Recovery of T Cell Subsets After Autologous Bone Marrow Transplantation Is Mainly due to Proliferation of Mature T Cells in the Graft

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In 22 patients with malignancies, treated with high-dose chemoradiotherapy and autologous bone marrow transplantation (BMT), peripheral blood T cell subsets and functions were studied. In ten cytomegalovirus (CMV)-negative patients, $CD4^+$ and $CD8^+$ T cells (representing T cells of the helper/inducer phenotype and T cells of the suppressor/cytotoxic phenotype, respectively), recovered slowly and simultaneously. In 12 CMV-positive patients, however, $CD8^+$ T cells recovered more rapidly than $CD4^+$ T cells and rose to increased counts. No T cells with an immature phenotype ($CD1^+$, $OKT6^+$) were observed. Lymphocyte stimulation by herpes simplex virus infected fibroblasts (and by CMV-infected fibroblasts in CMV-positive patients) in contrast remained high and even increased after BMT in both groups. These data indicate that T cell recovery after autologous BMT is mainly due to proliferation of mature T cells present in the BM graft and not to generation of new T cells from T cell precursors.

FUNCTIONAL RECOVERY of the immune system after high-dose chemoradiotherapy and bone marrow transplantation (BMT) is reported to be slow, with a long lasting immune deficiency.[1-5] Recovery of T cells, however, occurs more rapidly and with a preponderance of $T8^+$ T cells (comprised mainly of cytotoxic/suppressor T cells).[6-10] Recovery of T cells is generally considered as a generation of T cells from bone marrow stem cells. Alternatively, this is due to proliferation of mature T cells present in the bone marrow graft. Previously, we showed that recovery of T cell subsets, especially of the $T8^+$ subset is related to the presence or absence of cytomegalovirus (CMV) infections.[11] In this study, we will present additional data that support the hypothesis that recovery of the T cell subsets is mainly due to a proliferation of mature T cells present in the graft.

MATERIALS AND METHODS

Patients. Twenty-two patients undergoing high-dose chemoradiotherapy followed by autologous BMT were studied. Only patients with at least a follow-up of 60 days were included, while half of them had a follow-up of more than 180 days. Patients with solid tumors (three with a testicular carcinoma [TC], one with a small cell carcinoma of the lung [SCCL]) were treated with 1,500 mg/m^2 cyclophosphamide and 300 mg/m^2 etoposide for four days (day $^-$5, $^-$4, $^-$3, and $^-$2) while patients with a non-Hodgkin's lymphoma (NHL) of high-grade malignancy (n = 12) and patients with acute leukemia (AL) in complete remission (n = 6) were treated with cyclophosphamide (60 mg/kg for two days) and total body irradiation (800 rad, 16 to 18 rad/min) (linear accelerator on day $^-$1) before reinfusion of autologous bone marrow (day 0). Ten patients (six NHL, two AL, one TC, and one SCCL) had a relapse of their disease two to six months after BMT, two (one TC and one NHL) had a relapse after seven and nine months. One patient with a T cell NHL developed an acute myeloid leukemia after six months, which did not respond to chemotherapy. From the nine patients alive and well without a relapse, three (two AL and one TC) have had a follow-up of more than one year and three (two NHL and one AL) more than two years.

T cell subsets. T lymphocyte subsets were studied before and at regular intervals after BMT in the mononuclear fraction of peripheral blood obtained by Ficoll-Isopaque (Pharmacia, Uppsala, Sweden) density centrifugation. T lymphocytes and T cell subsets were determined by binding of the monoclonal antibodies Leu-1 (pan T cell), Leu-2a (cluster of differentiation [CD] 8, comprising the suppressor/cytotoxic T cells subset), Leu-3a (CD4, comprising the helper/inducer T cell subset)[12] from Becton Dickinson, Rutherford, NJ, and OKT6 (CD1 immature thymic phenotype) from Ortho Pharmaceutical Laboratories (Raritan, NJ). Absolute CD4- and CD8-positive T cell counts from the leukocyte count, percentage of lymphocytes in the differential count, and the percentages of $CD4^+$ and $CD8^+$ T cells were calculated.

