Potential for Cytokine and Product Manipulation to Improve the Results of Autologous Stem Cell Transplantation for Rheumatoid Arthritis

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ABSTRACT. The eradication of autoreactive T cells by high dose therapy and stem cell transplantation and the resultant alterations in the immunologic network, thymic reeducation, and peripheral tolerance provide treatment mechanisms for autoimmune and inflammatory diseases. One outcome of autologous stem cell transplantation is a significant decrease in the CD4:CD8 ratio due to a loss in CD4+ cells and a depression in T cell function. Mechanistically, the loss of T cell function is associated with an increased frequency of circulating monocytes, their expression of Fas ligand (FasL), and a high frequency of apoptotic CD4+ T cells. This suggests that activated Fas+ CD4+ lymphocytes interact with FasL+ monocytes, resulting in apoptosis, preferential deletion of CD4+ T cells, an inversion in the CD4:CD8 ratio, and depressed T cell function. These observations suggest the potential for immune regulation using stem cell manipulation or posttransplant cytokine administration as therapeutic strategies for autoimmune/inflammatory diseases. (J Rheumatol 2001;28 Suppl 64:32–8)

Key Indexing Terms:
PERIPHERAL TOLERANCE RHEUMATOID ARTHRITIS STEM CELL TRANSPLANTATION

INTRODUCTION

High dose chemotherapy (HDT) followed by stem cell transplantation (SCT) is used to treat a variety of advanced malignancies[1], as well as autoimmune and inflammatory conditions[2-8]. We and others have observed an immune dysfunction in the peripheral blood (PB) of patients following HDT and SCT despite restoration of T cell numbers[9-11]. This immunologic dysfunction includes an inversion in the CD4:CD8 T cell ratio and a depression of T cell function[9]. Studies from our laboratory and recently others have also revealed a cell mediated suppression of T cell function in stem cell products and PB of cancer patients[9,12-14]. This loss of function is associated with increased T cell apoptosis[15-17], which occurs predominantly with CD4+ T cell subpopulations. The induction of apoptosis is mediated, at least in part, by Fas ligand (FasL) expression on monocytes, which are found in significantly higher numbers following SCT[17]. In addition, high levels of type 2 associated cytokines are found in the infused T cells and monocytes as well as in PB posttransplant[18-20].

Apoptosis provides one mechanism for peripheral CD4+ T cell homeostasis. It is a highly regulated cell process dependent upon the expression of a family of death inducing ligands, including FasL. Enhanced monocyte dependent apoptosis of uninfected CD4+ T cells is postulated to contribute to CD4+ T cell depletion in individuals with human immunodeficiency virus infection, which leads to an inverted CD4:CD8 T cell ratio[21]. A similar profile of immune dysregulation is found following HDT and SCT and provides a potential therapeutic strategy for autoimmune and inflammatory diseases.

Preclinical models and anecdotal evidence[2-8] from patients transplanted for the treatment of malignant disease who had coincidental autoimmune or inflammatory disease have provided support for the concept that the clonal T cell populations responsible for the autoimmune/inflammatory disease processes may be altered by HDT and SCT. Eradication of autoreactive T cells by the conditioning regimen, redistribution of an altered immunologic network or thymic reeducation, and/or induction of peripheral tolerance provide possible mechanisms for the apparent clinical response[22-25]. Further, we and others have shown a type 2 cytokine profile following transplantation, perhaps in association with high levels and/or multiple cycles of chemotherapy, which may be critical in the maintenance of tolerance[19]. Thus, control of the immunoregulatory pathways may be multifactorial, including clonal deletion via activation induced cell death as well as regulation via type 2 cytokines for the maintenance of immune tolerance. If true, this suggests that mini-induction protocols might be less effective in the induction and maintenance of tolerance than more traditional myeloablative protocols. Support for the role

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of a type 2 response in tolerance was recently provided by a study whereby the prognostic significance of interleukin 10 (IL-10) production was studied in patients prior to an allogeneic bone marrow transplantation (BMT)[26]. High spontaneous IL-10 production was correlated with a subsequent low incidence of graft-versus-host disease (GVHD) and transplant related mortality compared to patients with low or intermediate levels of IL-10[26]. Support for the role of IL-10 is also provided by the observation that increased IL-10 production by mononuclear cells is associated with tolerance in patients with severe combined immunodeficiency disease following haplotype identical BMT[27].

