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Immune Reconstitution after Autologous Hematopoietic Transplantation with Lin⁻, CD34⁺, Thy-1^{LO} Selected or Intact Stem Cell Products*

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Abstract

In sequential studies, we compared immune reconstitution following high dose chemotherapy (HDT) and stem cell transplantation (SCT) using intact mobilized peripheral blood stem cell (PSC) in intermediate grade non-Hodgkin's lymphoma (NHL) patients and CD34⁺, lineage negative (Lin⁻), Thy-1^{LO} (CD34⁺Lin⁻Thy-1^{LO}) stem cells in low-grade NHL patients. Cytokine expression and cellular phenotype and function were used as the basis for comparison. Despite differences in cellular composition of the stem cell grafts, immune reconstitution in both groups was similar. Significantly higher levels of type 1 and 2-associated cytokine messenger ribonucleic acid (mRNA) were observed both prior to and following transplant in the peripheral blood (PB) of both cohorts as compared to normal individuals. Similar levels of interleukin (IL)-4, IL-10, interferon-gamma (IFN- γ), and tumor necrosis factor-alpha (TNF- α) mRNA were seen in PB mononuclear cells following transplant with either product. In contrast, patients receiving isolated CD34⁺Lin⁻Thy-1^{LO} cells expressed significantly higher IL-2 levels at all times examined post-transplant. Despite the high levels of cytokine gene expression and rapid restoration to pretransplant levels of CD3 cell number by day 30, T cell function and CD4:CD8 and CD4⁺CD45RA:CD4⁺CD45RO⁺ ratios were significantly depressed in both cohorts compared to normal donors, and significantly lower in patients transplanted with CD34⁺Lin⁻Thy-1^{LO} compared to patients receiving an intact PSC product. These data suggest that the peripheral tolerance in patients receiving HDT and an autologous SCT occurs independent

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of graft composition, although immune function and CD4 recovery is better facilitated by transplantation of an intact product.

Introduction

Autologous stem cell transplantation (SCT) is used increasingly for the treatment of disseminated lymphoma.¹⁻⁶ Like bone marrow (BM), peripheral blood stem cell (PSC) autografts from patients with lymphoma frequently contain malignant cells as revealed by sensitive immunocytochemical or molecular techniques.^{7;8} As tumor cells reinfused with the graft may contribute to relapse after autologous SCT,^{9;10} positive selection of CD34⁺ stem cells has been used to reduce the frequency of contaminating tumor cells.^{5;8;11-17}

Growth factor-mobilized PSC products, collected by leukapheresis, contain large numbers of lymphocytes and monocytes,¹⁸⁻²⁷ which may have therapeutic potential, as well as contribute to a rapid immune recovery.^{19-21;25;28-30} The role of the infused cells on long-term immunological reconstitution remains unclear.^{31;32} It has been reported that T and B cell function remained significantly impaired for a prolonged period (>6 months) following transplantation with a CD34⁺-enriched stem cell product.^{11;32-34} However, it is unresolved whether CD34⁺ cell selection, which results in extensive depletion of T lymphocytes in the graft, represents a risk factor for delayed immune recovery following transplantation.^{15;35}

In the present study, we compared immunological reconstitution in patients receiving high dose chemotherapy (HDT) and transplantation with either an autologous, intact PSC product or enriched CD34⁺Lin⁻Thy-1^{lo} stem cells. The immune reconstitution patterns were similar, irrespective of the stem cell grafts used, with early recovery of monocytes followed by CD8⁺ T cells. Significantly increased monocyte-dependent T cell inhibitory (MDTI) activity and cytokine-specific messenger ribonucleic acid (mRNA) levels, and depressed CD4⁺:CD8⁺ T cell ratios in the peripheral blood mononuclear cells (PBMC) of all transplant patients were observed. Despite the high levels of cytokine mRNA expression and rapid CD3 T cell recovery, T cell function (phytohemagglutinin [PHA] mitogenesis) and CD4:CD8 T cell ratios were depressed following HDT and SCT and were significantly lower in patients receiving CD34⁺Lin⁻Thy-1^{lo} products.

