were graft versus host disease (acute/chronic, grade, day of onset, affected organ), infections, survival, relapse, day of bone marrow regeneration and immunosuppression therapy. We found a statistically significant association between rising levels of HLA-G during transplantation and clinically relevant (grade II-IV) acute GvHD, infectious events after transplantation, in particular fungal infections, and development of chronic GvHD. Our preliminary data shows that sHLA-G molecules are involved in several complications after allogeneic hematopoietic cell transplantation.

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AN IN-VIVO MODEL OF HUMAN T CELL-MEDIATED REJECTION OF ALLOGENEIC MISMATCHED HEMATOPOIETIC CD34 + STEM CELLS USING NOD/SCID Γ^{NULL} (NOG) MICE

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Anti-human CD34+ cell T cell alloreactivity was previously shown in-vitro. In this study we transplanted human CD34+ cells and allogeneic T cells in a NOD/SCID γ^{null} (NOG) mouse model to evaluate the occurrence of stem cell rejection in-vivo. After sublethal irradiation NOG mice received 2×10^5 CB CD34+ cells and allogeneic blood T cells at 1:0 (control), 1:2, or 1:10 CD34+ cell: T cell ratio (n = 5-10 mice per group). Human cell engraftment was assessed in the bone marrow and in the spleen 6 weeks following transplantation. Marrow engraftment of huCD45+ cells was 60 ± 10% in control mice and included B cells (64 ± 4%), CD34+ cells (18 ± 1%), CD33+ myeloid cells (7 ± 1%), CD14+ monocytes (3 ± 1%). In contrast, mice that were transplanted with CD34+ cells and low dose (1:2 ratio) or high dose (1:10 ratio) allo-T cells had only 9 ± 2% and 3 ± 1% huCD45+ cells, respectively, in the bone marrow ($p = 0.01$) and >98% were huCD3 + T cells. Spleen engraftment of huCD45+ cells was lower (25 ± 8%) in control mice (1:0 ratio) as compared to 66 ± 10% and 36 ± 11% in 1:2 and 1:10 groups, respectively ($p = 0.05$). However, also the spleen of mice receiving CD34+ and T cells included >98% CD3 + T cells. Among the T cells, both in the marrow and in the spleen of mice in the 1:2 and 1:10 ratio groups, 60-70% were CD4 + CD8- cells, 22-25% CD8 + CD4- cells, 1-3% CD56+ cells, and 2-5% CD4 + CD25+ cells. Only in mice receiving low doses of T cells, on average 12 ± 6% of the T cells in the bone marrow and spleen were CD4 + CD8+. Mice receiving high doses of T cells had acute xenogeneic GVHD demonstrated by fur changes, reduced survival ($p = 0.02$) and weight loss ($p = 0.0001$) compared to control mice or mice receiving a lower dose of T cells (1:2 ratio). In-vitro mixed leukocyte cultures with irrad CD34+ cells and allogeneic T cell responders (R) at the same ratio as in in-vivo experiments were then performed w/wo the addition of CD4 + CD25+ regulatory T cells (Tregs) at 1:1 or 1:5 Treg: R ratio. Since a 60% T cell suppression was observed only with equal numbers of Tregs and allo-responders, we are currently testing whether auto or allo Tregs may prevent stem cell rejection in NOG mice transplanted with CD34+ cells, allogeneic T cells and Tregs at 1:2:2 or 1:1:2 ratio. NOG mice represent a useful model to study human T cell mediated bone marrow failure or stem cell rejection and will allow us to investigate new strategies of allogeneic transplantation with subsets of T cells or hematopoietic stem cells.

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BRONCHIOLITIS OBLITERANS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION – AN ANALYSIS OF 50 CASES OVER THIRTY YEARS AT A SINGLE BRAZILIAN INSTITUTION

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Introduction: Bronchiolitis obliterans (BO) is a late complication of hematopoietic stem cell transplantation (HSCT) usually associated with chronic graft versus host disease (C-GVHD) and often fatal. We describe here characteristics and clinical course of 50 patients at a brazilian transplant center who developed bronchiolitis obliterans as a manifestation of chronic graft versus host disease.

Patients and Methods: We searched the database and reviewed the medical records of 1645 patients who received HSCT between 1979 and 2009, and identified 50 patients who developed BO, whose clinical features and clinical course were analyzed retrospectively. The diagnosis of BO was determined by either the presence of an obstructive ventilatory defect on a pulmonary function test or a chest CT showing small airways wall thickening or air trapping associated with a typical clinical picture in the context of a patient with C-GVHD. NIH criteria were used to define the severity of C-GVHD.

