Applications of Flow Cytometry to Hematopoietic Stem Cell Transplantation

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Applications of flow cytometry to clinical and experimental hematopoietic stem cell transplantation (HSCT) are discussed in this review covering the following topics: diagnosis and classification of lymphohematologic disorders, quantitation of hematopoietic progenitors in the graft, lymphohematopoietic reconstitution following HSCT and animal models of human HSCT. At the end, the utilization of flow cytometry in clinical HSCT by Brazilian transplant centers is briefly reviewed.

Key words: flow cytometry - bone marrow transplantation - stem cell transplantation

During the last two decades, hematopoietic stem cell transplantation (HSCT) became the most efficient modality of treatment for a variety of lymphohematopoietic neoplasms and for some solid tumors and non-malignant disorders (Thomas 1994). Considerable progress in the technology of HSTC have expanded its clinical application and improved its survival rate, leading to an exponential growth in the numbers of transplants performed and of transplantation centers in operation, including in our country. This progress also caused a great diversification of HSCT in addition to the conventional HLA-identical sibling bone marrow transplantation (BMT): autologous, family related unmatched, family unrelated matched or unmatched, cord blood and peripheral blood transplantation have been introduced to medical practice. In the future, advances in immunotherapy, genetic engineering and cell selection will certainly lead to much better results of HSCT in curing a variety of diseases with otherwise fatal course.

In 1984, the Seattle group, which pioneered the field of human bone marrow transplantation, published a short review on the application of flow cytometry to BMT (Martin et al. 1984). Presentation was restricted to quantitative assays to assess the efficiency of T cell depletion from the marrow graft in order to prevent graft-versus-host disease (GVHD), concluding that we have found flow microfluorometry to be an indispensable tool in the development of methods of removing T cells from donor marrow. However, the prospective was that this application of flow microfluorometric technology to human marrow transplantation represents only one of several possible such applications. Flow microfluorometric technology will find utility in any situation requiring sensitive and specific methods for the identification of distinct cell populations in human bone marrow or blood.

In fact, two years later we disclosed in another review a broader range of applications of flow cytometry to BMT, including characterization of immunologic reconstitution, pathophysiologic and diagnostic investigation of GVHD, viral infections and minimal residual disease (Voltarelli & Stites 1986). A Medline search of the literature from October 1981 through January 1999 produced more than 500 papers in this area. Most of them focus on the same above mentioned subjects, but there were significant advances also in the detection of engraftment, of alloimmunization against blood cells, in the investigation of graft-versus-leukemia effect, in the quantitation of HSC and, more recently, in animal models of human transplantation and in gene therapy. Thus, technological improvements in equipments, methods, reagents and analytical software played a major role in the progress made in this area.

The purpose of this review is to summarize the participation of flow cytometry technology in the progress of HSCT during the last five years both at the clinical and experimental levels highlighting the contribution of our group to the field.

CLINICAL APPLICATIONS OF FLOW CYTOMETRY TO STEM CELL TRANSPLANTATION

In this section, current and potential utilization of flow cytometry to the management of HSCT candidates or patients will be discussed.
Diagnosis and classification of lymphohematologic disorders - Immunophenotypic characterization of lymphohematopoietic neoplasms was one of the first applications of flow cytometry to clinical medicine improving the precise diagnosis and staging of those diseases as well as the detection of their persistence or recurrence after HSCT (Jennings & Foon 1997). Other papers in this volume will certainly cover the subject. In Brazil, a survey of 225 patients with acute lymphoblastic leukemia (ALL) showed a higher frequency of the B-mature phenotype in the white population compared to developed countries. In the non-white population, there was a lower incidence of ALL in childhood, particularly of the common ALL (cALL) subtype, resembling the situation in non-white Americans before the 1970s and of British and American whites at the beginning of the century (Rego et al. 1996). In addition, among 117 patients with cALL, a high frequency of the CD19+CD10+strong phenotype was observed in both children and adults and overexpression of CD10 and/or CD19 occurred in 42% of the cases which may be helpful for diagnosis or detection of minimal residual disease (Rego et al. 1999). On the other hand, children up to four years of age have more than 65% of B cells in the lymphocyte window in the bone marrow. Most of these cells have the immature phenotype CD19+CD10+ which may be confounded with leukemic blasts (Rego et al. 1998a).