Materials and Methods

Patients and peripheral blood (PB) collection

A total of 35 patients diagnosed with primary refractory non-Hodgkin's lymphoma (NHL), relapsed NHL, high-risk NHL, and first complete or partial remission NHL, who had received multiple cycles of prior chemotherapy, were included in the cohort that received HDT and an intact product (Table 1). These samples were obtained using protocols approved by the appropriate Institutional Review Board and following written informed consent. Prior to the PSC collection, the patients received subcutaneous daily injections of 10 µg/kg/day granulocyte colony-stimulating factor (G-CSF) (Filgrastim, Amgen, Thousand Oaks, CA) for 4 to 5 days. PSC apheresis was started when the white blood cell (WBC) count reached 10,000 cells/µL (3 to 4 days after initiation of G-CSF administration). Leukapheresis was performed as an outpatient procedure with a COBE Spectra (COBE BCT, Inc., Lakewood, CO). Each patient underwent 3 to 15 leukaphereses (median 4). A cohort of low-grade NHL patients (n=18, Table 1) was subsequently recruited to receive HDT and transplants with Lin⁻CD34⁺Thy-1^{lo} cells isolated by Baxter isolation and high speed cell sorting (Systemix, Irvine CA). Following SCT with either product, all patients received G-CSF until their absolute neutrophil count was 1,500/mm³ on 2 consecutive days. PB samples were obtained prior to

mobilization and apheresis and on days 15, 30, 100 and 365, with day 1 the day of transplantation.

Cell Isolation

PB samples from NHL patients and normal healthy donors were diluted 1:2 in Hanks' balanced salt solution (HBSS) (Gibco BRL, Grand Island, NY), layered on Ficoll-Hypaque (Organon Teknika, Durham, NC), and mononuclear cells (MNC) isolated as described in previous studies.^{26;36} The cells were washed twice in HBSS and resuspended in RPMI-1640 (Gibco BRL, Grand Island, NY) containing 10% fetal bovine serum (FBS) (Hyclone, Logan, UT), HEPES (Research Organics, Cleveland, OH), gentamicin (Gibco BRL, Grand Island, NY) and L-glutamine (Gibco BRL, Grand Island, NY).

Flow cytometric analysis

For phenotypic analysis, contaminating red blood cells (RBC) were lysed with ammonium chloride lysis buffer. The WBC were counted and adjusted to 1×10^6 cells/ml in phosphate-buffered saline (PBS) (Gibco BRL, Grand Island, NY) containing 2.5% FBS to maintain cell viability. Aliquots of 1×10^5 cells were stained for 30 minutes at 4°C with each cocktail of monoclonal antibodies. The antibodies used in this study were phycoerythrin (PE)-labeled CD4, fluorescein isothiocyanate (FITC)-labeled CD8, and biotin-labeled CD14 obtained from Becton-Dickinson (San Jose, CA) and Coulter Corporation (Hialeah, FL). After incubating and washing, streptavidin allophycocyanin (APC) was added as the third fluorochrome. Data was acquired on a Becton Dickinson FACStar^{Plus}. Phenotypic analysis was performed using the Attractors and CellQuest software from Becton Dickinson as described earlier.^{23;25;29;36;37}

Mitogen response

MNCs (1×10^5 cells/well) were cultured in the presence or absence of an optimal concentration of PHA (0.5 µg/ml) in 96-well flat bottom microtiter plates (Falcon, Becton Dickinson Labware, Lincoln Park, NJ) for 96 h. For the final 18 h of culture, 1 µCi of ³H-thymidine (Amersham Life Sciences, Arlington Heights, IL; specific activity, 5 Ci/mmol) was added to each well. The cells were then harvested onto fiberglass filters using a 96-well harvester (Packard Instruments, Downers Grove, IL). The filters were allowed to air dry, scintillation cocktail added, and the samples counted in a Packard Multi-Well Beta Counter (Packard Instruments, Downers Grove, IL). Specific incorporation of ³H-thymidine was compared to control cells (no mitogen stimulus). Results are presented as a stimulation index calculated using the following formula (counts per minute [cpm]):

$$\text{Stimulation index} = \frac{\text{cpm in cultures with PHA}}{\text{cpm in cultures without PHA}} \times 100$$