RESULTS

T cell reconstitution. In a series of studies, we examined the immune phenotypic and functional reconstitution following high dose chemotherapy and autologous stem cell transplant for treatment of non-Hodgkin's lymphoma (NHL)[9,13,14,17,19,28]. The frequency and absolute numbers of CD3+ and CD4+, but not CD8+, cells were decreased pretransplant in the PB of NHL patients who were candidates for HDT and SCT. The frequency and absolute number of T lymphocyte subsets premobilization and on days 15, 30, 100, and 365 posttransplant are shown in Figure 1. These studies reveal that the frequency and absolute number of CD3+ cells were significantly lower on day 15 posttransplant compared to normal PB and pretransplant levels. The frequency of CD3+ cells returned to pretransplant and normal values on day 30 post-SCT (Figure 1). In contrast, no significant increase in the frequency or absolute number of CD4+ cells was observed post-SCT compared to pretransplant. Indeed, the frequency and absolute number of CD4+ cells were significantly lower pre- and posttransplant on all days measured compared to those observed in the PB of normal donors (all p values ≤ 0.001) (Figure 1). The absolute number and frequency of CD8+ cells were similar to those observed with CD3+ cells. Further, the absolute number and frequency of CD8+ cells were significantly increased on day 30 post-SCT compared to normal PB values ($p = 0.013$ and $p = 0.002$, respectively) (Figure 1). In association with the changes in CD4+ and CD8 cells, the CD4:CD8 T cell ratio was consistently decreased in SCT patients compared to normal donors (Figure 2).

Functional analysis of PB cells post-SCT compared to normal PB cells. The PB mononuclear cells (PBMC) from the SCT patients had significantly decreased phytohemagglutinin (PHA) mitogenic activity during the first 365 days after HDT and transplant compared to normal PBMC ($p \leq 0.001$) (Figure 3). In agreement with the loss of T cell mitogenic activity, PBMC from the SCT patients had significantly higher levels of cell dependent T cell inhibitory (CDTI) activity on days 15 and 30 posttransplant (Figure 3) compared to normal PBMC. The CDTI assay is a co-culture assay of allogeneic PBMC from a normal healthy donor with varying numbers of irradiated (500 cGy) PBMC from either stem cell product or PB of a transplant patient at various inhibitor to responder ratios in the presence or absence of PHA[13,14,17]. As reported, the CDTI activity is directly correlated with monocyte frequency[29] and can be partially blocked by establishing the co-cultures in the presence of neutralizing antibody to FasL (Figure 4).

Frequency of apoptotic T lymphocytes in PB of patients undergoing HDT and PSCT. In another series of studies using PBMC from patients who received HDT and SCT for treatment of metastatic breast cancer, we examined the potential mechanism of CD4+ T cell depression in PB. These studies revealed that the frequency of apoptotic CD4+ T cells was significantly higher on days 10, 14, and 26, but not day 100, in PB after HDT and SCT compared to normal individuals, and on days 10 and 14 relative to pretransplant levels (Figure 5). Further, the patients did not have a significant difference in the frequency of apoptotic CD4+ T cells prior to transplant compared to normal donors, suggesting that prior chemotherapy had no role in apoptosis. The frequency of apoptotic CD8+ T cells was also significantly higher on days 14 and 26 compared to pretransplant levels. However, on days 10 and 14, the frequency of apoptotic CD4+ T cells was significantly higher than that of CD8+ T cells, suggesting a preferential apoptosis of CD4+ T cells in patients undergoing HDT and SCT. Interestingly, the frequency of apoptotic CD8+ T cells before transplant was significantly lower than that observed in normal PB.

