Immune reconstitution after autologous hematopoietic stem cell transplantation in relation to underlying disease, type of high-dose therapy and infectious complications

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Background and Objectives. Autologous peripheral stem cell transplantation (APSCT) is increasingly used for various hematologic malignancies and solid tumors. The objective of this study was to analyze the immune reconstitution after APSCT and see if there was any correlation with diagnosis, age, type of high-dose therapy, CD34+ selection of the autograft and double vs single APSCT.

Design and Methods. Lymphocyte subset recovery was studied in 46 consecutive patients with hematologic malignancies and breast cancer, who underwent APSCT. Eleven patients with multiple myeloma received tandem autografts. Thirty-one patients were given total body irradiation (TBI) as part of the high-dose treatment. Eighteen patients received a CD34+ selected graft. The percentage and absolute numbers of lymphocyte populations, T-cells (CD2+, CD3+), B-cells (CD19+), NK cells (CD56+3– or CD16+3–), and T-cell subpopulations (CD4+, CD8+, CD4+CD45RA+, CD4+CD45RO+, CD4+DR+, CD8+CD45RO+, CD8+DR+), were monitored with flow cytometry during the first year after APSCT.

Results. The total B-cell (CD19+) and T-cell (CD3+) counts were reconstituted to normal levels 2-4 months after APSCT. All patients had a low CD4+/CD8+ ratio during the observation period, related to both a low number of CD4+ cells and elevated numbers of CD8+ cells. The low number of CD4+ cells was due to a persistently low level of naive CD4+CD45RA+ cells. A high proportion of the CD8+ cells displayed a phenotype compatible with activated T-cells (CD8+DR+) up to 10 months after autografting. The number of NK cells (CD56+3– or CD16+3–) reached normal values within one month post-transplant. No single variable, such as diagnoses, age, TBI as part of the high-dose treatment, tandem autografting or CD34+ selection of the graft, influenced the immune or hematopoietic reconstitution and no correlation with documented infectious complications was found.

Interpretation and Conclusions. Despite heterogeneity of diseases, age, initial treatment and high-dose regimens, lymphocyte subset analysis did not reveal any differences in hematopoietic or immune reconstitution. All patients had a low CD4+/CD8+ ratio during at least the first year post-transplant, caused by a persistent increase of CD8+ lymphocytes and a constant reduction of CD4+ lymphocytes, making the patients susceptible to infections for a prolonged period of time post-transplant.

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Key words: autologous stem cell transplantation, immune reconstitution, hematopoietic reconstitution, infectious complication, lymphocyte subset recovery

High-dose chemoradiotherapy followed by hematopoietic stem cell transplantation (HSCT) is increasingly used for various hematologic malignancies and solid tumors. Studies concerning the reconstitution of lymphocyte subsets have reported similar composition after allogeneic and autologous bone marrow/peripheral blood progenitor cell transplantation although many authors have observed differences in regard to reconstitution time1-6 and lymphocyte function in favor of peripheral progenitor cell transplantation.7 The B-cell recovery seems to parallel ontogeny in patients without chronic graft-versus-host disease as evidenced by the pattern of quantitative recovery of circulating B-cells, the increased B-cell size and the constellation of surface antigens on B-cells.8 However, to date, it is not completely understood along which pathway the T-cells regenerate. In the present study we analyzed immune reconstitution in 46 patients after APSCT in relation to diagnosis, age, type of high-dose therapy, CD34+ selection of the autograft and double vs single APSCT. We also related the recovery of lymphocyte subsets to incidence and type of infectious complications.