Monocyte-dependent T cell inhibitory (MDTI) activity

The methodology for the co-culture assay to measure MDTI activity has been previously described.³⁷ Briefly, normal Ficoll-Hypaque purified PBMCs as responder cells (1×10^5) were co-cultured with varying numbers of irradiated (500 cGY), putative inhibitory cells with an optimal concentration of PHA (0.5 µg/ml), and results from an inhibitor-to-responder (I:R) ratio of 2:1 are reported. Cells were then cultured at 37°C and 5% CO₂ for 96 h in RPMI 1640 medium containing 10% FBS. The mitogenic response of the responder cells was assayed by pulsing with 1.0 µCi/well of ³H-thymidine (Amersham Life Sciences, Arlington Heights, IL; specific activity, 5 Ci/mmol) over the final 18 h of culture. Cells were harvested with a 96-well harvester onto fiberglass filters and radioactivity counted in a Packard multi-well beta counter (Packard Instruments, Downers Grove, IL). Percent MDTI activity was calculated using the formula:

$$\% \text{ T cell inhibitory activity} = 1 - \frac{\text{cpm in co-cultures of Inhibitor plus Responders (I + R) with PHA}}{\text{cpm in responders only with PHA}} \times 100$$

Analysis of cytokine mRNA expression

Total cellular RNA was isolated from PBMC using Trizol® reagent (Gibco BRL, Gaithersburg, MD)³⁸ and reverse-transcription based polymerase chain reaction (RT-PCR) was performed as previously described.³⁹ First-strand complementary deoxyribonucleic acid (cDNA) was synthesized using total RNA (2 µg), oligo (dT)18 primer, and superscript RT (Gibco BRL, Gaithersburg, MD). Two µl of first-strand cDNA (1:10 dilutions) were amplified using PCR primer sets³⁹ and a DNA thermal cycler (Perkin Elmer, Foster City, CA). Each cycle set used a denaturing temperature (94° C) for 60 seconds, annealing temperatures (55° C for β-actin, interleukin (IL)-4, IL-8, IL-10, and tumor necrosis factor-α (TNF-α); and 53° C for IL-2 and interferon-gamma (IFN-γ) for 90 seconds; and extension temperature (72° C) for 90 seconds for a total of 20 cycles for β-actin and 40 cycles for the other genes. PCR fragments were separated on a 2% agarose gel containing ethidium bromide (0.25 µg/ml) and visualized and photographed using an ultraviolet (UV) transilluminator (Kodak, Rochester, NY). For quantitative studies, and to confirm the specificity of the amplified sequences, gels were blotted on GeneScreen membrane (NEN Research Products, Boston, MA) and processed for Southern blot analysis as described previously.^{26;39} The membranes were hybridized with ³²P-labeled specific oligonucleotide or cDNA probes for 4 h, washed under stringent conditions for each cytokine, and analyzed by digital autoradiography (Phosphor-Imager, Molecular Dynamics, Sunnyvale, CA). Relative amounts of radioactivity between samples blotted on each membrane were examined using the ImageQuant (Molecular Dynamics, Sunnyvale, CA) analysis system. Relative mRNA transcript levels were obtained by using an equal number of cells with simultaneous amplification within the linear range and by blotting and probing the samples to be compared. Cytokine signals are expressed as an expression index, the ratio of each cytokine signal to the signal from the housekeeping gene, β-actin.

Statistical Analysis

SPSS for Windows® (SPSS Inc. Chicago IL) was used for the independent samples *t*-test (two-tailed) to compare means. A *P* ≤ 0.05 was considered significant.

Results

Expression of type 1 cytokines in the PBMC of stem cell-transplanted patients

The levels of IL-2 and IFN-γ mRNA expression were significantly higher in the PBMC from patients receiving intact autografts or isolated progenitor cells, at all time points examined, than PBMC from normal donors. Further, PBMC from patients transplanted with Lin[−]CD34⁺Thy-1^{lo} cells expressed higher levels of IL-2 mRNA, but not IFN-γ mRNA, at all time points examined as compared to patients receiving intact autografts (Figure 1A). Indeed, the IL-2 mRNA levels in patients receiving Lin[−]CD34⁺Thy-1^{lo} were significantly increased on days 15 and 30 compared to pretransplant levels (Figure 1A), but were not significantly increased at any time point examined in patients receiving an intact product. There was, however, a significant increase in the IFN-γ mRNA levels on days 15, 30 and 100 following transplantation with an intact autograft (Figure 1B) as compared to pretransplant levels. Indeed, the IFN-γ mRNA levels in the PBMC of patients receiving intact autografts on days 15, 30, and 100 were significantly higher than that observed in PHA-stimulated normal PBMC (Figure 1B). In patients receiving Lin[−]CD34⁺Thy-1^{lo} cells, the expression of IFN-γ mRNA transcript levels was significantly higher pretransplant and on day 365 compared to patients receiving intact autografts. Further, at all time points examined, the IFN-γ mRNA levels in patients

receiving isolated progenitor cells were significantly higher than PHA-stimulated normal PB (Figure 1B).