FasL (CD95L) expression on CD14+ monocytes and frequency of monocytes. Studies examining the source of apoptosis were undertaken predicated on the prior *in vivo* studies, which suggested a role for FasL mediation (Figure 4). In these studies, we examined the expression of FasL on CD4+, CD8+, and CD56+ T cells and natural killer cells. These studies (results not shown) revealed no significant difference in the expression of FasL on T and natural killer cells. However, a significantly increased frequency of FasL expression was observed on CD14+ monocytes on days 10, 14, 26, and 100 after HDT and SCT compared to pretransplant levels and to monocytes from normal PB (Figure 6A). In addition to an increase in FasL+ monocytes, there were also significant increases in frequency and absolute number of monocytes in the PB at all time points examined following HDT and PSCT (Figure 6B). In contrast, there were no significant differences in frequency or absolute number of monocytes prior to HDT and SCT compared to normal donors (Figure 6B). A comparison of Figure 5 (apoptotic CD4+ cells) and Figure 6 (monocyte number and frequency and FasL+ monocyte) suggests an association between FasL expression and apoptosis.

DISCUSSION

The majority of autoimmune/inflammatory diseases are not life threatening, although with time they can induce functional disability despite therapeutic intervention. Chronic late stage diseases have severe and life threatening effects, as well as treatment related morbidity. Aggressive intervention,