Results: The prevalence of BO in these 30 years was 3%. Patient characteristics are summarized in Table 1. According to the severity of pulmonary involvement 14 patients (28%) had score 1, 12 (24%) had score 2, and 22 (44%) had score 3. Median time between transplantation and diagnosis of C-GVHD was 138 days (33-3738), while median time between the diagnosis of C-GVHD and the diagnosis of BO was 77 days (0 – 1752). Median time between transplantation and diagnosis of BO was 343 days (38 – 3877). Twenty-nine patients (58%) required second-line treatment due to lack of response. The main secondary treatments used were: thalidomide(11); azathioprine(18), tacrolimus (7); mycophenolate mofetil (7) and photopheresis (1). Median survival for this group was 1637 days (195 – 6102). At the time of this analysis 23 (46%) patients had died from BO related causes.

Conclusion: Bronchiolitis obliterans was a serious late complication, occurring in 3% of patients transplanted in our center over the past 30 years. Mortality rate was high (46%) and most patients (58%) did not respond to primary therapy. Better understanding of the pathophysiology of C-GVHD is necessary for the development of more effective therapeutic tools.

Bronquiolitis Obliterans- Patient Characteristics

CHARACTERISTICS	N = 50
age	25 (2 – 50)
Recipient M / Donor F	20(40%)
Donor and recipient match	
matched	40 (80%)
Source of stem cell	
BM	46 (92%)
other (PB or CB)	4(8%)
Donor	
related	45 (90%)
unrelated	5 (10%)
Diagnosis	
CML	24 (48%)
AML	8 (16%)
ALL	5 (10%)
Fanconi Anemia	5 (10%)
Others	8 (16%)
Chronic GVHD onset	
progressive	19 (38%)
quiescent	19 (38%)
de novo	8 (16%)
after DLI	3 (6%)
Risk characteristics	
platelets less than 100.000/mm3	21 (42%)
>3 organs	27 (54%)
<1500 lymphocytes	39 (78%)
NIH global scoring	
Moderate	14 (28%)
Severe	36 (72%)

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PKC$_\Theta$ IS REQUIRED FOR ALLOREACTIVITY AND GVHD BUT NOT FOR IMMUNE RESPONSES TOWARD LEUKEMIA AND INFECTION IN MICE

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When used as therapy for hematopoietic malignances, allogeneic bone marrow transplantation (BMT) relies on the graft-versus leukemia (GVL) effect to eradicate residual tumor cells through immunologic mechanisms. However, graft-versus-host-disease (GVHD) is a potentially lethal complication of allogeneic BMT. Thus, inhibition of GVHD, while preserving GVL and protective responses against infectious agents, can enhance the therapeutic potential of BMT. GVHD is initiated by alloreactive donor T cells that recognize mismatched major (MHC) and/or minor (MiHA) histocompatibility antigens and cause severe damage to hematopoietic and epithelial tissues. Protein kinase C theta (PKC_θ) is a key regulator of T cell-receptor signaling. We show here that T cell responses triggered by Listeria or following administration of antigen plus microbial adjuvant are relatively well preserved in the absence of PKC_θ. In contrast, we demonstrate an essential requirement for PKC_θ in alloreactivity and GVHD induction. Furthermore, absence of PKC_θ raises the threshold for T cell activation, which selectively impacts alloresponses. Most importantly, $PKC_\theta^{-/-}$ T cells retain both the ability to respond to virus infection and induce GVL post BMT. These findings validate PKC_θ as a potentially unique therapeutic target that is required for GVHD induction but not for GVL or protective responses to infectious agents.

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FEASIBILITY OF NIH CONSENSUS CRITERIA FOR CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD): SUCCESSFUL EFFORTS IN ESTABLISHING A MULTICENTER BRAZIL-SEATTLE COLLABORATIVE CONSORTIUM