Design and Methods

Patients and treatment

Immune reconstitution was studied in 46 consecutive patients who lived within Stockholm county and were referred for APSCT. The monitoring started after
the white blood cell (WBC) count reached 1×10⁹/L post-transplantation. The patients' characteristics are shown in Table 1. Twenty-five females and 21 males, with a median age of 53 (range 22-62) years were included in this study. Diagnoses were non-Hodgkin's lymphoma (NHL) (n=11), Hodgkin's disease (HD) (n=2), multiple myeloma (MM) (n=15), acute myeloid leukemia (AML) (n=10), acute lymphoblastic leukemia (ALL) (n=3), and breast cancer (BC) (n=3). The patients with AML received APSCT in first complete remission (CR) and the majority (n=9) were treated according to the ICE protocol.11 All patients with lymphoma received APSCT in second or third CR or partial remission (PR), except 2 patients with T-cell lymphoma who received high dose treatment in first CR. Four patients (3 NHL and 1 HD) received BEAM chemotherapy.10 Etoposide and cyclophosphamide in combination with either total body irradiation (TBI) or busulfan as myelosuppression for APSCT was given to 17 (8 NHL, 1 HD, 3 ALL, 1 AM L and 4 MM) and 3 (3 AML) patients respectively.11,12 Eleven patients with MM received double APSCT.13 Altogether 31 patients were given TBI as part of the high-dose treatment. Eighteen patients (10 MM, 7 NHL and 1 HD) received a CD34⁺ selected graft and 28 patients received an unmanipulated graft, with a mean dose of CD34⁺ cells at ABSCT of 3.4×10⁹/kg and 5.8×10⁹/kg respectively. Positive selection was performed with the Ceprate® SC biotin-avidin immunoadsorption method (CellPro Europe) achieving a mean purity of the CD34⁺ selected fraction of 79±12% and a mean number of CD3⁺ cells of 0.25×10⁹/kg. Granulocyte colony-stimulating factor (G-CSF) was given to 36 patients after autografting.

### Table 1. Patient characteristics according to diagnosis.

<table>
<thead>
<tr>
<th></th>
<th>AML (n=10)</th>
<th>ALL (n=3)</th>
<th>MM (n=15)</th>
<th>NHL (n=11)</th>
<th>HD (n=2)</th>
<th>BC (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age yrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>range</td>
<td>(30-60)</td>
<td>(36-60)</td>
<td>(30-63)</td>
<td>(36-60)</td>
<td>(32-50)</td>
<td>(36-62)</td>
</tr>
<tr>
<td>TBI (%)</td>
<td>8 (80)</td>
<td>3 (100)</td>
<td>14 (94)</td>
<td>8 (73)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tandem autograft (%)</td>
<td>0</td>
<td>0</td>
<td>11 (73)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>


the recovery of each lymphocyte subpopulation. The analysis of lymphocyte subsets was performed by flow cytometry.

The clinical and laboratory data were analyzed according to standard statistical methods using an Excel computer program. Comparisons of groups regarding infectious complications were performed by the chi squared test. Linear regression curves were established for all the patients in the study to analyze the recovery of each lymphocyte subpopulation. The statistical analysis is based on the figures derived from the analysis of 20-80 healthy adults.

### Infections

Infectious complications from day 30 to 12 months post-transplant were registered from the medical records and the patients were classified according to the following variables: clinical signs of upper respiratory tract infection; clinical signs of lower respiratory tract infection with positive pulmonary X-ray; clinical signs of septicaemia with fever and C-reactive protein elevation, positive culture results; herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections and clinical signs of CMV infection with positive polymerase chain reaction (PCR), indicating CMV reactivation or infection. Patients undergoing tandem autografting received trimethoprim-sulfa for 3 months after the treatment as prophylaxis against Pneumocystis carinii infection.
Table 2. Hematopoietic recovery in relation to purification of the autograft vs not.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Purified graft (n=18)</th>
<th>Not purified graft (n=28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM</td>
<td>10</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>NHL</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>NHL</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>NHL</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>BC</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>TBI (%)</td>
<td>14 (78)</td>
<td>19 (68)</td>
<td></td>
</tr>
<tr>
<td>Tandem autograft (%)</td>
<td>10 (55)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>PMN &lt;0.5 × 10^9/L, days, median</td>
<td>10 (7-12)</td>
<td>12 (5-27)</td>
<td>NS</td>
</tr>
<tr>
<td>PMN &lt;1.0 × 10^9/L, days, median</td>
<td>13 (8-12)</td>
<td>13 (5-30)</td>
<td>NS</td>
</tr>
<tr>
<td>Platelets &lt;20 × 10^12/L, days, median</td>
<td>13 (2-33)</td>
<td>15 (1-60)</td>
<td>NS</td>
</tr>
<tr>
<td>Platelets &lt;50 × 10^12/L, days, median</td>
<td>23 (9-110)</td>
<td>62 (10-365)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results