Expression of type 2 cytokines in the PBMC of stem cell-transplanted patients

Similar to IL-2 and IFN- γ mRNA expression, significantly higher levels of IL-4 and IL-10 mRNA were observed in the PBMC of stem cell-transplanted patients as compared to normal donors at all times examined (Figure 2A and B). However, when compared to pretransplant levels, there was no change in the IL-4 mRNA levels in patients receiving either intact autografts or Lin⁻CD34⁺Thy-1^{lo} cells (Figure 2A). In contrast, a significant increase in the IL-10 mRNA levels on day 30 was observed as compared to pretransplant levels in patients receiving intact autografts (Figure 2B). Regardless, no significant increase in the IL-10 mRNA levels was observed in patients receiving Lin⁻CD34⁺Thy-1^{lo} cells as compared to pretransplant levels (Figure 2B). Indeed, the levels of IL-10 mRNA transcripts in patients receiving intact autografts were similar to PHA-activated PBMC from normal donors (Figure 2B). With the exception of day 30, the IL-10 mRNA levels in patients receiving intact autografts were not significantly higher than patients receiving Lin⁻CD34⁺Thy-1^{lo} cells (Figure 2B). Taken together, these studies suggest that type 2 cytokines are significantly increased pretransplant compared to the levels in normal individuals and remain at higher levels for up to one year post-transplant with either an intact or Lin⁻CD34⁺Thy-1^{lo} transplant product.

Expression of TNF- α in the PBMC of stem cell-transplanted patients

The expression of the inflammatory monokine, TNF- α , was significantly higher in the PBMC of patients from both transplantation groups, both prior to and following HDT and PSCT, as compared to steady-state PBMC from normal individuals (Figure 3). Indeed, the TNF- α mRNA levels in patients following HDT and intact autografts were similar to PHA-stimulated normal PBMC (Figure 3). A significant increase in the TNF- α mRNA levels was observed on days 15, 30, 100, and 365 as compared to pretransplant levels in patients receiving Lin⁻CD34⁺Thy-1^{lo} cells (Figure 3). However, there was no significant increase in the expression of TNF- α mRNA in patients receiving intact autografts compared to pretransplant levels (Figure 3).

Immune cell phenotype, prior to and following SCT

To determine if the presence of immune cells in the transplant product affected cellular recovery, we followed immune cell phenotypic recovery post-transplant based on cellular frequency and absolute number. These studies revealed a significant depression in the frequency and absolute number of CD3⁺ T cells prior to and 15 days following HDT and PSCT as compared to normal donors (Figure 4A and B). Similarly, the frequency of CD3⁺ cells was significantly decreased on day 15 in patients receiving Lin⁻CD34⁺Thy-1^{lo} cells compared to pretransplant levels. The frequency of CD3⁺ cells was significantly increased on day 30 ($p \leq 0.05$) compared to pretransplant levels (Figure 4A) in patients receiving an intact product. Therefore, a significant difference in the frequency of CD3⁺ cells between the two transplant groups on days 15 and 30 was observed. Patients receiving intact autografts did not have significantly higher levels of CD3⁺ cells compared to patients receiving Lin⁻CD34⁺Thy-1^{lo} cells (Figure 4B) at the other time points examined. The absolute number of CD3⁺ T cells was similar at all time points examined in patients receiving intact autografts compared to pretransplantation levels (Figure 4B).

The frequency of CD4⁺ cells prior to transplantation and on days 15, 30, 100, and 365 was significantly lower in patients receiving intact Lin⁻CD34⁺Thy-1^{lo} enriched autografts as compared to normal PBMC ($p \leq 0.05$) (Figure 4C). In addition, prior to transplantation and on days 15, 30, and 365, the absolute number of CD4⁺ cells in both patient groups was significantly lower than in normal donors ($p \leq 0.05$). However, there were no significant differences in the

frequency of CD4⁺ cells after transplantation as compared to pretransplant in patients receiving intact grafts. In contrast, a significant decrease in the CD4⁺ T cell frequency following transplantation was observed in patients receiving Lin⁻CD34⁺Thy-1^{lo} cells as compared to pretransplant levels. Further, the frequency of CD4⁺ cells in patients receiving intact autografts was significantly higher than in patients receiving Lin⁻CD34⁺Thy-1^{lo} cells on days 15 and 30. Nonetheless, we did not observe any significant differences in the absolute number of CD4⁺ cells in patients receiving either transplant product before or after transplantation, although there was a trend for a nonsignificant increase in the absolute numbers of CD4⁺ cells in patients receiving intact grafts at similar time points.

