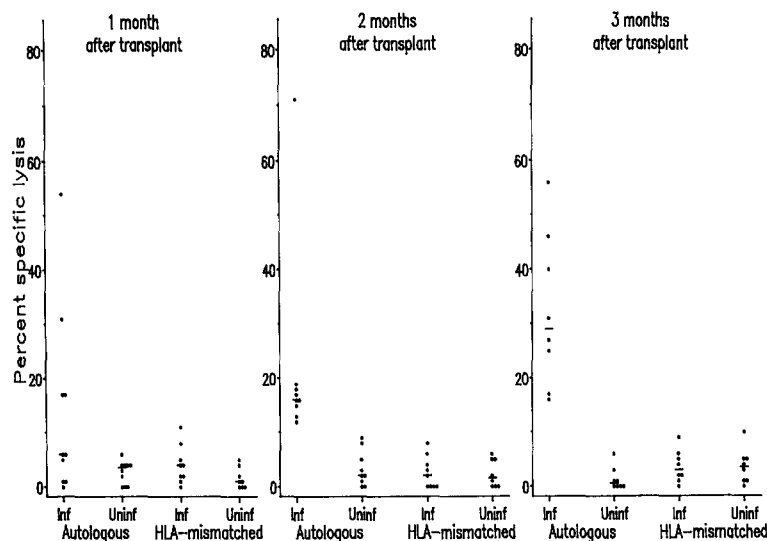
The kinetics of recovery of CMV-specific CTL activity was followed in one patient for 8 months after transplant (Fig 3). This patient was found to have asymptomatic CMV infection as reflected by urinary CMV excretion at 2 months after BMT, and concurrently demonstrated for the first time posttransplant a weak but detectable CMV-specific



**Fig 3. Restoration of CMV-specific CTL activity after allogeneic marrow transplant in a patient with long-term follow-up. CTL activity in the marrow donor is shown for comparison. Cytotoxicity was assayed against HLA-matched donor-derived (auto) and mismatched (mm) CMV-infected (▨) and uninfected (□) fibroblast targets.**

cytolytic response (Fig 3). This patient did not develop CMV disease, and demonstrated increased CMV-specific CTL activity by 3 months (CMV-specific lysis of 32%), which continued to increase and was similar at 8 months posttransplant to that detected in the healthy marrow donor (CMV-specific lysis of 56%).

The patients were monitored to determine if any clinical conditions correlated with the presence or absence of CTL activity. Moderate to severe (grade II to IV<sup>28</sup>) acute GVHD occurred in all 10 patients who failed to demonstrate a specific CTL response to CMV, but only in 4 of 10 patients with detectable CTL. Because more aggressive cyclosporine therapy might be given to patients with active GVHD, and this additional immunosuppression could influence the detection of CMV-specific CTL, trough serum levels of cyclosporine were obtained at or within 24 hours of the day on which a CTL culture was initiated. No correlation was discernible between the magnitude of cyclosporine levels



**Fig 2. Cytolytic activity of cells from 10 allogeneic marrow recipients with detectable CMV-specific CTL during the first 3 months after transplant. Cytotoxicity was assayed in this group at 1 month ( $n = 10$ ), 2 months ( $n = 9$ ), and 3 months ( $n = 8$ ) after transplant against autologous and HLA-mismatched CMV-infected (Inf) and uninfected (Uninf) fibroblast target cells at an E:T ratio of 15:1 (due to insufficient effector cell numbers in two patients, testing against HLA-mismatched fibroblast targets was incomplete at 1 and 2 months after BMT). Median indicated by horizontal bar.**

obtained on these isolated occasions, and the presence or absence of CMV-specific CTL activity. Thus, no clear relationship could be established between iatrogenic immunosuppression with cyclosporine and the generation of CTL in the BMT recipients.