Hematopoietic reconstitution

All patients but two had complete, trilineage hematopoietic recovery after 2 months. The median time to neutrophil recovery >0.5 × 10^9/L was 10 days (range 5-27), platelet count >20 × 10^10/L 11 days (range 1-30) and platelet count >50 × 10^10/L 16 days (range 9-365).

CD34+ selection of the autograft did not influence the time to hematopoietic recovery (Table 2). There was no significant difference in hematopoietic recovery related to diagnosis, type of high-dose treatment or tandem vs single autograft (data not shown).

The patients with MM who received TBI, tandem autografts and CD34+ selected grafts showed a similar pattern in hematopoietic reconstitution as other patients, with a median time to neutrophil count >0.5 × 10^9/L of 10 days (range 5-13) and platelet count >20 × 10^10/L of 11 days (range 2-60).

Primary graft failure was observed in two patients with AML. In one patient a second autograft was performed 200 days post-transplantation.

Immune reconstitution

All patients had B-cell (CD19+) counts below normal levels during the first 2-3 months. The T-cell (CD3+) count reached normal levels within 2 months after high-dose therapy. However, all patients had a low CD4/CD8 ratio during the 12 months of observation. This was related to both a relative decrease in CD4+ cells and an increase in CD8+ cells. A large proportion, up to 80%, of the CD8+ cells expressed HLA DR, a phenotype associated with activated T-cells. By 12 months post-transplant the CD8+ cell count tended to decrease to normal adult values. The low number of CD4+ T-cells was due to a persistently low level of immature naive CD4+CD45RA+ cells during the observation period. NK-cell counts (CD56+CD3- or CD16+CD3+) reached normal values within one month after APSCT. Two patients with MM had T-helper cells (CD4+) that were double positive for CD45RO and CD45RA, whereas all other patients had no overlap between the CD4+CD45RA and CD45RO populations.

The lymphocyte reconstitution was analyzed in relation to diagnosis, age, type of high-dose treatment and CD34+ selection of the graft. No difference in immune reconstitution was found in relation to diagnoses (Figure 1). Although the majority of the MM patients received TBI as part of the high-dose treatment, tandem autografting and CD34 selected grafts, the pattern of immune reconstitution did not differ from that of the other patient groups. The MM patients showed a trend towards a slower B-cell reconstitution and a higher number of CD8+DR+ cells compared to the remaining patients but these differences did not reach statistical significance. Age did not have any impact on the recovery of lymphocyte subsets (data not shown). Eighteen patients (10 MM, 7 NHL, 1 HD) received a CD34+ selected graft and 28 patients (10 AML, 3 ALL, 1 HD, 5 MM, 4 NHL, 5 BC) received unmanipulated grafts. The mean number of CD34+ cells at APSCT was 3.4 × 10^6/kg in the selected group and 5.8 × 10^6/kg in the non-selected group. The reconstitution pattern of lymphocytes after APSCT did not differ between these two groups (Figure 2). The influence of TBI on immune reconstitution was analyzed in 26 patients who received non-selected and single autografts. In this group 17 patients (8 AML, 3 ALL, 3 MM, 3 NHL) received TBI and 9 (2 AML, 1 HD, 1 MM, 5 BC) only chemotherapy. There was no difference in lymphocyte regeneration between these two groups (data not shown).