Simultaneous analysis of three or four antigens on the cell surface, employing one or two laser beams, and the quantitative evaluation of antigen density were two significant technological advances in the diagnosis of hematological malignancies. Thus, multiparameter flow cytometry was successfully employed to detect occult B cell malignancies in cytopenic patients (Wells et al. 1998a), to characterize acute myeloid leukemia with minimal differentiation (AML-M0) (Cohen et al. 1998) and to differentiate normal from leukemic blast cells after bone marrow transplantation (Wells et al. 1998b). Distinction between normal and leukemic cells can also be accomplished by quantitative flow cytometry, at least in ALL (Farahat et al. 1998, Rego et al. 1998b) and by intracellular markers in AML (Konikova et al. 1998). However, most efficient methods for detection of minimal residual disease combine fluorescent activated cell sorting with molecular methods such as in situ hybridization (Cotteret et al. 1998) or reverse transcriptase-polymerase chain reaction (RT-PCR) (Rasmussen et al. 1998).

Using multiparametric cytometry and well defined study protocols, the Salamanca group made significant contributions to the detection of minimal residual disease in hematologic neoplasms. A variety of diseases were studied, including ALL (Ciudad et al. 1998), AML (Macedo et al. 1995 San Miguel et al. 1997), chronic lymphocytic leukemia (Taberner et al. 1995), mast cell leukemia (Cervero et al. 1999) and multiple myeloma (Occuteau et al. 1996).

In addition, as we did earlier (Falcão et al. 1992), unusual NK-associated malignancies continue to be described with the help of flow cytometry (Drenou et al. 1997, Suzuki et al. 1997). The case we reported was a CD3+CD16+TCRγδ+ T cell leukemia with functional NK activity. The more recent ones were, respectively, two CD3-CD56+ non-Hodgkin’s lymphomas with no NK activity but aggressive behaviour related to a multi drug resistant (MDR) phenotype, and seven CD7+CD56+ leukemias which could represent an yet unrecognized common myeloid/NK primitive precursor.

Characterization and differential diagnosis of many nonmalignant disorders treatable by HSCT has also benefited from recent advances in the detection of specific disease markers by flow cytometry. Examples of those diseases are paroxysmal nocturnal hemoglobinuria (Doukas et al. 1998), Wiskott Aldrich syndrome (Yamada et al. 1999), leukocyte adhesion deficiency (Thomas et al. 1995), MHC class II deficiency (Klein et al. 1999), DiGeorge Syndrome (Matsumoto et al. 1998) and chronic granulomatous disease (Atkinson et al. 1997).

Quantitation of hematopoietic progenitors in the graft - One of the most significant breakthroughs for the success of HSCT was the phenotypic and functional characterization of the primitive cells responsible for lymphohematopoietic reconstitution in transplanted patients (Baum et al. 1992). Discovery of the CD34 molecule present on many of these cells caused an explosion of new data on the mechanisms of normal, pathological and transplanted hematopoiesis. It also provided a very powerful tool to evaluate the engraftment requirements and repopulating potential of different types of grafts now employed in clinical HSCT. More recently, however, a CD34neg human stem cell was also described (Bhatia et al. 1998).

With the exception of transfusion sensitized aplastic anemia patients, HLA-identical sibling BMT or PBSCT usually show very high engraftment rates. In other types of transplants, such as allogeneic HLA-mismatched, unrelated, T cell depleted, from cord blood and, particularly, in autologous transplantation, the numbers and function of HSC in the graft usually are more critical to achieve engraftment. Thus, qualitative and quantitative evaluation of HSC present in the graft be-
came a reliable predictor of engraftment in many modalities of HSCT and have been extensively used to select the best timing to harvest the cells to be transplanted. A minimum cell dose of 0.75 x 10^6 CD34+/kg was found to be necessary for engraftment of autologous PBSC and this dose could be achieved when the absolute CD34+ cell count was >5/µl in the peripheral blood (Perez Simon et al. 1998). Other studies, however, found higher thresholds for CD34+ cell dose (2 to 2.5 x 10^5/kg) and for absolute CD34+ cell counts in peripheral blood (10/µl) (Schots et al. 1996, Dansey et al. 1998). For unmanipulated allogeneic PBSC, the threshold cell dose was found to be 2.5 x 10^6 CD34+ cells/kg (Korbling et al. 1995) while for T cell-depleted marrow transplants it was 10^6 CD34+/kg (Mavroudis et al. 1996). Nucleated cell dose and CD34+ cell dose are major determinants in engraftment of cord blood transplants (reviewed by Cairo & Wagner 1997). However, the threshold number of CD34+ cells needed for engraftment could not be determined in a large cooperative study of cord blood transplantation in Europe (Eurocord) due to problems in the standardization of HSC quantitation (Gluckman et al. 1998).

