The influence of graft monocytes on the outcome of allogeneic bone marrow transplantation

The influence of graft monocytes on graft-versus-host disease (GVHD) has not yet been established in clinical trials. To understand this association better, we evaluated the influence of bone marrow graft monocytes aiming to analyze, primarily, the correlation with acute GVHD and chronic GVHD and, secondarily, the correlation with engraftment and survival.

Allogeneic peripheral blood progenitor cells (PBPC) contain at least 10 times more T-cells than bone marrow. Most studies have demonstrated that the incidence and severity of acute GVHD after allogeneic PBPC transplantation are not higher than those observed after allogeneic bone marrow transplantation.[1,2] This may be due to a direct effect of granulocyte colony-stimulating factor on T-cell function[3] or the presence of cells that can suppress donor T-cell responsiveness.[4,5,6] The $CD14^+$ monocytes in mobilized peripheral blood mononuclear cells suppress donor T-cell proliferation in a dose-dependent fashion. Normal $CD14^+$ cells, when used in comparable numbers, can also suppress T-cell response, suggesting a similar functional state as those present in mobilized peripheral blood mononuclear cells or normal bone marrow.[4,7] However, recent studies suggest that both acute and chronic GVHD are more frequent after PBPC transplants.[8,9]

We retrospectively analyzed our data from the Bone Marrow Unit in the State University of Campinas. Eligibility criteria were: age <60 years; patients with primary malignant or non-malignant hematologic disease receiving bone marrow from an HLA-identical sibling; availability of enumeration of $CD34^+$ cells, T-cell subsets, B-cells and monocytes in the graft. All patients had at least 100 days of follow-up after transplantation, except those who died of GVHD before day 100. The analysis was based on July 1st, 2001.

The Kaplan-Meier method was used for estimating the probability of GVHD, and overall survival. Each outcome was evaluated using Cox proportional hazard regression models or logistic regression, and the χ-squared test, when appropriate. The level of statistical significance was $p \leq 0.05$. S-Plus Software version 2000 was used. Cut-off values with simultaneously the best sensitivity and specificity for the variables analyzed were chosen according to the receiver operating characteristics curve (ROC) method. Patients were grouped according to these values for analysis of acute and chronic GVHD.

We analyzed 83 patients. The patients' and donors' characteristics, and graft composition are shown in Table 1. The median day to reach peripheral leukocytes $\geq 0.5\times10^9$/L and platelet count $>20\times10^9$/L was 20 (11-34) and 18.5 (10-60), respectively. In univariate analysis, no parameter was correlated with a faster engraftment.

The frequency of acute GVHD, grades 2-4 was 12/83 (14.5%). In univariate analysis, total nucleated cells (TNC) infused $\geq 2.31\times10^8$/Kg and $CD14^+$ cells $\geq 4.78\times10^6$/Kg were correlated significantly with lower rates of acute GVHD (p=0.04, p=0.02, respectively). Furthermore, patients >27 years old and those with a donor gender mismatch had higher rates of acute GVHD (p=0.03 and p=0.04, respectively) (Table 2). In a multivariate analysis, both TNC and age maintained their significance as predicting a lower risk of acute GVHD. The probability was 3.2% when the patient was < 27 years old and TNC infused $\geq 2.31 \times 10^8$/Kg. A higher risk of acute GVHD (51.5%) was found in patients aged > 27 years and when TNC infused $\leq 2.31 \times 10^8$/Kg (p<0.001). The number of $CD14^+$ cells showed a correlation with TNC (R=0.48, Spearman's correlation). This interaction might explain the loss of significance for monocytes in the multivariate analysis. Clinical chronic GVHD of any grade developed in 31/77 (40%) patients available for analysis. It was extensive in

Table 1. Features of the patients and donors, and composition of the graft (median and range).

Patients, no.	*83*
Patients' gender, no.(%)	M=62 (75%) F=21 (25%)
Donor gender→Patient gender, no.(%)	M→M=31 (37%) F→F=12 (15%) M→F= 31 (37%) F→M=9(11%)
Disease	
CML, no. (%)	39 (47%)
AA, no. (%)	18 (22%)
AML, no. (%	11 (14%)
Others, no. (%)	15 (17%)
Alive, no. (%)	58 (70%)
Dead, no. (%)	25 (30%)
Overall survival (days)	696 (33-2278)
TNC/kg (10^8)	2.64 (1.46-5.74)
TMNC/kg(10^8)	1.16 (0.45-2.25)
CD34/kg(10^6)	4.26 (0.57-29.36)
CD3/kg(10^6)	37.75 (13.21-170)
CD3/CD4/kg(10^6)	19.6 (8.04-78)
CD3/CD8/kg(10^6)	15.63 (5.19-97.48)
CD19/kg(10^6)	16.1 (3.36-179.2)
CD14/kg(10^6)	5.73 (0.4-49.86)

Abbreviations: (no.) number; M: male; F: female; CML: chronic myeloid leukemia; AA: aplastic anemia; AML: acute myeloid leukemia, TNC: total nucleated cells; TMNC: total mononuclear cells.

Table 2. Univariate analyses examining the influence of the characteristics of the transplant and GVHD.

