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Number of Nucleated Cells Infused During Allogeneic and Autologous Bone Marrow Transplantation: An Important Modifiable Factor Influencing Outcome

To the Editor:

Although variables relating to disease biology and patient characteristics are strong determinants of outcome after bone marrow transplantation (BMT), the identification of these *nonmodifiable* factors is often of limited practical use.¹ On the other hand, treatment- and transplant-related factors, which are modifiable, can potentially be manipulated in clinical practice to improve the results of transplantation.¹ The number of nucleated cells infused during transplantation is a variable which is usually controllable. Therefore, it is encouraging to see that increasing this number as much as possible improves survival by reducing transplant-related mortality in patients allografted from unrelated donors.²

We have also found in a number of different studies that the nucleated cell dose significantly affects transplant-related mortality,^{1,3-5} survival,^{1,3} and the speed as well as completeness of hematologic reconstitution after autologous^{1,6} and allogeneic³⁻⁵ transplantation for hematologic malignancies.

Among 74 patients with acute myeloid leukemia (AML) allografted in first remission after melphalan and single-fraction total-body irradiation (TBI),¹ 7 of 21 patients receiving $\leq 2 \times 10^8$ nucleated cells/kg body weight and 7 of 53 patients receiving $> 2 \times 10^8$ nucleated cells/kg body weight died of treatment-related toxicity ($P = .047$, χ^2 test). In multivariate analysis, patients receiving the higher cell dose had a significantly better disease-free survival (relative risk 2.17, $P = .045$).¹ The higher toxic death rate and poorer disease-free survival in patients receiving $\leq 2 \times 10^8$ nucleated cells/kg was only partially due to incomplete or delayed hematopoietic reconstitution with resultant increase in bleeding or infections because the cell dose did not affect the probability or rapidity of engraftment significantly in this group of 74 patients.¹ Because 2×10^8 /kg cells is our usual target for collection during an autologous marrow harvest, those receiving $\leq 2 \times 10^8$ /kg nucleated cells may

have represented a group of patients with compromised marrow reserve due to either high-risk disease or previous chemotherapy who subsequently had problems during the transplant.

However, in a study of factors affecting hematologic recovery after unpurged autologous blood or marrow transplantation in 240 patients with acute leukemia (which included the previous 74 patients),⁶ a nucleated cell dose of $< 2 \times 10^8$ /kg was independently associated with slower recovery to 0.5×10^9 /L neutrophils, and with slower and incomplete recovery to 50×10^9 /L platelets. There was a strong correlation between neutrophil recovery and five increasing nucleated cell dose levels (< 1.5 , $1.5-2$, $> 2-2.5$, $> 2.5-3.5$, and $> 3.5 \times 10^8$ /kg).

Long-term follow-up of 85 first-remission AML patients allografted from HLA-identical siblings after cyclophosphamide and single-fraction TBI with cyclosporine for graft-versus-host disease (GVHD) prophylaxis showed that infusion of a lower nucleated cell dose ($\leq 2.6 \times 10^8$ /kg) was independently predictive of increased transplant-related mortality (relative risk 2.69, $P = .002$).³ The higher cell dose was associated with better disease-free survival (relative risk 2.01, $P = .045$) in multivariate analysis.³ Figure 1 shows the effect of the cell dose in this group of patients.

Similarly, in a study of transplant-related mortality in 138 acute leukemia patients allografted after melphalan and TBI with cyclosporine \pm methotrexate,⁴ a high infused marrow cell dose ($\geq 2.5 \times 10^8$ total nucleated cells/kg or $\geq 0.6 \times 10^8$ mononuclear cells/kg) protected against fatal interstitial pneumonitis compared with lower cell doses (risk ratio 0.47, $P = .023$).

After allogeneic transplantation in 712 patients with hematologic malignancies, a low total leukocyte count in the third week was found to be the most significant predictor of death due to infections, hemorrhage, or graft failure within the first 3 months.⁷ A low nucleated cell dose ($< 2.5 \times 10^8$ /kg) was independently associated with increased risk of death due to these causes (relative risk 2.2, $P = .025$).⁷