**Lymphoproliferative response to CMV and PHA in marrow donors and recipients.** A lymphoproliferative assay was performed on PBMC obtained from marrow donors and recipients at the time of initiation of the CTL cultures to provide an index of CD4<sup>+</sup> T-helper cell function present in these individuals. Nineteen of the 20 marrow donors were evaluated and all 19 donors had a proliferative response to CMV antigen (median stimulation index of 26, range 4 to 197) and to PHA (median stimulation index of 36, range 3 to 432) (Fig 4). A proliferative response to CMV antigen was detected in 9 (50%) of 18 recipients tested at 1 month, in 8 (50%) of 16 patients at 2 months, and in 8 (73%) of 11 patients at 3 months after transplant. The proliferative response to CMV antigen among patients was significantly depressed at 1 month ( $P = .0009$ ) and at 2 months ( $P = .0002$ ) after BMT when compared with the response among marrow donors (Fig 4). Similarly, the proliferative response to PHA was significantly weaker among transplant recipients at 1 month ( $P = .0008$ ), 2 months ( $P < .002$ ), and at 3 months ( $P < .02$ ) after BMT when compared with the response among marrow donors (Fig 4). These results are consistent with a global defect in IL-2-producing CD4<sup>+</sup> T-cell function during this 3-month period post-BMT.

Deficient helper function provided by CD4<sup>+</sup> T cells could potentially influence the ability of the host to develop a CTL response; therefore, we compared the CMV-specific proliferative response between patients who had detectable CMV-specific CTL activity and patients with no demonstrable CTL activity. Patients with detectable CMV-specific CTL responses had greater CMV-specific proliferative responses than those without CMV-specific CTL activity, with a median stimulation index of 9 versus 2 at 1 month, 8

versus 1 at 2 months, and 20 versus 2 at 3 months after transplant. The presence of both CMV-specific CTL activity and a proliferative response among transplant recipients was assessed simultaneously on 45 occasions. There was a significant association between the results of these assays, with CMV-specific CTL and proliferative responses being simultaneously present or absent on 19 occasions each ( $P < .0001$ ). Thus, although the presence of a proliferative response to CMV was not necessarily associated with the development of a CTL response, all patients who developed CTL activity to CMV had a proliferative response to CMV at the time CMV-specific CTL were detected.

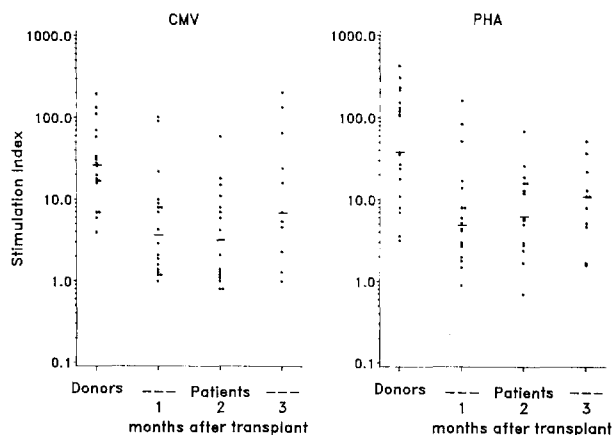
**CMV infection and the CMV-specific CTL response.** CMV infection occurred in 13 (65%) of the 20 patients at a median of 57 days after transplant (range 49 to 87 days). CMV disease developed in 7 of these 13 patients demonstrating active infection, with six having CMV pneumonia and one having CMV esophagitis. Pneumonia developed at a median of 56 days after transplant (range 53 to 87 days). Pneumonia was the direct cause of death in five of the six patients despite treatment with ganciclovir and CMV Ig,<sup>5</sup> and was a concomitant cause of death in the remaining one patient who died from *Pseudomonas* septicemia.

The 10 patients who developed a detectable CMV-specific CTL response were all CMV-seropositive before and after transplant. Seven of the 10 patients had evidence of CMV infection, but none developed CMV pneumonia, and only one developed evidence of CMV disease, as reflected by esophagitis that subsequently resolved. Three of the 10 patients with specific CTL activity had no documented evidence of CMV infection during the first 3 months after transplant.