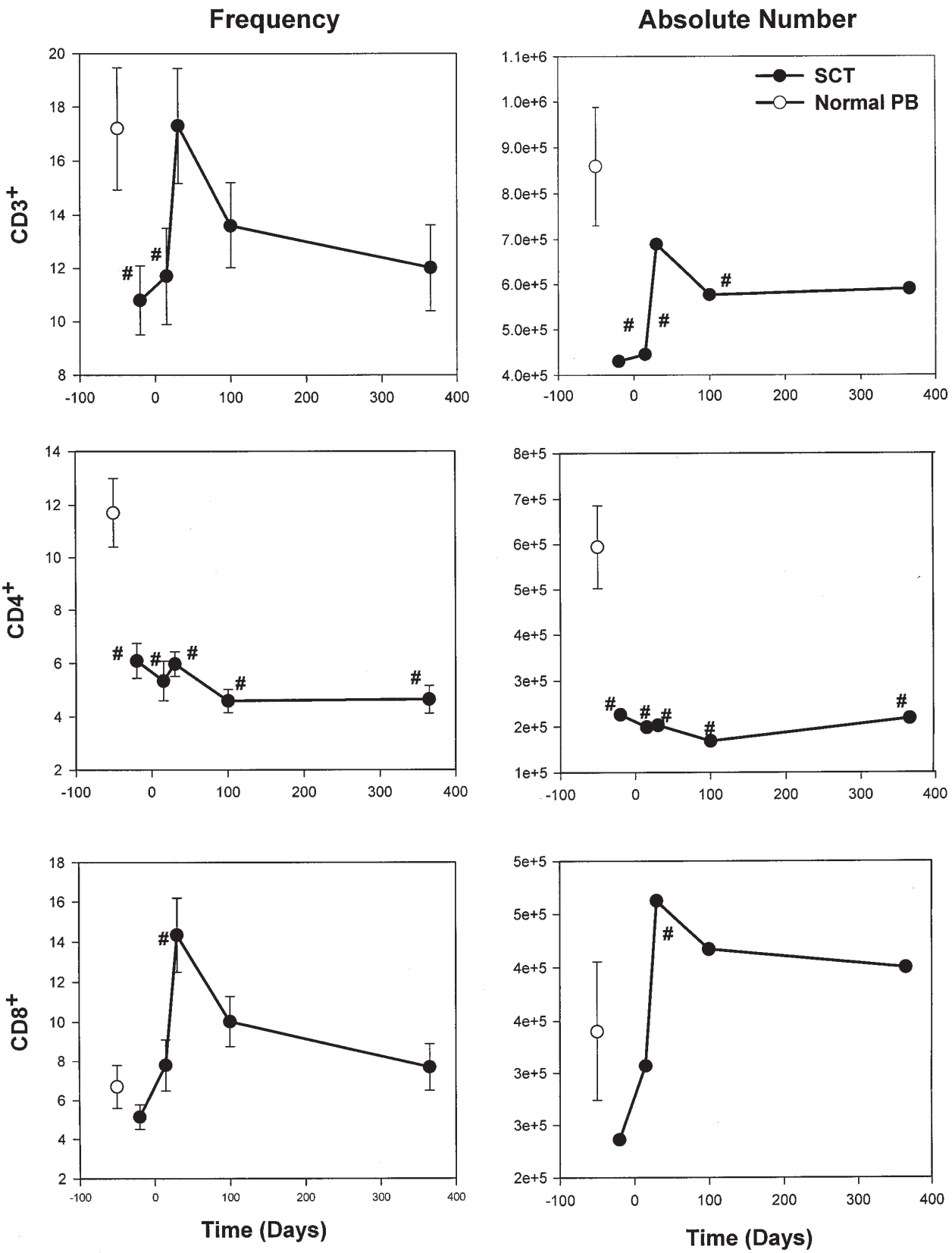

Figure 1. The frequency and absolute number of CD3+, CD4+, and CD8+ premobilization and on days 15, 30, 100, and 365 posttransplant. #Significantly different from normal peripheral blood leukocytes ($p \leq 0.005$). Values represent the mean percentage of positive cells ± standard error of the mean (SEM).

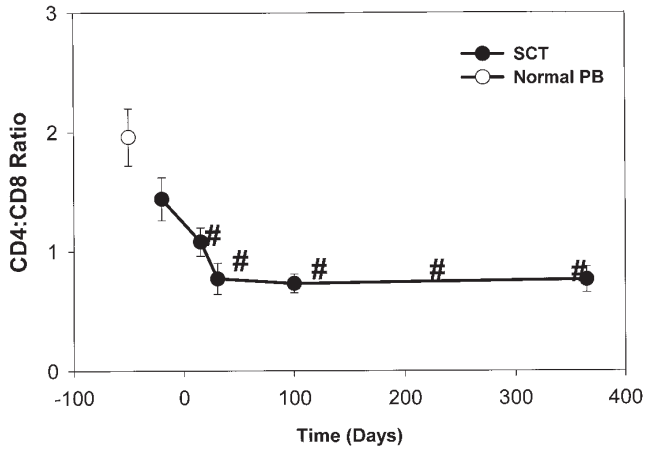

Figure 2. The CD4:CD8 ratio in SCT patients and normal PBL, premobilization and on days 15, 30, 100, and 365 posttransplant. Values represent the mean percentage of positive cells ± SEM. #Significant difference between lymphocyte levels in SCT patients and normal donors ($p \leq 0.005$).

including HDT supported by SCT, appears to be a promising approach. Although autologous SCT has become an established treatment for some malignant diseases, it has yet to become an established treatment for autoimmune and inflammatory diseases. Recent advances in stem cell mobilization and manipulation and supportive care have significantly reduced the toxicity of this therapy, making it amenable for less life threatening diseases. Anecdotal case reports have suggested that allogeneic and autologous SCT, performed for other indications, also have resulted in coincidental objective responses in patients with autoimmune or inflammatory diseases[3-7]. However, early relapses have been observed following autologous SCT in patients with autoimmune and inflammatory diseases[30]. Nonetheless, it should be noted that some patients became resensitized to nonsteroidal antiinflammatory drugs to which they were resistant prior to SCT, suggesting the utility of autologous SCT despite disease relapse.

Autologous stem cell products, particularly ones obtained following mobilization and apheresis, contain significant numbers of T cells, which are reinfused into the patient. Presumably, these infused T cells include immunoreactive cells, which can contribute to the autoimmune/inflammatory conditions. A report by Euler[30] described the early recurrence/persistence of autoimmune disease after transplant with unmanipulated autologous stem cell products. Since that time, several techniques have been employed to reduce the infusion of T cells. These include depletion of T cells (negative selection) or positive selection of the hematopoietic (CD34+) stem cells. Given the rigor of both techniques, questions remain regarding the level of T cell depletion that should be targeted. Experimental studies have shown that allograft patients who receive less than 10^5 T cells/kg body weight develop no GVHD.[31] While allogeneic studies may not be directly applicable, the results suggest that the infusion of less than 1×10^5 T cells/kg may be a reasonable target dose, although the role of T cell depletion of an autologous stem cell product remains debatable[22,23,30]. Further, the observed response failure in patients who received T cell depleted grafts and responses in patients who received unmanipulated products suggest that disease status may be more relevant than T cell purging of the graft.

