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Immune reconstitution in patients with Fanconi anemia after allogeneic bone marrow transplantation

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Abstract

Background aims. Fanconi anemia is an autosomal recessive or X-linked genetic disorder characterized by bone marrow (BM) failure/aplasia. Failure of hematopoiesis results in depletion of the BM stem cell reservoir, which leads to severe anemia, neutropenia and thrombocytopenia, frequently requiring therapeutic interventions, including hematopoietic stem cell transplantation (HSCT). Successful BM transplantation (BMT) requires reconstitution of normal immunity. *Methods.* In the present study, we performed a detailed analysis of the distribution of peripheral blood subsets of T, B and natural killer (NK) lymphocytes in 23 patients with Fanconi anemia before and after BMT on days +30, +60, +100, +180, +270 and +360. In parallel, we evaluated the effect of related versus unrelated donor marrow as well as the presence of graft-versus-host disease (GVHD). *Results.* After transplantation, we found different kinetics of recovery for the distinct major subsets of lymphocytes. NK cells were the first to recover, followed by cytotoxic $CD8^+$ T cells and B cells, and finally $CD4^+$ helper T cells. Early lymphocyte recovery was at the expense of memory cells, potentially derived from the graft, whereas recent thymic emigrant ($CD31^+$ $CD45RA^+$) and naive $CD4^+$ or $CD8^+$ T cells rose only at 6 months after HSCT, in the presence of immunosuppressive GVHD prophylactic agents. Only slight differences were observed in the early recovery of cytotoxic $CD8^+$ T cells among those cases receiving a graft from a related donor versus an unrelated donor. Patients with GVHD displayed a markedly delayed recovery of NK cells and B cells as well as of regulatory T cells and both early thymic emigrant and total $CD4^+$ T cells. *Conclusions.* Our results support the utility of post-transplant monitoring of a peripheral blood lymphocyte subset for improved follow-up of patients with Fanconi anemia undergoing BMT.

Key Words: *bone marrow, Fanconi anemia, immune system, transplantation*

Introduction

Proliferation and maturation of blood cells are tightly regulated processes that involve a large number of growth factors and cytokines (1,2). In addition, the bone marrow (BM) stroma, which comprises vascular and mesenchymal cells, is critical to provide the ideal microenvironment for proper hematopoiesis (3). Therefore, changes in cytokine levels and/or the hematopoietic microenvironment may lead to altered hematopoiesis, including BM aplasia (4). Fanconi anemia (FA) is an inherited disease characterized by BM failure (5). Patients with FA have a defect in DNA repair that progressively leads to the accumulation of chromosomal and genetic alterations. Apart from anemia, patients with FA display congenital malformations, deafness and skin hyper-pigmentation (eg, "café au lait" spots), among other symptoms (6). Regarding outcome, FA is an unpredictable disease, with some patients having development of leukemia and solid tumors at different stages in life (7). Among the available therapeutic options, hematopoietic stem cell transplantation (HSCT) is the most effective approach, significantly extending patient lifespan (8–12). One of the major caveats associated with HSCT is the transient immune deficiency state that follows transplantation, which is associated with significant patient morbidity and mortality (13). The duration and severity of immune

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deficiency vary according to graft manipulation, the choice of the type of graft, the development of graft-versus-host disease (GVHD) and the level of residual thymic activity, among other variables (14,15).

Assessment of lymphocyte-associated markers can be a useful tool in the clinical setting and is currently under research (i) to monitor response of patients with FA to therapy, (ii) to measure disease activity, and (iii) to predict chronic GVHD (16–19). Successful allogeneic HSCT (allo-HSCT) requires reconstitution of normal T-cell immunity; key factors involved in this process include thymic activity of the HSCT recipient, biological features of the allograft (eg, degree of histocompatibility, number and type of infused donor T cells) and preparative regimens. Specific suppression of allo-reactive T cells, without inhibiting the entire T-cell repertoire, remains an important goal of transplantation immunology (20–22).

In the present study, we evaluated the number and immunophenotype of circulating T, B and natural killer (NK) cells and their subsets in peripheral blood (PB) samples from 23 patients with FA who underwent allogeneic BM transplantation (BMT) after a myeloablative conditioning regimen. Our goal was to better understand the kinetics of immune reconstitution in patients with FA who received an allogeneic BMT and to identify potential factors associated with normal versus altered immune recovery.

Methods

Patients

A total of 23 patients with FA (12 male and 11 female; age range, 4–21 years) who underwent allogeneic BMT between 2009 and 2011 were studied. Before entering the study, informed consent was obtained for all subjects, and the study was approved by the Institutional Review Board of the Federal University of Clinics Hospital (Curitiba, Brazil). Twelve patients (52%) received a transplant from related donors, whereas in the other 11 cases (48%) the graft was of unrelated origin. The conditioning regimen consisted of cyclophosphamide (60 mg/kg) alone for those cases that received a BMT from related donors, whereas patients who received a BMT from an unrelated donor were treated with a combination of cyclophosphamide (60 mg/kg), fludarabine (125 mg/m^2) and thymoglobulin (5 mg/kg). For both groups, immunoprophylactic treatment consisting of cyclosporine and methotrexate (Table I) was given for a mean time of 12 months (range, 4–22 months). Successful engraftment was observed in 21 of 23 patients (91%) after a minimum follow-up of 1 year (range, 1–5 years). Overall, 13 patients had acute (n = 5) and/or chronic (n = 8) GVHD. Causes of death (n = 2) were acute GVHD occurring on day +30 in one patient and transplant rejection occurring on day +375 after HSCT in the second patient (Table I). Complete chimerism was defined when 100% of donor cells were detected, indicating complete hematopoietic replacement by donor cells; in turn, mixed chimerism was defined when host cells were detected within specific cell populations (eg, the lymphocytes) at percentage values ≥10% and ≤95% of all cells (Table I); the presence of mixed chimerism did not influence therapy. No patient received Rituximab or manipulated (eg, *in vitro* T-cell–depleted) bone marrow grafts; however, 56% of patients (Table I) received *in vivo* T-cell depletion with a low dose of rabbit thymoglobulin (5 mg/kg) during the conditioning regimen.

Table I. Patient characteristics (n = 23).

Age at transplantation (y)	8 (4–21)
Sex (male/female)	12/11 (54%/46%)
Donor recipient histocompatibility	
HLA identical	20/23 (87%)
One (HLA-A, B or DR) mismatch	3/23 (13%)
Donor	
Related	12/23 (52%)
Unrelated	11/23 (48%)
Conditioning regimen	
FLU/ATG/CFA	13/23 (56%)
Cyclophosphamide	9/23 (35%)
FLU/TBI	1/23 (4%)
Immunoprophylaxis	
Cyclosporine + methotrexate	21/23 (91%)
Mycofenolate + cyclosporine + CFA	2/23 (9%)
GVHD	13/22 (59%)
Acute GVHD	5/22 (23%)
Chronic GVHD	8/21 (38%)
Chimerism on day +100	
Complete	12/23 (52%)
Mixed	11/23 (48%)
Cause of death	
Acute GVHD	1/23 (4%)
Rejection	1/23 (4%)

Results are expressed as number of cases and percentages in parentheses or as median (range).
ATG, thymoglobulin, 5 mg/m^2; CFA, cyclophosphamide, 60 mg/kg; FLU, fludarabine, 125 mg/m^2; HLA, human leukocyte antigen; TBI, total body irradiation, 200 cGy.

Multiparameter flow cytometric analysis of lymphocyte subsets

PB samples were collected into tubes containing 7.5% K3 ethylenediaminetetra-acetic acid for both flow cytometry and complete blood cell count