Antibodies to CMV and HSV. Antibodies to CMV (CMV-late antigen) were determined by a sensitive enzyme-linked immunosorbent assay (ELISA) as described before.[13]

Antibodies to herpes simplex virus (HSV) were determined in the complement fixation (CF) test and in an indirect immunofluorescence (IF) test using a laboratory isolate of HSV type 1 and HSV type 2 strain MS.[14] Titers $\geq$1:8 in the CF or IF test and $\geq$1:40 in the ELISA test were considered positive. Two patients suffered from a primary CMV infection as demonstrated by seroconversion, positive cultures for CMV, and in one case cytopathologic effects in the lung.[15] Ten patients had antibodies to CMV-LA with negative cultures before BMT (a latent CMV infection). Only three of the patients showed CMV reactivation after BMT (titer rise of more than fourfold, presence of IgM antibodies and/or positive cultures). These 12 patients are referred to as CMV-positive patients. Ten patients were negative for CMV in antibody and lymphocyte stimulation tests and viral cultures and remained so with a deliberate transfusion policy.[15] This policy included thrombocyte concentrates from CMV antibody-negative donors, filtered leukocyte-free erythrocytes, and avoidance of granulocyte concentrates.

Lymphocyte stimulation by virus-infected cells. To determine cell-mediated immunity to viral antigens, lymphocytes were cultured with fibroblasts infected with CMV (strain AD 169)[16] and fibroblasts similarly infected with HSV (type 1 strain MS).[17] Control cultures included noninfected fibroblasts. Quadruplicate cultures were set up with 1×10^5 mononuclear cells in round-bottom microtiter plates, with noninfected fibroblasts, CMV-infected fibroblasts, and HSV-infected fibroblasts in three ratios of fibroblasts to lymphocytes. Low control cultures and good stimulation was gener-

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ally observed with 10^5 mononuclear cells and 10^4 fibroblasts. These data will be presented. On the last day (day 6) of culture 1 μCi of ^{3}H-thymidine was added and the cultures were harvested eight hours later with a multisample culture harvester. The cpm of quadruplicate cultures with noninfected and CMV- or HSV-infected fibroblasts are presented. Lymphocyte stimulation by virus-infected cells was considered positive if it was at least 3 x and 1 x 10^3 cpm higher than the control cultures with noninfected fibroblasts.

Statistical analysis. Differences were evaluated by student's *t* test. *P* values < .05 were considered significant.

RESULTS

T cell subsets after BMT. As shown in Fig 1, the recovery of CD8$^+$ T cells was very rapid in the CMV-positive patients and reached higher counts than before BMT, whereas CD4$^+$ T cells recovered much more slowly. At any time after BMT the CD8$^+$ T cell count was higher than the CD4$^+$ T cell count and thus the ratio CD4/CD8 was <1.0. Recovery of CD8$^+$ T cells in the three patients with CMV reactivation was not more rigid than in the seven patients without signs of reactivation (data not shown). It was, however, more rapid in the two patients with a primary CMV infection.[11] In contrast CD8$^+$ T cells and CD4$^+$ T cells recovered simultaneously in the CMV-negative patients and did not reach pre-BMT values at 180 days after BMT (Fig 2). CD4$^+$ T cell counts reached higher values in the CMV-positive patients at day +180 than in CMV-negative patients, while CD8$^+$ T cells counts were significantly higher in CMV-positive patients at any time after BMT ($P < .005$). No CD1$^+$ (OKT6$^+$) T cells were found in the patients and the sum of CD4$^+$ and CD8$^+$ T cell counts never exceeded the total T cell counts (indicating the absence of T cells bearing CD4 and CD8; data not shown).

Immunity to HSV. In 14 patients (eight CMV$^+$ and six CMV$^-$) CF titers to HSV were positive (1:16 to 1:64). In 12 of 14 patients, lymphocyte stimulation with HSV-infected fibroblasts was positive before BMT (Fig 3). After BMT only two patients showed reactivation of HSV, one with clinical symptoms, one with a titer rise only. All 12 patients kept positive lymphocyte stimulation after BMT (Fig 3), with very strong lymphocyte stimulation in the two patients with HSV reactivation (mean cpm before BMT, 6.8 x 10^3; maximum after BMT, 133.4 x 10^3 cpm). Lymphocyte stimulation after BMT was significantly higher than pre-BMT values at day +30 ($P < .01$), day +100 ($P < .001$), and day +180 ($P < .005$). One of the two patients with CF antibodies but without lymphocyte stimulation by HSV became positive in the latter test after BMT. Two patients had only anti-HSV antibodies in IF; one of them became positive in the lymphocyte stimulation test after BMT. Six patients, negative in all tests for HSV immunity before BMT, remained negative in these tests after BMT.