Recently, Tyndall, *et al*[32] published interim results from an ongoing multicenter, prospective phase I-II trial of patients

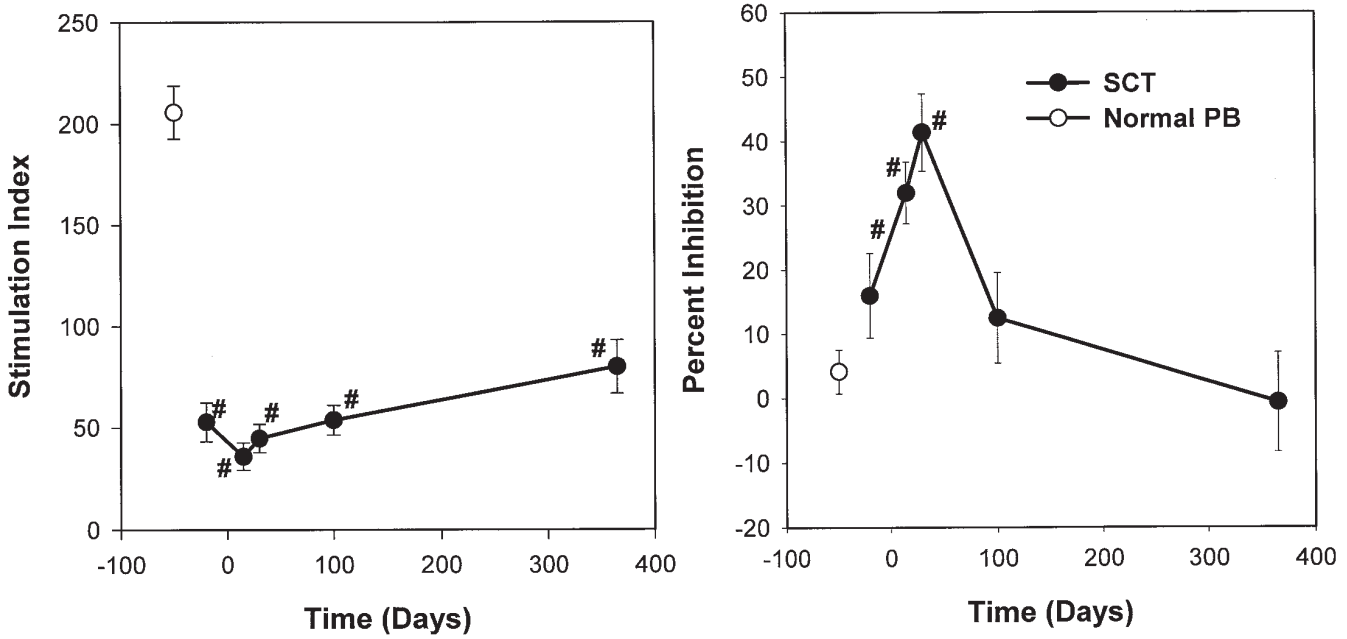

Figure 3. Functional analysis of peripheral blood mononuclear cells (PBMC) from SCT compared to normal PBMC. Assays include PHA mitogenesis and a coculture assay of normal PBMC with irradiated effector cells from an apheresis product in a CDTI assay. #Significantly different from normal PBMC ($p \leq 0.005$). Values represent the mean ± SEM.