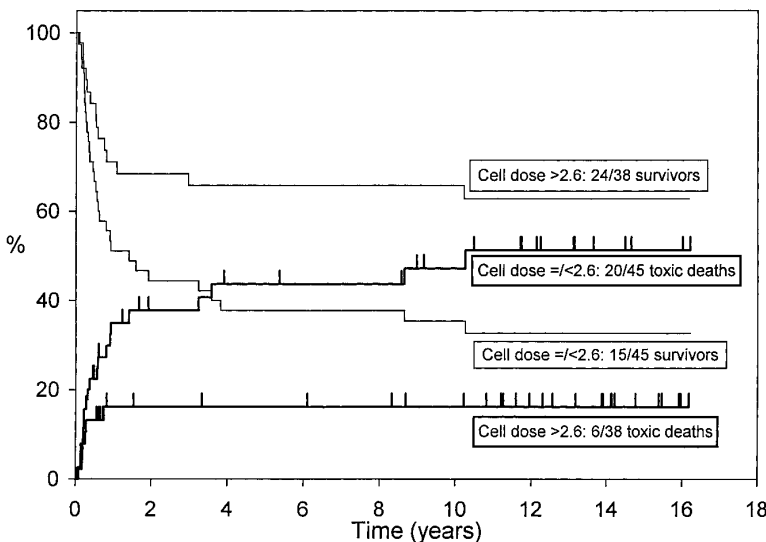


Fig 1. The effect of cell dose on disease-free survival and transplant-related mortality in 85 AML patients allografted in first remission (cell numbers not available for two patients). Higher cell doses are significantly better.

Table 1. Comparison of Marrow and Peripheral Blood Stem Cell Yields From 21 Healthy Donors

	Marrow	Blood	Blood:Marrow Ratio	P
Total nucleated cells (10 ⁹ /kg)	3.2 (2.4-4.4)	7.5 (2.6-14.1)	2.3 (1.1-3.9)	<10 ⁷
CD34 ⁺ cells (10 ⁶ /kg)	1.6 (0.3-4.2)	6.2 (1.5-19.0)	4.2 (1.0-9.3)	<10 ⁻⁴

All donors underwent marrow as well as blood stem cell harvests. Values are calculated by patient weight.

Because of their impact on transplant-related mortality, the number of infused cells could potentially affect conclusions of important transplant studies. For example, two large, recently concluded randomized studies (United Kingdom MRC AML 10,⁸ and the European Organization for Research and Treatment of Cancer [EORTC] and Gruppo Italiano Malattie Ematologiche Maligne Dell'Adulto [GIMEMA] cooperative group⁹) which showed no advantage for autologous BMT (ABMT) in first remission AML, had recommended 1×10^8 nucleated cells/kg as the target collection for ABMT. It is possible that inadequate cell doses may have compromised engraftment and increased mortality in the ABMT arm in these studies, negating any beneficial effect ABMT may have had. However, details on the effect of cell doses are not available for either of the two studies.^{8,9} The late survival advantage that appears to have emerged in the MRC AML 10 study for the ABMT arm¹⁰ may well have emerged earlier if the cell doses had been better controlled.

Using peripheral blood stem cells may be a better way of obtaining faster and more complete engraftment in patients autografted for AML,^{6,11} but may be associated with a higher relapse rate.^{12,13} The high relapse rate may be a result of reinfusion of more residual disease secondary to infusion of nucleated cell numbers that are much larger than those infused during marrow transplantation.¹² Because of these concerns, we do not use blood-derived stem cells for autografting patients with AML, but aim for a target collection of 2.5 to 3×10^8 nucleated marrow cells/kg after two courses of consolidation chemotherapy.¹

After appreciating the importance of cell dose, in order to at least partially eliminate this issue from a double-blind randomized comparison of peripheral blood versus marrow for allogeneic transplantation which has just finished recruitment,¹⁴ we chose 3×10^8 nucleated donor marrow cells per kilogram patient weight as the target for collection. Despite this, and the fact that all the marrow donors underwent just one apheresis procedure, the difference between marrow and blood-derived nucleated and CD34⁺ cell numbers remained highly significant (Table 1).¹⁴

A more precise measure of the progenitor cell content of a hematopoietic stem cell graft is the number of CD34⁺ cells. Mavroudis et al¹⁵ showed that a low CD34⁺ cell dose was associated with increased mortality after T-cell-depleted allogeneic BMT. The only problem with using CD34⁺ cell numbers is that this may not measure the contribution of accessory cells including lymphocytes to engraftment and immune recovery.

Until more definitive data are available on the effect of CD34⁺ cell numbers on transplant-related mortality, it would be reasonable to aim for 2.5 to 3×10^8 nucleated marrow cells for autografting in acute leukemia, at least 3×10^8 nucleated marrow cells for allogeneic transplantation from HLA-identical siblings, and at least 4×10^8 nucleated marrow cells for allogeneic transplantation from unrelated donors.