Several recent studies assessed the content, phenotype and function of HSC from different transplantable tissues using flow cytometry. Compared to peripheral blood, cord blood contains a higher proportion of primitive HSC (CD34+HLA-DR-) and of immature and non-functional T cells (Cairo & Wagner 1997, Madrigal et al. 1997, Dimitriou et al. 1998). These features suggest that cord blood cells may be more efficient and cause less GVHD than other sources of HSC. On the other hand, bone marrow cells aspirated from iliac crests contain a high proportion of HSC with B cell markers while most HSC from peripheral blood mobilized with either high dose chemotherapy or myeloid growth factors have myeloid markers (Fritsch et al. 1996). This difference may explain the faster myeloid engraftment after mobilized PBSCT compared to non-mobilized BMT (Talmadge et al. 1997, Vigorito et al. 1998). Cadaveric vertebral bodies have also been used in some protocols to promote hematopoietic reconstitution or to increase donor cell chimerism in solid organ transplantation. Vertebral bodies have more nucleated cells than bone marrow aspirated from iliac crests of normal allogeneic donors and equivalent content of HSC, as evaluated by clonogenic assays or flow cytometry (Rybka et al. 1995). Finally, the content of megacaryocytic precursors (CD34+/CD61+) in the graft could be correlated with time of platelet engraftment in patients submitted either to allogeneic (Bojko et al. 1998) or to autologous (Johnsen et al. 1998) peripheral blood HSCT.

The very low frequency and unremarkable morphologic characteristics of HSCT in various tissues make it very difficult to precisely quantify these cells. Many factors interfere with their enumeration, such as sample storage (Gutensohn et al. 1996), cell washing (Menendez et al. 1998), red cell lysing method (Cassens et al. 1998) and the quality of the monoclonal antibody used (Macey et al. 1997). In fact, the latter and other reports have shown that mAbs to class II and III epitopes of the CD34 molecules are less affected by lysis and fixation procedures than class I antibodies. Several protocols have been devised to standardize the flow cytometric enumeration of HSC and to minimize artifacts and count errors. Recent comparisons between most popular protocols showed superiority of a volumetric method employing reference beads over the International Society of Hemotherapy and Graft Engineering (ISHAGE) four-parameter methodology (Leuner et al. 1998) and over institutional in-house methods when CD34 cell counts are very high (Olivero et al. 1999).

Fewer studies have investigated functional aspects of HSC containing transplants by flow cytometry. Chalmers et al. (1998) showed that cord blood T lymphocytes produced less intracellular proinflammatory cytokines (mainly IFN-γ and TNF-α) than peripheral blood lymphocytes. In addition, most cytokine producing cells in cord blood were naive T helper cells (CD4+RA+) while in peripheral blood they were both T helper (CD4+RO+) and T cytotoxic cells (CD8+RO+) memory cells. These results could explain in part the lower incidence of GVHD after cord blood transplantation. In another study, the rate of the rhodamine-123 vital dye efflux from CD34+ cells was correlated with cell immaturity and with repopulating capacity of human HSC (Uchida et al. 1996). The most primitive CD34+Thy1+-Lin- self-renewing cells had low or medium levels of Rh-123 retention while CD34+ cells with high level of dye retention lacked long-term engraftment potential.

**Lymphohematopoietic reconstitution following HSCT** - Immunologic reconstitution after HSCT is a very complex and important series of phenomena which could not be properly investigated until the advent of flow cytometry. Low numbers of circulating cells with exquisite phenotypes and multiple functional abnormalities seen in the posttransplant period are very difficult to characterize with conventional fluorescence microscopy or cell culture based immune function methods. While immune reconstitution followup is not routinely performed after HSCT, information provided by its investigation has great predictive value for infection, GVHD, relapse and immunoprofilaxis.
On the other hand, hematopoietic engraftment following HSCT is routinely monitored by rising blood cell counts following the pancytopenic state caused by the conditioning regimen. However, fluoro-cytometric techniques may detect early and more subtle signs of myeloid engraftment and may help to distinguish between several types and degrees of chimerism that may occur in the event of graft failure or recurrent disease. We have found that graft failure after allogeneic bone marrow transplantation is associated with defective expression of IL-2R (CD25) on activated CD8 T cells (Voltarelli et al. 1989) and that a mild T cell depletion with anti-CD6 mAb may explain its low rate of graft failure compared to other methods (Voltarelli et al. 1990). More recently, fluoro-cytometric reticulocyte counts detect engraftment earlier than neutrophil counts (SMSGHR 1994, Greinix et al. 1994) and a panel of Mabs against erythrocyte antigens could be used to uncover both early engraftment and chimeric states after HSCT (Blanchard et al. 1995, Nelson et al. 1996, Hendriks et al. 1997). Finally, monitoring CD34+ cells after HSCT showed that their numbers correlated with platelet recovery (Gebauer et al. 1996) and that donor CD34+/HLA-DR+ capable of long term hematopoietic repopulation are present in the BM of patients receiving allogeneic PBSCT (Briones et al. 1998).