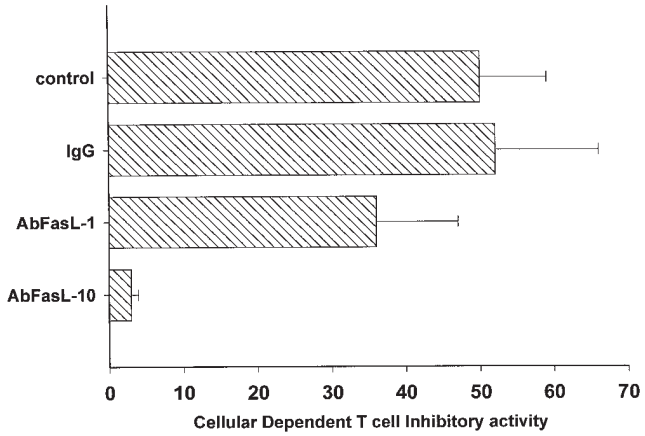

Figure 4. Effect of the addition of anti-FasL with neutralizing antibody (1 or 10 µg/ml) on cellular dependent T cell inhibitory activity compared to antibody isotype control cultures (10 µg/ml) and control assays. Functional analysis of PB cells from PBMC from SCT patients compared to normal PBMC. *Significantly different from control culture without antibodies. Values represent the mean ± SEM.

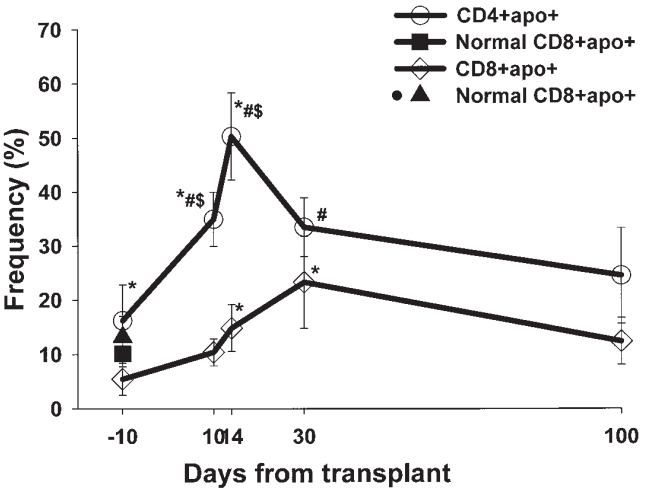

Figure 5. The frequency of apoptotic CD4+ or CD8+ T cells in a total cell gate of CD4+ or CD8+ T cells determined using 3 color flow cytometry. Results are reported as the mean ± SEM. *Significantly different from pretransplant levels ($p \leq 0.05$). #Significantly different from normal PB ($p \leq 0.05$). $Significant difference in the frequency of apoptotic CD4 and CD8 T cells ($p \leq 0.05$).

