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


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+, OKT8+) 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.

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Supported by a grant from the Stichting Koningin Wilhelmina Fonds, Nederlandse Organisatie voor de Kankerbestrijding (UUKC-Haem 81).

Submitted Aug 30, 1984; accepted Feb 20, 1985.

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ally observed with $10^5$ mononuclear cells and $10^6$ fibroblasts. These data will be presented. On the last day (day 6) of culture 1 nCi 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 $3 \times 10^4$ 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 CD$^8^+$ T cells was very rapid in the CMV-positive patients and reached higher counts than before BMT, whereas CD$^4^+$ T cells recovered much more slowly. At any time after BMT the CD$^8^+$ T cell count was significantly higher than the CD$^4^+$ T cell count and thus the ratio CD4/CD8 was $<1.0$. Recovery of CD$^8^+$ 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. In contrast CD$^8^+$ T cells and CD$^4^+$ T cells recovered simultaneously in the CMV-negative patients and did not reach pre-BMT values at 180 days after BMT (Fig 2). CD$^4^+$ T cell counts reached higher values in the CMV-positive patients at day +180 than in CMV-negative patients, while CD$^8^+$ T cell counts were significantly higher in CMV-positive patients at any time after BMT ($P < .005$). No CD$^1^+$ (OKT6+) T cells were found in the patients and the sum of CD$^4^+$ and CD$^8^+$ 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, $6.8 \times 10^4$; maximum after BMT, $133.4 \times 10^4$ 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.
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-DR) in the CMV-positive 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 to about 80% in allogeneic BMT.18 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 allologic 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.2 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-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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre-BMT</th>
<th>Day +30</th>
<th>Day +60</th>
<th>Day +100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultures with CMV-infected fibroblasts</td>
<td>8.0 ± 2.4</td>
<td>8.2 ± 3.3</td>
<td>10.9 ± 3.6</td>
<td>14.4 ± 5.1</td>
</tr>
<tr>
<td>Cultures with non-infected fibroblasts</td>
<td>0.9 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.2</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

Values are expressed as mean cpm x 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, 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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