The first in depth fluoro-cytometric analysis of recovering lymphocyte subpopulations after HSCT was published fifteen years ago (Ault et al. 1985). They followed four patients submitted to BMT (three allogeneic T cell-depleted and one syngeneic) during the first 90 days after transplantation and found few mature T cells and large numbers of functional NK cells and non-functional CD5+ B cells. These findings were later confirmed by other studies (reviewed by Voltarelli & Stites 1986 and by Lum 1990). More recently, immunologic reconstitution has been investigated in different types of HSCT. Thus, Small et al. (1999) showed that adult patients submitted to T cell-depleted unmanipulated grafts experienced a slower CD4 T cell recovery than patients receiving unmanipulated BMT. This delay in CD4 recovery was not associated with an increased risk of severe opportunistic infections which correlated negatively with the rate of recovery of CD4 T cells. This correlation was also observed by Trensche et al. (1998) comparing related and unrelated allogeneic BMT with peripheral blood HSCT which showed faster myeloid and CD4 reconstitution and lower frequency of opportunistic infections. In other set of studies, human dendritic cells identified by the Mab CMRF-44 were found to be low in the peripheral blood of patients submitted to PBSCT harvesting for allogeneic or autologous HSCT and in patients with acute GVHD (Fearnley et al. 1999). In this condition CD4 cells were activated (HLA-DR+Ox40+) and displayed Th2 phenotype (CD30+/CD7-)(Grimley et al. 1999) while eosinophils showed signs of selective activation as judged by the expression of the IL2-Rα (CD25) (Rumi et al. 1998). Finally, the presence of residual thymic function was shown to be required for the reconstitution of the CD4 T helper subset, but not for the CD8 T cytotoxic/suppressor subset (Heitger et al. 1997). On the other hand, in autologous BMT, hematopoietic growth factors favored the recovery of activated CD8 T cells (GM-CSF) or of memory and naive CD4 T cells (G-CSF) (San Miguel et al. 1996). In addition, autologous transplantation of FACS-sorted CD34+ cells from peripheral blood resulted in delayed immune reconstitution, decreased diversity of Vβ TCR expression in all patients and an increase of TCR γδ T cells and of CD5+ B cells in one third of patients compared to unmanipulated grafts (Bomberger et al. 1998). Immune recovery after autologous transplantation was reviewed by Guillaume et al. (1998).

FUTURE AND POTENTIAL APPLICATIONS

In the future, flow cytometric methods will certainly replace much of the current technology employed in the practice of human HSCT. New areas of development include detection of cytomegalovirus infection (Honda et al. 1997, Imbert-Marcille et al. 1997), of neutrophil (Maher & Hartman 1993) or platelet (Kohler et al. 1996) alloimmunization, documentation of chimerism by fluorescence in situ hybridization (Arkesteijn et al. 1995, van Tol et al. 1998), functional activation of T cells detected by intracellular cytokine production (Tarantino et al. 1998) and selection of HSC or immunocompetent cells for transplantation or gene therapy (Korbling et al. 1994, Sasaki et al. 1995). In fact, both the human multidrug resistance (MDR) gene transfected to HSC (Richardson & Bank 1995) and the herpes simplex virus-thymidine kinase gene transfected to human T cells (Bonini et al. 1997) express cell surface proteins (a MDR associated p-glycoprotein and the low affinity receptor for nerve growth factor respectively) that can be detected and selected by flow cytometry. In addition, FACS selection of T cells containing TCR-Vβ families which lead to GVHD or GVL (Epperson et al. 1999) or of T cells which bind to MHC-leukemic peptide tetramers (Dunbar et al. 1998) may be used in the future as immunotherapeutic approaches combined to HSCT. Finally, cell cycle analysis of normal stem cells (Gothot et al. 1998) or neoplastic tissues (Orfao et al. 1994) by DNA staining may also be helpful for engraftment or prognosis studies after HSCT.
APPLICATIONS OF FLOW CYTOMETRY TO ANIMAL MODELS OF HUMAN STEM CELL TRANSPLANTATION