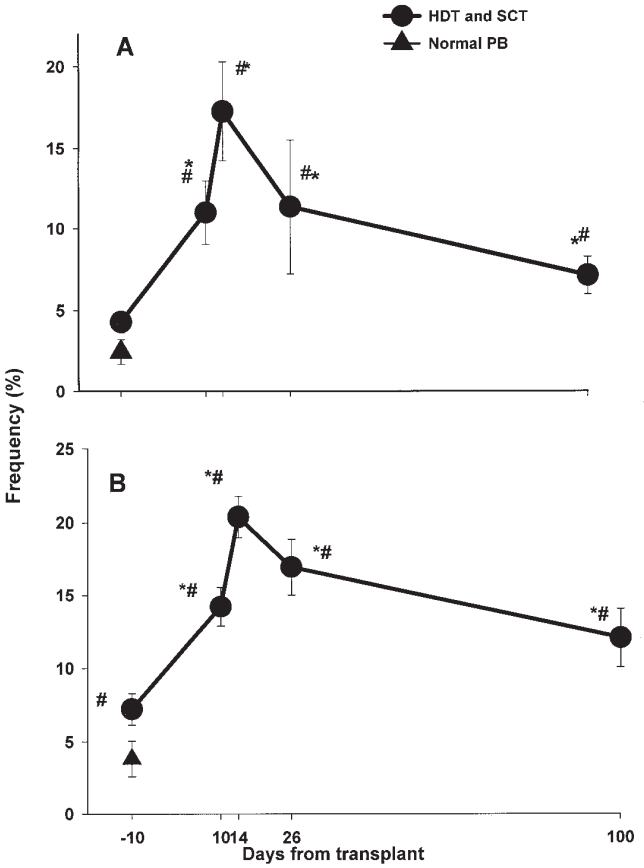

Figure 6. A. The frequency of CD14+ monocytes in the PB of patients undergoing HDT and SCT was determined by 3 color flow cytometry. Results are reported as the mean ± SEM. *Significantly different from pretransplant levels ($p \leq 0.05$). #Significantly different from normal PB ($p \leq 0.05$). B. Flow cytometry analysis of FasL expression on CD14+ monocytes in the PB of breast cancer patients following HDT and SCT. The results are shown as frequency of FasL+ cells in the total monocyte cell gate. Results are reported as the mean ± SEM. *Significantly different from pretransplant levels ($p \leq 0.05$). #Significantly different from normal PB ($p \leq 0.05$).

with autoimmune diseases who received an autologous SCT. Of these consecutive patients treated with autologous SCT, 60 patients were evaluable for response, of whom 65% showed improvement. Perhaps the most important finding was that these patients had a transplant related mortality rate similar to that observed in patients with non-Hodgkin's lymphoma, suggesting that autologous SCT is relatively safe.

The selective depletion of CD4+ T cells is one mechanism associated with the peripheral tolerance observed following HDT and SCT. In our studies, we associated the loss of T cell function with both frequency of monocytes and monocytes expressing FasL in the PB[17]. We suggest that the preferential deletion of CD4+ T cells is due to their increased expression of Fas compared to CD8+ T cells, which have a normal frequency of Fas expression, and high frequency of monocytes expressing FasL in the PB. These studies suggest a possible mechanism for immune dysfunction, peripheral tolerance, and depressed CD4:CD8 T cell ratio that are observed after HDT and SCT[9,16,28]. We hypothesize that the high frequency of FasL expression on monocytes is associated with the secretion of high levels of monocyte-activating cytokines by T cells after transplant[20]. *Ex vivo* studies have revealed that monocytes in mobilized PSC products, as well as in PB following transplant, inhibit T cell function[9,13,14,28] by inducing T cell apoptosis[13]. While these studies do not provide a definite cause and effect relationship, the high frequency of apoptotic CD4+ T cells in the PB of patients following HDT and SCT

might contribute to the development of tolerance for epitopes critical to disease expression. Reports by Donnenberg, *et al*[33,34] also suggest that T cell apoptosis parallels lymphopoiesis in patients who have had bone marrow transplants. The requirement for T cell activation appears to be a common feature of monocyte dependent T cell apoptosis mediated by Fas-FasL interaction[35]. Our previous results suggest that T cells and monocytes in the PB of HDT and SCT patients are highly activated based on the expression of immunoregulatory cytokines[19,20]. Thus, we suggest that activated CD4+ T cells in the PB of HDT and SCT patients undergo apoptosis after encountering monocytes expressing FasL. This provides a hypothetical mechanism and a strategy to dampen the activated immune response and control autoimmune or inflammatory diseases.

In summary, we suggest that activated Fas+ CD4+ T lymphocytes interact with activated monocytes that express FasL, resulting in apoptosis, which leads to the deletion of clonal populations of CD4+ T cells. This interaction provides a potential mechanism to induce peripheral tolerance with therapeutic implications in patients with autoimmune or inflammatory diseases. Further, manipulation of stem cell products or cytokine support posttransplant may be an important adjuvant therapeutic strategy. One such manipulation might include the removal of T cells from the product with the retention of monocytes/dendritic cells. If used with a granulocyte-colony stimulating factor mobilized product, which biases to a DC2 and type 2 response, this technique might help induce tolerance. Early data suggest some clinical benefit to HDT and SCT for autoimmune disease, although there is the question of what form the product should take. In addition to maximal protocols, objective criteria for treatment responses as well as analysis of nonspecific and specific immunologic reconstitution are needed to help determine strategies for future trials including low dose conditioning, use of monoclonal antibodies for the depletion of lymphocyte subsets, and blockade of costimulatory factors. It appears that manipulation of the stem cell product has the potential to control immunologic reconstitution. Further, both the conditioning regimen and the actual transplant product have immunosuppressive characteristics, and it is likely that these can be optimized to induce peripheral tolerance.

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