Ten patients had no detectable CMV-specific CTL activity during the posttransplant study period. Four of these 10 patients were CMV-seronegative before BMT, and 2 of these 4 patients, despite receiving marrow from a CMV-seropositive donor, remained seronegative and were without other evidence of CMV infection throughout the posttransplant course. Eight of the 10 patients were CMV-seropositive after transplant, with six developing CMV infection. In three of these patients, the initial manifestation of CMV infection was interstitial pneumonia, and in the other three CMV was first isolated at other sites, but in all cases infection progressed to interstitial pneumonia within 7 days. Thus, 6 of 10 patients who did not have detectable CMV-specific CTL responses posttransplant developed CMV pneumonia, whereas none of the 10 patients with detectable CMV-specific CTL responses developed CMV pneumonia ( $P < .025$ ).

## DISCUSSION

The temporal relationship between the early period after BMT, in which patients are profoundly immunodeficient,<sup>16-21</sup> and the occurrence of CMV infection and severe CMV disease, has been well documented.<sup>1-3</sup> Various methods to enhance host immune function post-BMT are being explored, including administration of cytokines and growth factors that might nonspecifically hasten immunologic reconstitution. Our group has extensively evaluated in murine



**Fig 4.** Lymphoproliferative responses of marrow donors and patients to CMV antigen (left panel) and to PHA (right panel). Patients were evaluated at 1 month, 2 months, and 3 months after transplant. The stimulation index was calculated as described in Materials and Methods. A stimulation index  $\geq 4.0$  was considered positive.<sup>23</sup> Median indicated by horizontal bar.

models adoptive T-cell therapy of tumors by the transfer of tumor-specific T cells,<sup>34,35</sup> and has recently proposed to apply specific adoptive immunotherapy to the treatment of human CMV disease by transferring CMV-specific T-cell clones derived from the HLA-matched bone marrow donor into the BMT recipient. The rationale for an immunotherapeutic approach for human CMV disease in BMT recipients is supported by studies in immunosuppressed mice in which the transfer of CD8<sup>+</sup> CMV-specific CTL was shown to protect hosts from life-threatening CMV infection.<sup>9</sup> In this study, we evaluated class I MHC-restricted CMV-specific CD8<sup>+</sup> CTL responses in healthy seropositive bone marrow donors with latent CMV infection and in marrow recipients during the first 3 months after allogeneic BMT. The results demonstrate that healthy, immunocompetent seropositive hosts have detectable cytolytic responses. However, after allogeneic BMT, the class I-restricted, CMV-specific CTL response is usually absent and restoration of this response frequently requires an extended period of time. Moreover, patients without detectable CMV-specific CTL activity during the first 3 months posttransplant appear to be at a significantly increased risk of developing severe CMV disease if they develop active CMV infection.

These results extend previous observations in BMT recipients that demonstrate the presence of cytotoxic responses of lymphocytes assayed directly from the peripheral blood for CMV-infected target cells correlate with an improved outcome from CMV infection.<sup>14,15</sup> In past studies it proved difficult to reliably detect CD8<sup>+</sup> class I-restricted virus-specific CTL by direct assay of PBMC, and to determine whether lytic activity measured was mediated by class I-restricted CTL or by non-MHC restricted effector cells.<sup>14,15</sup> To more precisely study the contribution of CD8<sup>+</sup> CTL to posttransplant immunity to CMV, we have used a short-term in vitro culture system to selectively activate and expand CD8<sup>+</sup> CMV-specific CTL. This approach has been used to study the class I-restricted CTL response to CMV in healthy CMV seropositive individuals and we now demonstrate the application of this technique to study CTL reconstitution in immunocompromised BMT recipients.