Over the last years, there was considerable progress towards the development of animal models to support human stem cell engraftment. Most of these models involve mutant/inbred strains of immunodeficient mice which allow long term proliferation and differentiation of primitive human hematopoietic stem cells from different sources. They provide invaluable research tools not only to dissect the physiology of human hematopoiesis but also to investigate multiple aspects of hematopoiesis-based diseases, including the effects of genetic manipulation on the treatment of these diseases. The contribution of flow cytometry for the establishment and investigation of animal models of human hematopoiesis is outlined in this section.

Early attempts to transplant human bone marrow into irradiated mice, into mice deprived of T cells (with the nude mutation) or into fetal sheep resulted in low levels of human stem cell engraftment. Other immunodeficient murine models such as beige, xid (X-linked immunodeficient) and Rag1 or 2 (deficient in the recombination activating gene-1 or 2) were not successful as well and the most suitable host was found to be mice homozygous for the scid mutation (reviewed by Greiner et al. 1998). The scid mutation was first described in 1983 in the C.B-17 strain of mice which lack both cellular and humoral immunity in the homozygous state. The first reports of engraftment of human hematopoietic cells in scid mice appeared in 1988, including intraperitoneal transplantation of PBL (Mosier et al. 1988) and transplantation of fetal bone marrow or fetal liver with or without fetal thymus fragments under the renal capsule of unirradiated recipients (McCune et al. 1988) followed by intravenous transplantation of bone marrow into irradiated animals (Lapidot et al. 1992). Models of hematopoietic engraftment employing human fetal tissues (the SCID-hu mice) have ethical and availability limitations while in the hu-SCID model with postnatal tissues the engraftment levels of human cells are low (0.5-5 % of the scid marrow). This is due to the lack of species cross-reactivity of growth factors and cytokines required for human stem cells and also to residual host innate immunity present in the scid mice. These animals have elevated levels of hemolytic complement and NK activity and normal granulocyte and macrophage function which interfere with the engraftment of human hematopoietic cells. Backcrossing the C.B-17-scid mice with several inbred strains carrying specific defects in innate immunity led to the generation of the non-obese diabetic (NOD)-scid mice which exhibit multiple defects in innate immunity but do not develop diabetes. In this NOD-scid strain the levels of engraftment of human stem cells from peripheral blood or spleen were always 5- to 10-fold higher than in any of the other scid strains examined and it became the most useful animal model of human hematopoiesis and HSCT available. Additional modifications of the NOD/scid model with the introduction of the β2-microglobulin deficiency and consequent absence of MHC class I further increased the level of human cell engraftment and allowed the development of T cells with normal CD4:CD8 ratio (Christianson et al. 1997).

Phenotypic characteristics of various sources of human lymphohematopoietic cells engrafting NOD-scid mice have been investigated. Initially, nonmobilized peripheral blood or spleen cells were injected intraperitoneally into unirradiated scid mice (Greiner et al. 1995). As mentioned earlier, the degree of engraftment was higher in NOD-scid than in C.B-17-scid and it was similar for PBL and spleen transplants; most circulating or splenic cells grafted in the mouse were CD4 or CD8 T cells, less than 5% expressed monocyte or B cell markers. When selected CD34+ cells mobilized from the peripheral blood by G-CSF were administered intravenously to irradiated NOD-scid mice, engraftment of human cells could be detected up to 6.5 months after transplantation and comprised as high as 96% of bone marrow cells (van der Loo et al. 1998). The phenotypes of these cells differ significantly between different organs of the host animal: mature CD20+10- B cells predominate in the spleen whereas myeloid cells (CD33+HLA-DR+) predominate in the BM, and the thymus contained a large percentage of human immature T cells (CD4+CD8+ and CD8+CD7+). However, other studies showed that T cells developed in NOD/SCID mice transplanted with the more primitive CD34neg SC and not with the CD34+ ones (Bhatia et al. 1998). Human bone marrow cells capable of repopulating NOD-scid mice are present in the CD34+38- fraction and generated significant numbers of human CD34+38- cells in the marrow of the animals and multiple lineages of human cells (Larochelle et al. 1996).