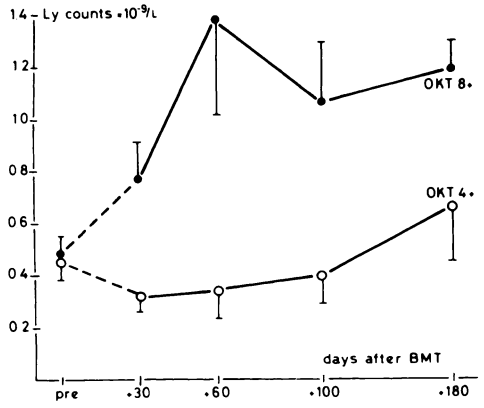

Fig 1. Recovery of T cell subsets after autologous BMT in CMV-positive patients. In 12 CMV antibody-positive patients (two with a primary CMV infection), a rapid increase of CD8$^+$ T cells (OKT8$^+$, Leu 2a$^+$) occurred, while CD4^4 T cells (OKT4$^+$ and Leu-3a$^+$) recovered slowly. Results are expressed as means ± SE.

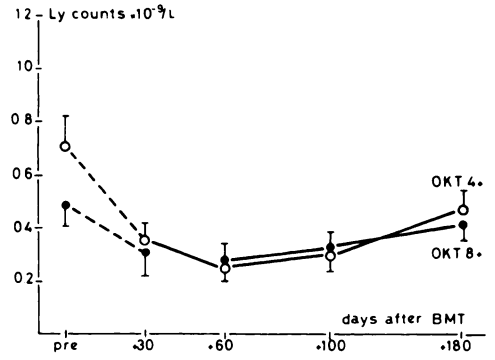

Fig 2. Recovery of T cell subsets after autologous BMT in CMV-negative patients. In ten CMV-negative patients, CD4$^+$ T cells (OKT4$^+$ and Leu-3a$^+$) and CD8$^+$ T cells (OKT8$^+$ and Leu-2a$^+$) recovered simultaneously and slowly. CD8$^+$ T cell counts were significantly lower than in the CMV-positive patients. Results are expressed as means ± SE.

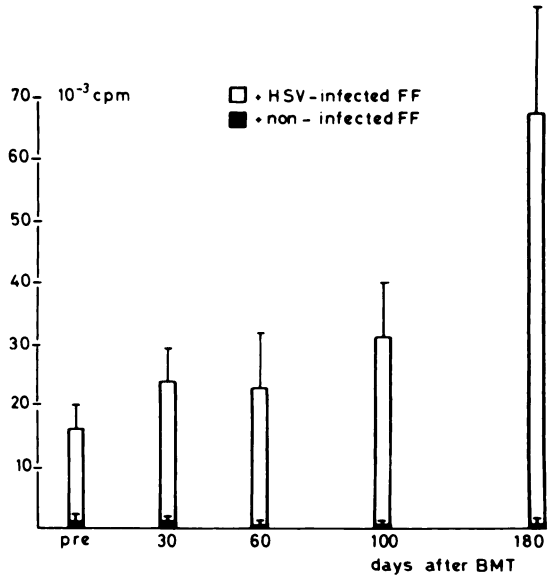

Fig 3. Lymphocyte stimulation by HSV-infected fibroblasts after autologous BMT. Lymphocyte stimulation by HSV-infected fibroblasts (open bars, mean cpm ± SE) increased significantly after BMT, while control cultures with noninfected fibroblasts (solid bars, mean cpm ± SE) remained low in the 12 patients with positive stimulation before BMT.

Immunity to CMV. As presented in Materials and Methods, two patients had a primary CMV infection after BMT; only one showed lymphocyte stimulation at day +180 after BMT. Ten patients had a latent CMV infection before BMT, with reactivation of CMV in three patients. Six of the ten patients with a latent CMV infection showed positive lymphocyte stimulation by CMV-infected fibroblasts before and after BMT (Table 1). The three patients with CMV reactivation did not show higher lymphocyte stimulation than the patients without reactivation. The ten CMV-negative patients remained negative in antibody and lymphocyte stimulation tests.