Infectious complications

Of the 15 MM patients, 14 received TBI, 10 purified grafts and 11 double APSCT. These patients showed a tendency, although not statistically signif-

Table 3. Infectious complications in relation to diagnosis.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>AL (n=13)</th>
<th>MM Lymphoma (n=15)</th>
<th>BC (n=5)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients infected (%)</td>
<td>4 (31)</td>
<td>9 (60)</td>
<td>3 (31)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Herpes zoster (%)</td>
<td>3 (23)</td>
<td>4 (31)</td>
<td>1 (8)</td>
<td>0</td>
</tr>
<tr>
<td>Generalized varicella-zoster (%)</td>
<td>0</td>
<td>1 (6.7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CMV (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Upper resp. tract infection (%)</td>
<td>3 (23)</td>
<td>5 (33)</td>
<td>1 (8)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Bacteremia (%)</td>
<td>0</td>
<td>2 (13)</td>
<td>0</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Pneumonia (%)</td>
<td>0</td>
<td>4 (27)</td>
<td>1 (8)</td>
<td>0</td>
</tr>
<tr>
<td>Urinary tract infection (%)</td>
<td>0</td>
<td>1 (6.7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other bact. infection (%)</td>
<td>0</td>
<td>2 (13)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The p value describes the heterogeneity between the groups.
Autologous stem cell transplantation

significant, to develop more infectious complications during the first year post-transplantation compared to the other patients, not taking into account the 30 days immediately after high-dose treatment. Nine of the 15 patients with MM developed infections during the observation period. Herpes virus infections, respiratory tract infections and bacteremia were more frequent in this group of patients (Table 3). The risk of infectious complications was not predicted by the immune reconstitution (Figure 3). No deaths caused by infectious complication were observed during the first year post-transplantation.

Discussion

This study describes hematopoietic and immune reconstitution during 12 months following APSCT in 46 patients. The patients form a heterogeneous group with regards to diagnoses, extent of previous treatment and type of high-dose regimen. Despite this variability, hematopoietic recovery was remarkably uniform and did not correlate with the underlying disease. TBI as part of the high-dose regimen, single vs double APSCT or CD34+ selection of the graft. It has been shown that the time to neutrophil and platelet recovery is inversely related to the number of reinfused CD34+ cells with a threshold number of 2.5 × 10^6 CD34+ cells/kg. All patients in this study were autografted with more than 2.5 × 10^6 CD34+ cells, except three who received between 1.5 and 2 × 10^6/kg CD34+ cells and they did not differ, with regard to hematopoietic and immune reconstitution, from the remaining patients.

The total number of B-cells reached the normal range approximately 2 months post-transplantation in all patients, irrespective of diagnosis, age or high-dose regimen (Figure 1). In studies of B-cell development after HSCT, the B-cell reconstitution seems to parallel ontogeny and derive from the stem cells rather than from grafted mature B-cells. This is compatible with our results in APSCT, in which intensification of the high-dose treatment and CD34+ selection of the graft did not influence the B-cell recovery. Other investigators have shown that serum Ig levels
remain low during the first 3 months after allogeneic bone marrow transplantation and that proliferative responses to T-cell independent antigen are blunted, probably due to inadequate B-cell function.17,18 NK-cells were observed among the first cells to recover, reaching normal levels within one month post-APSCT which is in line with other reports.6 The activity of NK cells also returns to normal shortly following transplantation, which may be relevant for anti-tumor immune responses post-transplantation.19 The maturation of NK-cells is not dependent on residual thymus tissue and this may account for the rapid recovery.

