Immunobiology 219 (2014) 611–618

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Evolution of peripheral blood T lymphocyte subsets after allogenic or autologous hematopoietic stem cell transplantation

Bénédicte Puissant-Lubrano[a,b], Anne Huynh[c], Michel Attal[c], Antoine Blancher[a,b,*]

[a] Laboratoire d'Immunogénétique Moléculaire (EA3034), Université Paul Sabatier, Toulouse 3, France
[b] Laboratoire d'Immunologie, CHU de Toulouse, France
[c] Service d'Hématologie Clinique, CHU de Toulouse, France

ARTICLE INFO

Article history:
Received 6 January 2014
Received in revised form 13 March 2014
Accepted 14 March 2014
Available online 21 March 2014

Keywords:
Allogenic HSCT
Autologous HSCT
Hematopoietic stem cell transplantation
T lymphocyte reconstitution

ABSTRACT

With the aim to search for differences in T cell reconstitution after allogenic or autologous hematopoietic stem cell transplantation (HSCT), we characterized peripheral blood T-cell subsets by means of flow cytometry, in adult patients who had undergone either allogenic ($n = 23$) or autologous ($n = 29$) HSCT for the treatment of hematological malignancies. The patients were followed every 3 months for 21 months after HSCT.

Compared to healthy controls ($n = 20$ blood donors), the two transplanted groups displayed (i) a CD4 lymphopenia, (ii) a low percentage of naive T cells, (iii) high percentages of memory T cells and of activated T cells (HLA-DR+, CD25+) and high percentages of CD4 T cells with a high expression of CD25. The levels of TRECs (TCR rearrangement excision circles) were not significantly different between the two groups. In total, the differences of the nature and the speed of T lymphocyte reconstitution observed between the two patient groups were minor. This leads us to conclude that in allografted patients, lymphocyte activation as well as many other disturbances of subpopulations of peripheral blood lymphocytes are probably not related to the allogenicity of the graft, but are due to the expansion of T cells transfused with HSC and slow differentiation of T lymphocytes in the thymus progressively colonized by bone marrow-derived T-cell precursors.

Introduction

Both allogenic and autologous hematopoietic stem cell transplantations (HSCT) result in a transitory severe immune deficiency, mainly due to the conditioning regimen of the recipient required to obtain the engraftment of stem cells and to prevent the risk of rejection. The immune reconstitution after HSCT is a stepwise process. Recovery of innate immunity occurs rapidly, within 100 days after HSCT, with the production of monocytes, granulocytes and natural killer lymphocytes (Storek et al. 2004). The reconstitution of adaptive immunity is much slower. The B lymphocytes reach normal values within 6–12 months after HSCT while the CD4 T lymphocytes are the latest subset to normalize. The progressive increase of T cell numbers involves different mechanisms such as the peripheral expansion of mature donor memory T cells transferred together with HSC and the differentiation of naïve T cells inside the thymus colonized by precursors differentiated from the grafted HSC (see Mackall et al. 1997; Guillaume et al. 1998 for general reviews). CD8 T cells normalize within 3 months after HSCT, earlier than the CD4 T cells which remain low even two years post HSCT (Guillaume et al. 1998). Indeed, it was shown that the reconstitution of CD4 T cells relies more on thymic production than the reconstitution of CD8 T cells, which depends mainly on the expansion of mature grafted T lymphocytes (Mackall et al. 1997). Thus, combined immune deficiencies following allogenic or autologous HSCT are responsible for severe impairment of the adaptive cellular and humoral immune responses, which contribute to the morbidity and mortality of recipients through infectious complications (Storek et al. 1995).

The graft versus host disease (GvHD), which often complicates allogeneic transplantation, increases the difficulties of physiological immune restoration by driving the repertoire of T cells toward pathogenic alloreactive T cells. In addition, prophylaxis and

Abbreviations: GvHD, graft versus host disease; HSCT, hematopoietic stem cell transplantation; TCM, central memory T cells; TEM, effector memory T cells; TRECs TCR, rearrangement excision circles.

* Corresponding author at: Laboratoire d'Immunologie du CHU de Toulouse, Hôpital Rangueil, TSA 50032, 31059 Toulouse Cedex 9, France. Tel.: +33 5 61 32 34 32; fax: +33 5 61 32 34 24.
E-mail address: blancher.a@chu-toulouse.fr (A. Blancher).

http://dx.doi.org/10.1016/j.imbio.2014.03.012
0171-2985/

treatment of GvHD by immunosuppressive drugs impair the reconstitution of the adaptive immune system (see Ferrara et al. 2009 for review).

Allo- and auto-HSCT recipients differ by the nature of the graft, the immunosuppressive treatment and the occurrence of GvHD. Therefore, immune reconstitution may occur differently in these two types of transplantation. We asked whether the T cell reconstitution of allo-HSCT patients differed significantly from that of auto-HSCT. Three previous studies have analyzed the recovery of peripheral CD4+ and CD8+ T lymphocyte counts in allo- and auto-HSC recipients. A faster recovery was observed after allogenic HSCT in two studies performed in children (Kalwak et al. 2002; Schwinger et al. 2006) and after autologous HSCT in the third study performed in adults (Roberts et al. 1993). In the present investigation we refined the analysis of T lymphocyte subsets (including naïve, memory and activated T lymphocytes) after HSCT in adults and, by means of TRECs, we explored the contribution of the thymus to the reconstitution of peripheral T cells. Peripheral blood T-cell reconstitution in adult patients with hematological malignancies was surveyed during the first 21 months after allogenic or autologous hematopoietic stem cell transplantation. The blood T cell phenotype was analyzed by flow cytometry performed on whole blood labeled with monoclonal antibodies specific for classical markers. We discussed the mechanisms that drive T lymphocyte reconstitution after allogenic and autologous HSCT.