Most transplantation models of normal human stem cells into scid mice involved the use of cord blood cells. These studies showed that, like the BM, the long term repopulating cell is also present in the CD34+38- fraction and that predominant cell generated in the marrow and in peripheral tissues are B cells, expanded from the CD34+CD19+ precursor compartment (Pflumio et al. 1996, Hogan et al. 1997). Culture of purified CD34+CD38- cord blood cells with bone marrow stromal cells reduced
significantly the in vivo repopulating capacity of transplanted cells (Gan et al. 1997) while the expansion of those cells with growth factors (IL-3, IL-6 and stem cell factor) markedly delayed lymphohematopoietic engraftment (Guenechea et al. 1999). Finally, repopulation of NOD-scid mice with CD34+ purified cord blood cells was found to be dependent on the expression of the chemokine receptor CXCR4 which binds to the stromal cell-derived factor-1 (SDF-1) (Peled et al. 1999).

The scid mouse model has also been extensively used to investigate the biology and treatment alternatives for human leukemias (reviewed by Uckun 1996) and other hematological malignancies such as multiple myeloma and lymphomas. Flow cytometric methods were very instrumental in these investigations, including the few studies involving infusion of immunocompetent cells from allogeneic or autologous donors. Detection of engraftment of human malignant cells in the animal host can be achieved by a panel of monoclonal antibodies similar to that used for diagnosis of the disease in humans (Baersch et al. 1997) or to characterize human tumor cell lines as in multiple myeloma (Rebouissou et al. 1998). On the other hand, infusion of in vitro antigen stimulated cytotoxic T cells caused regression of human autologous EBV-induced B cell lymphoproliferative disease (Lacerda et al. 1996) or of allogeneic acute myeloid leukemia (Warren et al. 1997) or acute lymphoblastic leukemia (Nijmeier et al. 1998).

Protocols of gene transfection of human HSC which could be employed in clinical transplantation (reviewed by Richter 1997, and by Brenner et al. 1998) have also benefited from the convenience and quickness of fluorocytometric technology. Most of these protocols use oncoretroviral vectors such as the murine leukemia virus which integrates into the chromosomes of replicating target cells resulting in stable expression of transgenes. However, few studies have succeeded in transducing retroviral vectors to human stem cells (Yurasov et al. 1997, Cheng et al. 1998, Rill et al. 1997) possibly due to the quiescent nature of human HSC and the requirement of cell division for retroviral integration. Very recently, human resting CD34+ cells were transduced with an HIV-derived vector and were able to repopulate NOD-scid mice (Miyoshi et al. 1999). Levels of engraftment, detected in the FACS by the expression of the green fluorescence protein coupled to the viral vector, were as high as 27% of human CD45+ cells in the BM (Figure), 22% in the spleen and 20% in the peripheral blood. The use of this new class of vectors provide an innovative approach to study hematopoiesis and to human gene therapy if the safety issues are properly addressed.

FLOW CYTOMETRY APPLIED TO HSCT IN BRAZIL

In a recent survey among 12 stem cell transplant centers active in Brazil, we found that the majority of them (8) use a flow cytometer for clini-
cal or investigation purposes. Four centers have equipments restricted to cell analysis and in other four centers equipments were also capable of cell sorting, but they are used mainly for analysis. Most centers utilize flow cytometry to quantitate stem cells contained in autografts or T cells present in donor lymphocyte infusions and in investigations related to stem cell mobilization or immune reconstitution (Souza et al. 1994, Diamond et al. 1995, Vigorito et al. 1998). In our center, we have been using fluorocytometric methods to compare cell content between different types of grafts (bone marrow, peripheral blood and cord blood) (Lemos et al. unpublished), to assess alloimmunization against neutrophils (Corsini et al. unpublished) or platelets (Palma et al. unpublished) and to characterize the immune response of donor lymphocyte infusions (DLI) against chronic myelogenous leukemia relapsed after allogeneic BMT (Castro et al. unpublished). In a preliminary analysis of this latter study, we found that some patients receiving DLI show signs of lymphocyte activation in the peripheral blood, specially upregulation of HLA class II molecules in various lymphocyte subsets, which correlated with functional assays of allostimulation. These findings are particularly relevant given the reduced expression of HLA class II molecules on target (Dermime et al. 1997) and effector (Castro et al. 1999) cells from patients with leukemia which seem to impair their immune response against tumor cells.

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