	Acute GVHD (p) [OR (CI)]	*Chronic GVHD (p) [OR (CI)]*
Age	0.03 [0.26(0.07-0.93)]	0.13 [2.22(0.79-6.26)]
Patients' gender	0.50 [1.59(0.43-5.93)]	0.52[1.45(0.46-4.53)]
Donors' gender	0.26 [2.05(0.57-7.44)]	0.10[2.38(0.83-6.82)]
Donor-gender mismatch	0.04 [6.83(1.40-33.45)]	0.45 [2.37(0.82-4.05)]
TNC	0.004 [0.15(0.04-0.58)]	0.84 [1.11(0.40-3.08)]
TMNC	0.26 [0.49(0.14-1.70)]	0.17 [0.48(0.17-1.39)]
CD34	0.26 [0.49(0.14-1.70)]	0.09 [0.42(0.14-1.18)]
CD3	0.30 [1.91(0.55-6.60)]	0.95 [0.97(0.35-2.67)]
CD4	0.90 [0.97(0.28-3.30)]	0.39 [0.64(0.3-1.78)]
CD8	0.39 [1.71(0.50-5.89)]	0.95 [0.97(0.35-2.67)]
CD19	0.19 [0.49(0.14-1.71)]	0.74 [0.84(0.30-2.32)]
CD14	0.02 [0.24(0.07-0.87)]	0.39 [0.64(0.23-2.32)]
Malignant vs. Non-malignant	0.94[0.94(0.23-3.89)]	0.23 [2.21(0.57-8.50)]
Previous acute GVHD		0.008 [9.17(1.62-52)]

Abbreviations: OR: odds ratio; CI: confidence interval.

20 cases and limited in 11 cases. In univariate analyses there was a correlation between previous acute GVHD and a higher risk of chronic GVHD ($p<0.001$). $CD14^+$ cells did not influence the development of chronic GVHD.

The estimated 6-year overall survival was 66% (95% CI: 55%-79%). In univariate analyses, the absence of acute GVHD was correlated with a higher survival ($p<0.001$). Furthermore, there was a trend for a better survival in patients receiving more $CD34^+$ cells ($p=0.06$), as described by others.[10] The CD14 cells had no impact on overall survival. These preliminary data suggest that $CD14^+$ cells (monocytes) may have a protective effect in allogeneic BMT. Further controlled studies should be done in order to clarify this important point.

Francisco J.P. Aranha, Afonso C. Vigorito, Cármino A. De Souza, Gislaine B. Oliveira, Roberto Zulli, Irene Lorand-Metze

Bone Marrow Transplantation Unit, State University of Campinas, SP/Brazil, Bone Marrow Transplantation Unit, P.O. Box 6198, Barão Geraldo, Campinas, São Paulo, Brazil

Key words: allogeneic bone marrow transplant, graft-versus-host disease, monocytes, $CD14^+$ cells.

Correspondence: Francisco JP Aranha, State University of Campinas, SP, Brazil, Bone Marrow Transplantation Unit, PO Box 6198, Campinas, Brazil.
Phone: international +55.19.7888729.
Fax: international +55.19.7888600.
E-mail: aranha@unicamp.br

References

1. Bensinger WI, Martin PJ, Storer B, Clift R, Forman SJ, Negrin R, et al. Transplantation of bone marrow as compared with peripheral-blood stem cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med 2001; 344:175-81.
2. Vigorito AC, Comenalli Marques JF Jr, Penteado Aranha FJ, Oliveira GB, Martins Miranda EC, De Souza CA. A randomized, prospective comparison of allogeneic bone marrow transplantation in the treatment of hematologic malignancies: an update. Haematologica 2001; 86:665-6.
3. Pan L, Delmonte J Jr, Jalonen CK, Ferrara JL. Pretreatment of donor mice with granulocyte colony-stimulating factor polarizes donor T lymphocytes toward type-2 cytokine production and reduces severity of experimental graft-versus-host disease. Blood 1995; 86:4422-9.
4. Mielcarek M, Martin PJ, Torok-Storb B. Suppression of alloantigen-induced T-cell proliferation by $CD14^+$ cells derived from granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells. Blood 1997; 89: 1629-34.
5. Ino K, Singh RK, Talmadge JE. Monocytes from mobilized stem cells inhibit T cell function. J Leukoc Biol 1997; 61: 583-91.
6. Tanaka J, Mielcarek M, Torok-Storb B. Impaired induction of the CD28-responsive complex in granulocyte colony-stimulating factor mobilized CD4 T cells. Blood 1998; 91: 347-52.
7. Tanaka J, Imamura M, Kasai M, Asaka M, Torok-Storb B. The role of accessory cells in allogeneic peripheral blood stem cell transplantation. Int J Hematol 1998; 69:70-4.
8. Cutler C, Giri S, Jeyapalan S, Paniagua D, Viswanathan A, Antin JH. Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. J Clin Oncol 2001; 19: 3685-91.
9. Schmitz N, Beksac M, Hasenclever D, Bacigalupo A, Gorin N, Szer J, et al. A randomised study from the European Group for Blood and Marrow Transplantation comparing allogeneic transplantation of filgrastim-mobilised peripheral blood progenitor cells with bone marrow transplantation in 350 patients with leukemia. Blood 2000; 96: Suppl 11:2068a[abstract].
10. Morariu-Zamfir R, Rocha V, Devergie A, Socie G, Ribaud P, Esperou H, et al. Influence of $CD34^+$ dose on outcome of HLA-identical sibling allogeneic bone marrow transplants in patients with chronic myeloid leukemia. Bone Marrow Transplant 2001; 27:575-80.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received October 8, 2001; accepted December 3, 2001.