Patients, materials and methods

Patients

Fifty-two patients with advanced hematological malignancies who had undergone either allogenic ($n = 23$) or autologous ($n = 29$) HSCT were studied. Clinical parameters of patients including conditioning regimen, GvHD prophylaxis (for allo-SCT recipients) and clinical outcome are described in Table 1. Each patient was studied every three months until 21 months after transplant. As it was not always possible to obtain all blood samples, the number of patients varied depending on the period. Details are provided in the figures. Twenty blood donors were studied as healthy controls (HC). The study was approved by the local institutional review board. All patients gave written informed consent.

Quantification of TREC (TCR rearrangement excision circle) levels

Fresh PBMCs from patients or healthy controls were separated into CD4+ and CD8+ populations using magnetic microbeads (Miltenyi Biotec). DNA was isolated from PBMC, CD4+ and CD8+ enriched fractions by using the Wizard® Genomic DNA Purification Kit (Promega, Charbonnières-Les-Bains, France). The DNA concentrations were measured using the Picogreen DS-DNA quantitation kit (Molecular Probes, Interchim, Montluçon, France). Signal-joint TRECs were quantified by real-time quantitative PCR (ABI 5700 system, Applied Biosystem, Courtaboeuf, France) (Douek et al. 2000).

From the average TREC content, as measured per microgram DNA, the TREC content per 10^6 cells was calculated considering that 1 μg DNA corresponds to 150,000 cells. The TREC/10^6 cells were corrected by the percentage of CD3+ cells found by flow cytometry in the three cell samples studied (PBMCs, CD4+ and CD8+ PBMC-sorted fractions), yielding the number of TREC/10^6 CD3, TREC/10^6 CD3+CD4+ and TREC/10^6 CD3+CD8+.

Flow cytometry

Absolute numbers of T lymphocyte subsets (CD3+CD4+, CD3+CD8+) were determined by flow cytometry using TruCount tubes (BD Biosciences, Le Pont de Claix, France).

Various monoclonal antibody combinations were used to characterize T lymphocytes: (i) activated T cells (CD25-PE and HLA-DR-PE from BD Biosciences), (ii) naïve T cells and memory T cells (CD45RO-PE (Dako, Trappes, France), CD45RA-FITC, CD62L-PE, CD3-PerCp, CD4-APC (all from BD Biosciences)). The naïve and memory T cell subsets were defined as follows: central memory T cells (TCM) as $CD45RA^{neg}CD62L^{high}$, effector memory T cells (TEM) as $CD45RA^{neg}CD62L^{low}$ and T naïve T cells were defined as $CD45RA^{pos}CD62L^{high}$ (Sallusto et al. 1999). Acquisitions were performed on a Facs Calibur cytometer (BD Biosciences). Lymphopenia was defined according to reference ranges (95% confidence) provided by BD Biosciences: CD3+CD4+<400/mm^3 and CD3+CD8+ <200/mm^3. The CD25, HLA-DR and CD45RO markers have been added after the start of the study. This explains why the number of patients at different time points varied from one lymphocyte subset to another.

Statistical analysis

Qualitative patient characteristics were compared using Fisher's exact test while quantitative patient characteristics were compared using the *t* test. T lymphocyte subset reconstitution between groups was compared using the Mann–Whitney test. Longitudinal analysis was performed using the Kruskall–Wallis test. Values of $p < 0.05$ were taken to be statistically significant. In all the figures, the statistical results are detailed: NS: >0.05; *: 0.01–0.05; **: 0.0001–0.01; ***: <0.0001.

Results

Patient characteristics and outcome

The patient characteristics (summarized in Table 1) showed that the allo-HSCT recipients were younger than the auto-HSCT recipients ($p < 0.0001$). The two groups did not differ significantly in the proportion of patients suffering from infectious events during the follow-up (19 out of 23 allo-HSCT recipients and 19 out of 29 auto-HSCT recipients; $p = 0.2$) nor in terms of the overall survival 24 months after transplantation ($p = 0.99$). The total number of infections tended to be higher in allografted patients ($n = 33$ infectious events) compared to autografted recipients ($n = 26$, $p = 0.065$). The number of fungal infections was higher in allografted patients than in autografted recipients ($p = 0.02$, Table 1). The frequencies of CMV sero-positive individuals before HSCT were almost identical in the two groups of patients (11 out of 23 allografted patients and 12 out of 23 autografted patients). After graft, nine out of 11 CMV+ allografted patients experienced CMV reactivation between the first and the third months (M1 and M3), among them, seven were grafted with HSC from a CMV+ donor and two were grafted with HSC from a CMV− donor. The two CMV− recipients who received allo-HSC from a CMV+ donor were not infected by CMV (as demonstrated by PCR monitoring after HSCT). None of the 12 CMV+ patients treated by auto-HSCT experienced CMV reactivation, they thus differed significantly from their allo-HSCT counterparts ($p = 0.0015$). Although most blood samples were obtained from patients free of infection, one allografted patient suffered from a thoracic zona at M12 and from a viral cold at M18, and one autografted patient suffered from a *Haemophilus influenzae* bronchopathy at M15. As for GvHD, seventeen allo-HSCT recipients (74%) developed acute GvHD during the first two months after transplantation; and one of them developed mild chronic GvHD. The frequency of acute GvHD is thus comparable to those described in a previous study (Ferrara et al. 2009).