Jayesh Mehta
Ray Powles
Jennie Treleaven
Samar Kulkarni

Clive Horton
Seema Singhal
Leukaemia Unit
Royal Marsden Hospital
Surrey, UK

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Granzyme A mRNA Expression in Mycosis Fungoides Progression

To the Editor:

In a recent issue of *Blood*, Oudejans et al¹ showed the presence of CD8⁺, granzyme B⁺ lymphocytes, considered to be activated cytotoxic T cells, in Hodgkin's disease. Interestingly, the presence of a high percentage of granzyme B⁺ lymphocytes was found to be an unfavorable prognostic marker. This is surprising because cytotoxic lymphocytes mediated lysis of tumor cells, predominantly achieved by perforin and granzymes, is considered to be of major importance for antitumor response.² Oudejans et al suggested that their findings reflect an inability of activated cytotoxic T lymphocytes to kill the malignant Hodgkin's Reed-Sternberg cells. They speculated that certain cytokines, mainly interleukin-10 (IL-10), known to be produced by the malignant cell, could be responsible for this phenomenon, as it may induce a local T-cell anergy.

We have very recently shown IL-10 overexpression in mycosis fungoides (MF), a frequent cutaneous T-cell lymphoma.³ Although there is evidence for a local⁴ and a systemic antitumor cell-mediated immune response,⁵ granzyme A mRNA expression was studied only in MF skin samples by *in situ* hybridization and was not detected.⁶ Therefore, we investigated whether cutaneous granzyme A mRNA is detectable in this non-Hodgkin's lymphoma, by using a sensitive competitive reverse transcriptase-polymerase chain reaction (RT-PCR) technique as recently described.³

Granzyme A mRNA was detectable in all cutaneous T-cell lymphoma skin samples. A stage-dependent increase of granzyme A mRNA expression was detected (Table 1). Remarkably, correlation with a stage-dependent increase of IL-10 mRNA levels was found ($r = .645$, $P = .003$). Moreover, in parallel an increase of CD3 mRNA expression, indicating increasing T-cell infiltration, was observed (Table 1).

Our findings indicate that the presence of granzyme, a marker

of activated cytotoxic T lymphocytes (CTL)/natural killer cells, in lymphoma is not restricted to Hodgkin's disease. Moreover, similar to the findings in Hodgkin's disease, granzyme expression seems to be a negative prognostic sign in MF, because a stage-dependent increase was found and advanced stages are known to have a less favorable prognosis than early stages.⁷ Finally, the concomitant increase of cutaneous IL-10 and granzyme A mRNA levels further support the hypothesis by Oudejans et al that this cytokine may be involved in the development of some kind of CTL resistance at the site of the tumor.

In contrast to Oudejans, who performed immunohistological double-staining experiments, we could not conclude from our mRNA data that CD8⁺ cytotoxic T cells are the source of the granzyme expression in non-Hodgkin's lymphoma, although a T-cell source may be suggested because the CD3 mRNA levels rise in parallel. Moreover, it has been reported on the poor prognosis of granzyme B-expressing peripheral T-cell lymphomas.⁸ Remarkably, MF progression is often characterized by increasing numbers of malignant CD4 but decreasing CD8⁺ T cells in the skin lesions. Therefore, the increasing granzyme levels we found may reflect such a shift in the cellular pattern of the T-cell infiltrate, if the malignant MF cell could produce granzyme. Such a scenario would be of major importance, because it may indicate the capacity of the malignant cells to induce apoptosis in antitumor immune cells and escape from immune control. Therefore, further investigations to clarify these observations should be performed.

Khusru Asadullah
Markus Friedrich
Antje Haeußler
Wolfram Sterry
Department of Dermatology
Wolf-Dietrich Döcke
Hans-Dieter Volk
Department of Medical Immunology
University Hospital Charité
Berlin Humboldt University
Berlin, Germany

Table 1. Expression Values (AU; mean \pm SD) for Granzyme A, CD3, and IL-10 mRNA in Skin Samples

	CD3	Granzyme A	IL-10
Mycosis fungoides			
Patch stage (n = 11)	387 \pm 186*	132 \pm 131	76 \pm 73
Plaque stage (n = 5)	407 \pm 217*	196 \pm 135	140 \pm 95
Tumor stage (n = 3)	620 \pm 417	360 \pm 200	283 \pm 115
Pleomorphic TCL (n = 5)	1,337 \pm 599*	457 \pm 181	574 \pm 591
Psoriasis (n = 7)	145 \pm 164	49 \pm 62	45 \pm 16
Atopic dermatitis (n = 5)	147 \pm 61	44 \pm 51	346 \pm 302*
Normal skin (n = 8)	64 \pm 55	59 \pm 60	44 \pm 25

Abbreviation: TCL, T-cell lymphoma.

* $P < .01$ (Mann-Whitney test) compared with normal skin. Granzyme A and IL-10 mRNA expression of the advanced stages of MF (plaque and tumor) but not of the early (patch) stage was significantly higher than in healthy and psoriatic skin ($P < .01$ Mann-Whitney test).

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