Both CD4+ and CD8+ lymphocyte populations comprise functionally distinct subsets, based on the expression of different isoforms of the CD45 antigen. At birth most T-cells express CD45RA which are considered to be immature and naive. T-cells may convert to CD45RO+ after stimulation and become mature cells of the memory type. Previous reports show that the regenerating CD4+ T-cells after HSCT in adult life are mainly CD45RO+ as the generation of CD4+CD45RA- T-lymocytes is thymus-dependent.3,6,20,21,22 In this series of patients CD45RO was consistently expressed on essentially all CD4+ T-lymphocytes during the first year post-transplant. The CD8+ T-lymphocytes, however, appear to regenerate by a thymus-independent pathway,23 explaining the rapid CD8+ cell reconstitution and absolute number exceeding the normal values for the first 8 months post-transplant. A large proportion of the CD8+ cells, up to 80%, expressed HLA-DR, a phenotype associated with activated T-cells (Figure 1). This was observed in all patients, post-APSCT, regardless of diagnosis or type of high-dose regimen. Interestingly, the MM patients showed a similar pattern of immune reconstitution to that of the other groups of patients. However, there was a trend to more infectious complications during the first year post-transplant in this group of patients. It is possible that functional analysis of the lymphocyte subpopulations might have detected some differences in the immunologic recovery.

Figure 2. Reconstitution of lymphocyte subtypes in relation to CD34 selection of the autograft vs. not. □ = purified graft; ● = not purified graft. ——— indicates upper (90th percentile) and lower (10th percentile) level of normal values. The figures show mean values.
non-CD34+ tumor cells, perhaps limiting the risk of post-transplant relapse. However, it has been postulated that purification of the autograft delays immune reconstitution, which might result in higher infectious morbidity and relapse rate post-transplantation, potentially neutralizing the beneficial effects of stem cell selection. In this study there was no correlation between purification of the autograft and delayed immune reconstitution or increased risk of infectious complications post-transplantation. These results and the observation that the CD4+/CD8+ ratio is normal in the graft, indicate that persisting mature T-cells in the graft have a minor impact on T-cell regeneration. It might be expected that intensification of the high-dose treatment with TBI and/or tandem autografting would delay the T-cell recovery post-transplantation. However, in this study, TBI and/or tandem autografting did not influence immune reconstitution. In the light of the above observations, we suggest that progenitors in the graft account for the post-transplant T-cell reconstitution along a non-thymic dependant pathway, resulting in an inverted CD4+/CD8+ ratio and low numbers of CD4+CD45RA+ cells for at least one year following APSCT.

In conclusion, despite the heterogeneity of diseases, age, initial treatment and high-dose regimens, lymphocyte subset analysis did not reveal any differences in hematopoietic or immune reconstitution. As in previous studies low CD4+/CD8+ ratios were observed during at least the first year post-transplant. This is caused by a persistent increase of CD8+ lymphocytes and a constant reduction of CD4+ lymphocytes. The slow reconstitution of certain lymphocyte subsets following intensive treatment makes the patients susceptible to infections, in particular viral infections, for a prolonged period of time and might affect the course of the underlying disease.

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HS: Clinical data, analyzed data, wrote the manuscript. AG: Clinical data, contributed to writing of the manuscript. MB: Analysis of data, contributed to writing the manuscript. AS: Laboratory work. MH: formulated the questions asked and designed the study and analyzed data.

Disclosures
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Figure 3. Reconstitution of lymphocyte subtypes in MM patients in relation to infection episodes vs. no infection during the first year after APSCT excluding the neutropenic period.  Infection; ☐ = no infection. ----- indicates upper (90th percentile) and lower (10th percentile) level of normal values. The figures show mean values.
Potential implications for clinical practice

- Patients receiving tandem autografting and TBI could be prone to more severe infectious complications post-transplant. Low CD4/CD8 ratios were observed during at least the first year post-transplant. This slow reconstitution of certain lymphocyte subsets following intensive treatment makes the patients susceptible to infections, in particular viral infections, for a prolonged period of time and might affect the course of the underlying disease. These observations may have important implications for approaches considering vaccine strategies post-transplant as well as strategies for immunotherapeutic models.

References