A significant increase in the frequency of CD8⁺ cells in patients receiving an intact graft was observed on day 30 compared to pretransplant levels (Figure 4E). In contrast, the frequency of CD8⁺ cells was significantly decreased on day 15 in patients transplanted with Lin⁻CD34⁺Thy-1^{lo} cells compared to pretransplant levels (Figure 4E). Similar to CD4⁺ cells, the frequency of CD8⁺ cells in patients receiving intact grafts was higher on days 15 and 30 compared to patients transplanted with Lin⁻CD34⁺Thy-1^{lo} cells at similar time points. In addition, the absolute number of CD8⁺ cells in the PB post-transplant was similar in both groups. However, a significant increase in the absolute numbers of CD8⁺ T cells was found on day 100 in patients transplanted with an intact product compared to pretransplant levels.

The CD4⁺:CD8⁺ T cell ratios in patients receiving intact or Lin⁻CD34⁺Thy-1^{lo} autografts were significantly decreased on days 30, 100, and 365 compared to pretransplant levels or to normal donors (Figure 5). Further, there were no differences in the CD4⁺:CD8⁺ T cell ratios between patients receiving intact or Lin⁻CD34⁺Thy-1^{lo} autografts before or after transplantation (Figure 5A). Further, an analysis of the ratios of naïve and memory T cells and found that naïve:memory CD4 cell ratios (CD4⁺CD45RA⁺:CD4⁺CD45RO⁺) in the PB of patients transplanted with Lin⁻CD34⁺Thy-1^{lo} cells were significantly lower than in the PB from patients receiving an intact autograft (Figure 5B). However, there was no significant decrease in the CD4⁺CD45RA⁺:CD4⁺CD45RO⁺ ratio following transplantation of either product compared to pretransplant observations due, in part, to the large standard deviation observed pretransplant.

The frequency of CD14⁺ monocytes on day 30 post-transplant in patients receiving intact grafts was significantly higher than pretransplant levels, yet returned to pretransplant levels by day 100 (Figure 4). However, the frequency of CD14⁺ monocytes was significantly higher on day 15 and returned to the pretransplant level on day 30 in patients transplanted with Lin⁻CD34⁺Thy-1^{lo} cells (Figure 4G). In addition, the frequency of CD14⁺ monocytes was significantly higher at all time points examined regardless of the product infused except on day 365 in patients receiving intact grafts compared to normal donors. The absolute number of CD14⁺ monocytes was also significantly higher on day 100 in patients receiving intact autografts as compared to pretransplant levels (Figure 4H). However, no significant difference was observed in the absolute numbers of CD14⁺ monocytes in patients receiving Lin⁻CD34⁺Thy-1^{lo} cells before and/or at any time after transplantation (Figure 4).

Immune function of PBMC prior to and following transplantation

The PHA proliferative response of PBMC from patients undergoing HDT and SCT, both prior to and following transplantation was compared to the response by PBMC from normal donors, which were used as a control. At all time points analyzed, the PBMC from patients receiving either intact or Lin⁻CD34⁺Thy-1^{lo} autografts exhibited a significantly depressed mitogenic response to PHA as compared to PBMC from normal, healthy donors (Figure 6A), suggesting a depressed immune function. There was also a significant decrease in the PHA mitogenic response in the PBMC on days 15 and 30 in patients receiving intact autografts as compared to pretransplant levels (Figure 6A). Further, there was a significant decrease in the PHA

mitogenic response in patients transplanted with a Lin⁻CD34⁺Thy-1^{lo} autograft on days 15, 30, and 100 compared to pretransplant levels (Figure 6A). In addition, the mitogenic responses by PBMC from patients receiving intact autografts were significantly higher than from patients transplanted with Lin⁻CD34⁺Thy-1^{lo} autografts.