DISCUSSION

Our previous data showing that T cell recovery after autologous BMT is dependent on CMV infection,[11] are extended in this study comprising more patients and a longer follow-up. Remarkably, $CD4^+$ T cells and $CD8^+$ T cells recovered at the same speed in CMV-negative patients, while $CD8^+$ T cell recovery was much quicker than $CD4^+$ T cell recovery in CMV-positive patients, and reached levels above normal shortly after BMT. No T cells with an immature phenotype (CD1, $OKT6^+$) were observed, while a majority of the T cells had markers of activation ($HLA\text{-}DR^+$) in the CMV-positive patients, but not in the CMV-negative patients. Concomitantly, a rather rapid recovery of phytohemagglutinin (PHA) induced stimulation was observed in the CMV-negative patients, being nearly normal at day +180, while in the CMV-positive patients this remained low.[11] The lower reactivity of lymphocytes from CMV-positive patients may be explained by the lower percentage of $CD4^+$ T cells and the higher percentage of $CD8^+$ T cells in the CMV-positive patients. A high suppressor T cell activity and low helper T cell activity in pokeweed mitogen-induced B cell differentiation has been reported by us in these patients, of which the high suppressor cell activity may contribute to lower PHA reactivity.[11] It has to be emphasized that in the CMV-positive patients, reactivation of CMV was not always demonstrated after autologous BMT, while it generally occurs after allogeneic BMT.[18,19] We assume that these T cell alterations are the first signs of subclinical CMV reactivation and even occur before a titer rise or positive cultures for CMV can be demonstrated. A more rapid recovery of $CD8^+$ T cells than of $CD4^+$ T cells has been reported after allogeneic, syngeneic, and autologous BMT, but the relationship to CMV infections was not investigated in these studies.[6-10]

Surprisingly, lymphocyte stimulation by HSV-infected fibroblasts was not decreased after BMT and even significantly increased independent of the presence or absence of CMV infection. After allogeneic BMT, an increase in lymphocyte stimulation by HSV antigen has been described.[17] In our study, symptoms of HSV infection were only found in one of 14 patients with CF antibodies. Reactivation of HSV occurs often after organ transplantation in patients with CF antibodies, the incidence varying from about 50% in renal and cardiac transplantation[20,21] to about 80% in allogeneic BMT.[19] The higher degree of reactivation of herpes virus infections (HSV, varicella-zoster virus [VZV], and CMV) in these studies compared to autologous BMT may be due to an allogeneic effect since the treatment regimen in allogeneic and autologous BMT is similar, and includes total body irradiation (except in the four patients with solid tumors). The increase in lymphocyte stimulation by HSV antigen after BMT is probably caused by subclinical HSV infection similar to that proposed by Hope-Simpson for VZV infections.[22] Clearly, the presence of CF antibodies and lymphocyte stimulation was not protective against clinical HSV infection. Similar to cell-mediated immunity to HSV, lymphocyte stimulation by CMV-infected fibroblasts was as high after BMT as before in those patients who showed this stimulation already before BMT.

These data point to reactivity of mature sensitized T cells present in the BM graft to viruses present in the body in latent form, which probably are reactivated by the immune deficiency created by high-dose chemoradiotherapy. A defense mechanism against these herpes viruses shortly after BMT is of utmost importance to the host, as these viruses cause high rates of morbidity and mortality after allogeneic BMT.[18,19] It seems very unlikely that these T cells are newly formed T cells from BM precursors, which are sensitized shortly after BMT, because cellular reactivity to primary antigens is decreased for a very long time.[3] Our data suggest that resident mature (memory) T cells in the bone marrow graft are responsible for the recovery of peripheral T cell populations in the BMT recipient and that the recovery of T cell subsets is greatly influenced by the presence of CMV in the host. An additional argument for the proliferation of already sensitized mature T cells and not newly formed T cells is the absence of T cells with an immature phenotype ($CD1^+$).

In conclusion, the presence of a CMV infection in patients undergoing high-dose chemoradiotherapy and autologous BMT did result in a preponderance of $CD8^+$ T cells, which were mainly activated ($HLA\text{-}DR^+$), accompanied functionally by high suppressor T cell activity, low helper T cell activity,[11] and low mitogen-induced T cell proliferation.[11] Virus-induced proliferation (HSV and CMV) remained high or even increased after autologous BMT, suggestive of a

Table 1. Lymphocyte Stimulation With CMV-Infected Fibroblasts Before and After BMT in Six Patients

	Pre-BMT	Day +30	Day +60	Day +100
Cultures with CMV-infected fibroblasts	8.0 ± 2.4	8.2 ± 3.3	10.9 ± 3.6	14.4 ± 5.1
Cultures with non-infected fibroblasts	0.9 ± 0.3	0.7 ± 0.3	0.7 ± 0.2	0.4 ± 0.2

Values are expressed as mean cpm $\times 10^{-3}$ ± SEM.

proliferation of already sensitized mature T cells present in the graft.

Studies of allogeneic or autologous BMT, in which T cells are removed from the BM graft,[23,24] may give a definitive answer as to whether T cell recovery is due to proliferation of mature T cells, generation of new T cells from BM precursors, or a combination of these two pathways.

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