The modified in vitro culture system developed in our laboratory readily detects MHC class I-restricted, CD8<sup>+</sup>, CMV-specific CTL precursors in the peripheral blood of immunocompetent CMV seropositive human subjects.<sup>26,27</sup> The T-cell nature of this cytolytic activity generated by short-term in vitro culture was demonstrated by the lysis of autologous but not HLA-mismatched CMV-infected targets, the inhibition of lysis by anti-class I MoAb, and the abrogation of lysis after selective depletion of the CD8<sup>+</sup> T-cell subset. Although these healthy CMV-seropositive individuals demonstrate no evidence of active CMV infection, the frequency of CMV-specific CTL precursors has been estimated by limiting dilution analyses to be between 1 in 5,000 and 1 in 20,000 peripheral blood T cells.<sup>12</sup> This relatively high frequency of CTL precursors persistently present throughout life suggests that this CD8<sup>+</sup> response

may play an active role in protecting the host from viral reactivation.

In contrast to healthy, immunocompetent marrow donors, CD8<sup>+</sup> class I-restricted CMV-specific CTL precursors were detectable only in 10 of 20 BMT recipients during the first 3 months after transplant. The kinetics of recovery of this response was variable in this group, with responses comparable in magnitude with those detected in donors usually present by 3 months, but potentially delayed for a much longer time (Figs 2 and 3). Moreover, no CMV-specific CTL activity was demonstrable in 16 of 20 patients during the immediate posttransplant period. The cause of absent or delayed recovery of CMV-specific CTL activity is presumably multifactorial.

Acute GVHD has been demonstrated to contribute to delayed restoration of cellular immunity,<sup>16,36,37</sup> and grade II through IV GVHD was present in all 10 patients who lacked detectable CTL activity, but in only 4 of 10 patients with a detectable CTL response. The delayed recovery of CTL activity in this situation could reflect both direct effects of GVHD and secondary consequences of the immunosuppression induced by the treatment of GVHD.

Defective T-helper cell functions in BMT recipients could also contribute to delayed or incomplete recovery of CTL activity. Impaired functioning of T-helper cells in BMT recipients has been documented by low IL-2 production,<sup>20,38,39</sup> decreased PHA-induced proliferation,<sup>17,19,38</sup> and decreased proliferation after stimulation by CMV antigen.<sup>33</sup> In our study, the proliferative response to PHA and to CMV antigen in BMT recipients was significantly weaker than among the marrow donors. Although recovery of the proliferative response to CMV has been shown not to correlate with protection from CMV disease in BMT recipients,<sup>33</sup> we observed a significant association between the presence or absence of CMV-specific T-helper cell proliferation and the development of CTL activity. Thus, the T-helper cell response may play an important role in the generation of a CTL response after BMT.

Analysis of the clinical outcome of this cohort of BMT recipients showed that a lack of detectable CMV-specific CTL activity after BMT was associated with fatal CMV pneumonia in 6 of 10 patients, whereas the presence of a specific CTL response appeared to protect from severe CMV disease. This overall frequency of CMV pneumonia in 6 of 20 patients is consistent with predictions of disease occurrence in unselected recipients of BMT from seropositive donors.<sup>1</sup> Although the contribution of CMV-specific CTL to disease protection can only be indirectly inferred from these results, the importance of antigen-specific, MHC class I-restricted CTL in controlling CMV infection and CMV disease has been clearly demonstrated in a murine CMV model. During primary infection, activated murine CMV-specific CTL can be detected in increased numbers in draining lymph nodes,<sup>8</sup> and adoptive transfer of CMV-specific, CD8<sup>+</sup> CTL into immunocompromised mice with acute CMV infection can mediate control of virus multiplication in tissues and protection from virus-induced disease and tissue destruction.<sup>8,9,40</sup> These findings raise the



possibility that similar adoptive transfer of human CMV-specific CTL clones might protect immunocompromised BMT recipients from severe CMV disease. Our laboratory has recently developed the culture methods to clone CD8<sup>+</sup> CMV-specific T cells from healthy CMV seropositive donors and to expand these cells to large numbers in long-term culture.<sup>26,27</sup> We are presently evaluating specific adoptive immunotherapy for CMV infection occurring in BMT

recipients, an approach that could have implications for the treatment of many infectious diseases occurring in immunocompromised hosts.

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