Recent reports from our laboratory have suggested that there are high levels of MDTI activity in PSC products³⁷ and PBMC post-transplantation.^{26;29} In the present comparison, we observed significantly higher MDTI activity on days 15 and 30 in patients receiving intact autografts as compared to pretransplantation and to the PBMC of normal donors ($p \leq 0.05$) (Figure 6B). The MDTI activity levels in patients transplanted with Lin⁻CD34⁺Thy-1^{lo} autografts were significantly higher on day 30 than pretransplant levels and on days 30 and 100 compared to normal PBMC activity. By day 100, the MDTI activity levels in patients receiving intact autografts returned to the levels observed in PBMC from normal donors, while the patients receiving a Lin⁻CD34⁺Thy-1^{lo} product required 365 days to normalize (Figure 6B). Further, the MDTI activity levels in patients receiving a Lin⁻CD34⁺Thy-1^{lo} transplant were significantly higher on days 15 and 30 than that of PBMC from patients transplanted with an intact autograft (Figure 6B).

Discussion

In the present study, we compared the expression of immunoregulatory cytokine mRNA levels prior to and following SCT with intact or Lin⁻CD34⁺Thy-1^{lo} autografts in intermediate and low-grade NHL patients. Significant increases in the levels of all cytokines examined were observed in both groups of transplanted patients compared to normal PBMC. The difference in the cytokine mRNA levels was not due to an increase in the frequency of T cells in PBMC. Indeed, following HDT and SCT, the CD4⁺:CD8⁺ T cell ratios were significantly lower than that observed in the PB of normal donors. In addition, despite the increased cytokine expression, which suggests that the immune cells were activated, the mitogenic T cell responses in the PB following transplantation were significantly depressed. This may be due to the high levels of MDTI activity levels observed following HDT and SCT as compared to normal donors, which normalized by days 100 or 365 for patients transplanted with intact or Lin⁻CD34⁺Thy-1^{lo} products, respectively, providing one mechanism for the depressed immune function.^{26;29;37} These results suggest that despite high levels of immune cells in intact autografts, the immune reconstitution pattern was abnormal in both patient groups.

An earlier report from Dreger et al¹⁵ suggested that transplantation with highly enriched CD34⁺ PSC products did not inhibit immune recovery. These studies included patients receiving either unmanipulated PSC products or CD34⁺ cells enriched using the Isolex 300 device. Both patient groups had similar hematological and immune recovery,¹⁵ although CD4⁺CD45RA⁺ cell recovery was delayed in patients receiving the enriched CD34⁺ cell product. Similar observations have been made in patients receiving allogeneic hematopoietic transplantation using a selected CD34⁺ PSC or an unmanipulated graft.⁴⁰⁻⁴² Our data support the observation that the infusion of enriched autologous stem cell products causes only marginal effects on immune reconstitution post-transplantation as compared to the infusion of an intact product. However, we did observe some differences in T cell subset recovery between patients receiving either intact or Lin⁻CD34⁺Thy-1^{lo} autografts. These differences in T cell subset recovery and immune reconstitution might be due to differences in the stem cell grafts. However, as these studies were performed in two different NHL patient populations, the effect of the different patient population cannot be eliminated. Further, unlike the study by Dreger et al.,¹⁵ our studies used Lin⁻CD34⁺Thy-1^{lo} cells, which is a more rigorous depletion of T cells than the isolated CD34⁺ cells.

Our studies begin to address previous speculation that accessory cells might be necessary for immune reconstitution⁴³ and the results suggest that “stem cells can do it all.” However, the rapid expansion of T cells from progenitors, or the infusion of a few T cells, may be associated with restricted T cell repertoire diversity.^{44;45} Further, immune function in both transplant cohorts remained depressed despite the rapid reconstitution of CD3⁺ T cells, and the molecular mechanism for immune dysfunction remains unclear, although there is a consistent observation of heightened MDTI activity. Earlier reports suggest that cytokine expression abnormalities might contribute to the immune dysfunction in autologous BMT patients, including decreased IL-2 levels^{19;46-50} or increased IL-10 levels.⁵¹ The high levels of both type 1 and 2 cytokine mRNA that we observed in the PBMC of NHL patients prior to transplantation suggested an abnormal cellular activation, which may be associated with previous cycles of chemotherapy or tumor burden and may contribute to the induction of T cell anergy.²⁶ Recent reports demonstrating that chemotherapeutic agents such as Taxol and Adriamycin enhance the levels of cytokine (IL-1, IL-8, TNF- α) mRNA from monocytes *in vitro* and IL-8 expression in a subset of ovarian cancer cells *in vivo* support this hypothesis.^{52;53} We showed in a previous report that the number of prior chemotherapy cycles inversely correlated with PHA response, suggesting an association between prior chemotherapy and T cell function.^{25;54} Further, tumor infiltrating lymphocytes (TIL) from patients with renal cell carcinoma express IL-4 and IL-10, which may inhibit an effective immune response.⁵⁵ Similarly, in hairy-cell leukemia, tumor burden has been reported to correlate with the plasma or serum levels of IL-1 α , IL- β , IL-6, and soluble IL-2R.⁵⁶ Reports also suggest that pretreatment with G-CSF polarizes donor T lymphocytes towards type 2 cytokine production and induces T cell unresponsiveness.⁵⁷⁻⁵⁹ However, it remains unclear which mechanism or if multiple mechanisms, such as chemotherapy, tumor burden, or mobilizing cytokines, regulate cytokine gene expression in the PBMC of NHL patients undergoing HDT and SCT.

Studies from our laboratory suggest that despite the high levels of cytokine mRNA expression lymphocyte function in transplant patients remains depressed.^{26;36;37} Recently, high levels of IL-2 mRNA, but not IL-2 protein expression, were observed with leukocytes from breast cancer patients.⁶⁰ The lack of IL-2 translation and secretion despite IL-2 mRNA transcription with these TILs might be due to a translational control that contributes to T cell anergy.^{60;61} However, it is not clear from our studies whether higher levels of mRNA transcripts in the PBMC of these patients result in increased cytokine production. Aberrant cytokine production may arise from defective transcription and/or instability of the cytokine mRNA transcript. Alternatively, defective translation and reduced cytoplasmic protein half-life could result in decreased secretion. However, in our preliminary studies of MNC from PSC products, we found that the levels of cytokine mRNA transcripts directly correlated with mitogen-stimulated cytokine secretion.^{36;39} Additionally, a defect in the signaling pathways might contribute to the inability of cytokines to function. One report demonstrated that BMT patients exhibited such a deficit, affecting the signaling cascade downstream of second messenger generation and resulting in impaired cytokine production.⁴⁷ Alternative studies have suggested that high IL-10 levels,^{26;51} impaired co-stimulation,^{62;63} or depressed zeta chain expression⁶³⁻⁶⁶ and presence of immunoregulatory cells^{67;68} may also contribute to the immune dysfunction.

The effects of HDT and SCT on immune cellular reconstitution are not as marked as the functional immunosuppression that occurs regardless of transplantation with either an intact or Lin⁻CD34⁺Thy-1^{lo} autograft product. Therefore, we suggest that the presence of T cells and potentially monocytes within the stem cell product may be of secondary importance to immune recovery following transplantation. While there were differences in the patient populations receiving the two different types of stem cell products, i.e. intermediate grade NHL patients received intact products while low-grade NHL patients received the Lin⁻CD34⁺Thy-1^{lo} cell products, we suggest that this is unlikely to have a major impact on the results described herein. Further, the rapid immunologic recovery following transplantation with the

Lin⁻CD34⁺Thy-1^{lo} autografts suggest that it is the infused stem cells that are responsible for rapid immunologic recovery and not the presence of “accessory” cells within the product, such as the lymphocytes and monocytes.²³ We conclude, therefore, that greater attention should be focused on the manipulation of the reconstituting cells using possibly cytokine or growth-factor administration to regulate immune recovery as opposed to autograft manipulation. It should be emphasized, however, that autograft manipulation to reduce tumor-cell contamination as demonstrated by gene marking studies^{69;69;70} remains a highly relevant objective.

Recovery of a functional immune system is essential for the effective eradication of minimal residual disease and control of opportunistic infections following HDT and SCT³². The present report demonstrates that a similar pattern of immune reconstitution exists in patients receiving intact and Lin⁻CD34⁺Thy-1^{lo} intact autografts, suggesting that “stem cells can do it all.” Despite the differences in the composition of infused grafts, the immune cells in the PB of stem cell-transplanted patients were activated (as supported by cytokine gene expression), yet functionally depressed. A better understanding of the mechanism of cytokine-gene transcription/translation and signaling events will allow us to effectively design adjuvant immunotherapy and overcome the peripheral tolerance observed post-transplant.

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Figure 1A

Figure 1B

Figure 1. A&B. Expression of IL-2 and IFN-γ

The mRNA expression of IL-2 (A) and IFN-γ (B) was analyzed by RT-PCR. Expression indices were calculated by comparing the values of IL-2 and IFN-γ with the housekeeping gene β-actin. The values are mean ± standard error (SE) for each group. *Significantly different from pretransplantation (p ≤ 0.05). #Significantly different from patients receiving CD34⁺Lin⁻Thy1^{lo} stem cells at corresponding time points (p ≤ 0.05).

Figure 2A

Figure 2B

Figure 2. Expression of IL-4 and IL-10

The mRNA expression for IL-4 (A) and IL-10 (B) was analyzed. The expression indexes for IL-4 and IL-10 were calculated by comparing them with the housekeeping gene β -actin. The values are mean $\pm$ SE for each group. *Significantly different from pre-transplantation ($p \leq 0.05$). #Significantly different from patients receiving CD34⁺Lin⁻Thy-1¹⁰ stem cells at corresponding time points ($p \leq 0.05$).

Figure 3. Expression of TNF- α

The expression of TNF- α was determined. Expression indices for TNF- α were calculated by comparing with the housekeeping gene β -actin. The values are mean $\pm$ SE for each group.

*Significantly different from PHA stimulated normal PBMC ($p \leq 0.05$). *Significantly different from pre-transplantation ($p \leq 0.05$). #Significantly different from patients receiving CD34⁺Lin⁻Thy1^{lo} stem cells group at corresponding time points ($p \leq 0.05$).

Figure 4. Cellular Frequency of CD4⁺, CD8⁺ T Cells and Monocytes

The frequency and absolute numbers of immune cells by immunophenotyping was analyzed by FACS. The values are mean $\pm$ SE for each group. (A & B) CD3⁺ cells; (C & D) CD4⁺ cells; (E & F) CD8⁺ cells; (G & H) CD14⁺ monocytes. \$Significantly different from normal PBMC ($p \leq 0.05$). *Significantly different from pre-transplantation ($p \leq 0.05$). #Significantly different from patients receiving CD34⁺Lin⁻Thy-1^{lo} stem cells at corresponding time points ($p \leq 0.05$).

Figure 5A

Figure 5B

Figure 5.

A. The ratio between CD4⁺ and CD8⁺ cells. The frequency of CD4⁺ and CD8⁺ cells by immunophenotyping was analyzed by FACS and ratio was determined. The values are mean $\pm$ SE for each group. **B. The ratios between CD4⁺CD45RA⁺ and CD4⁺CD45RO⁺.** Cellular frequency and absolute numbers of naïve and memory CD4⁺ T cells as determined by immunophenotyping and ratios was determined. The values are mean $\pm$ SE for each group. \$Significantly different from normal PBMC ($p \leq 0.05$). *Significantly different from pre-transplantation ($p \leq 0.05$). #Significantly different from patients receiving CD34⁺Lin⁻Thy1^{lo} stem cells at corresponding time points ($p \leq 0.05$).

Figure 6A

Figure 6B

Figure 6.

A. Mitogenic Response in PB of Pre- and Post-SCT Patients. T cell mitogenic response was analyzed using optimal concentration of PHA. The values are mean counts per minute (cpm) $\pm$ SE from each group. **B. Cellular-dependent T cell inhibitory activity.** T cell inhibitory activity at I:R ratio of 2:1 was determined. The values are mean percent T cell inhibitory activity $\pm$ SE from each group. §Significantly different from normal PBMC ($p \leq 0.05$). *Significantly different from pre-transplantation ($p \leq 0.05$). #Significantly different from patients receiving CD34⁺Lin⁻Thy1^{lo} stem cells at corresponding time points ($p \leq 0.05$).

Table 1**NHL patient characteristics**

Number of patients		PSCT 35	CD34 ⁺ Lin ⁻ Thy-1 ^{lo} 18
Age (year)	Median (Range)	50.50 (27.3-64.2)	51.9 (35.7-62-8)
Sex (%)	Male	53	61
	Female	47	39
No. of infused CD34 ⁺ cells/kg	×10 ⁶	10.5 ± 1	0.7 ± 0.5
No. of prior chemotherapy cycles	Median (Range)	2 (1-5)	1.5 (1-5)