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Advances in clinical trials for treatment of chronic GVHD have been hampered by slow accrual of patients (pts) and by inconsistency and limitation of historical systems for grading used prior to the 2005 NIH consensus criteria for diagnosis and classification of chronic GVHD (Filipovich AH et al BBMT 2005;11:945-956). In an effort to foster collaboration and expand the network for future clinical trials in chronic GVHD, a Brazil-Seattle consortium was established in 2008 to conduct chronic GVHD studies in Brazil. The Brazil-Seattle chronic GVHD consortium included 15 multidisciplinary participants (i.e., hematopoietic cell transplant (HCT) physicians, dermatologist, dentist, psychiatrist, psychologist, physiotherapist and one data manager) from 5 Brazilian institutions and two experts in chronic GVHD from Seattle. The consortium held monthly teleconferences and met at least twice yearly during national and international HCT and Hematology meetings in Brazil and USA. After training and translation of the NIH consensus criteria tools into Portuguese, the group initiated a feasibility study using the NIH consensus criteria after approval by each of the 5 participating centers' institutional review boards. So far, 34 pts with NIH chronic GVHD after allogeneic HCT in Brazil have been enrolled between June 2006 and May 2009 and prospectively followed. Of the 34 pts with NIH chronic GVHD, 26 (76%) met the overlap syndrome subcategory and 8 (24%) the classic subcategory. The overall severity of NIH chronic GVHD was moderate in 21 pts (62%) and severe in 13 pts (38%). The median time from HCT to onset of NIH chronic GVHD *overlap syndrome* subcategory was 5.9 months (2.5-10) and from transplant to NIH chronic GVHD *classic* subcategory was 7.3 months (3.0-16). At a median follow up of 16.5 months, the overall survival was 70%. At least 5 pts were enrolled from each center. In conclusion we demonstrate the feasibility of the NIH consensus criteria for the diagnosis and scoring of chronic GVHD in a prospective Brazilian multicenter study and, more importantly, in establishing a collaborative HCT network in Brazil that offers new opportunities for future intervention, validation, and biomarkers clinical trials in chronic GVHD.

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INCIDENCE OF GRAFT-VERSUS-HOST DISEASE (GVHD) IN PATIENTS WITH ALLOGENEIC PERIPHERAL HEMATOPOIETIC STEM CELL TRANSPLANTATION AFTER A NON-MYELOABLATIVE CONDITIONING

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Introduction: Graft-versus-host disease (GVHD) is the most common complication of allogeneic hematopoietic cell transplantation (alloHCT) and may affect the transplant outcome. Its incidence is higher when the preparative regimen used is a non-myeloablative and the stem cell's source is peripheral blood after mobilization.

Objective: To demonstrate the 10 years incidence of GVHD in 2 Mexican transplant centers using peripheral hematopoietic stem cell transplantation (PHSCT) in related donors after non-myeloablative conditioning.

Patients and methods: Three hundred and fifty six patients with hematological and non-hematological malignancies that underwent PHSCT after non-myeloablative conditioning between October 1998 and November 2008 were included. The age ranged between 1 and 71 years (median of 29). Two hundred and nineteen patients were men and 137 women. They received cyclosporine 4 mg/kg per day and intramuscular methotrexate 5 mg/m^2 in days +1, +3, +5 and +11 for GVHD prophylaxis.

Results: Three hundred and fourteen (88%) patients were successfully engrafted. One hundred and fifty six patients (49%) developed acute and/or chronic GVHD. Of this last number of patients, 68 (44%) developed acute GVHD, 44 (28%) developed chronic GVHD, and 44 (28%) developed both. Twenty three percent (26) of the patients who developed acute GVHD were grade III or IV, and 35% (31) of chronic GVHD patients presented in the extensive way. GVHD was the cause of dead in 33 patients (12%), even when immunosuppressive therapy (high dose steroids and rituximab) was used. Last death was 24 months ago; subsequently, alemtuzumab was included in GVHD treatment.

Conclusion: The incidence of acute and chronic GVHD in our patients is lower than the reported in the literature, even though the source of hematopoietic stem cells was peripheral blood. The mortality rate has decreased due to the introduction of new are more aggressive immunosuppressive agents like alemtuzumab.

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MOBILIZING CELLS WITH A TOLEROGENIC PHENOTYPE USING PEGYLATED RECOMBINANT MURINE GM-CSF: IN VITRO AND IN VIVO EFFECTS

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Granulocyte-macrophage colony stimulating factor (GM-CSF) is a growth factor regulating proliferation and differentiation of hematopoietic stem and progenitor cells. Murine GM-CSF (mGM-CSF) has a very short half-life and pegylation of mGM-CSF (peg-mGM-CSF) has been shown to prolong its half-life. Based on our clinical observations that patients transplanted with stem cell products mobilized by GM-CSF have a lower incidence of acute GvHD, we hypothesized that peg-mGM-CSF may be protective of acute GvHD in a MHC mismatched murine model.

We have shown that C57/Bl6 mice treated with peg-mGM-CSF led to increased CFU-C by 26.5 fold compared to PBS treated mice (n = 6, 95% CI = 10.25-42.73). (ASH 2009) In addition, we observed that peg-mGM-CSF increased CD4 + FoxP3 + Tregs in both blood and spleen by 2 fold. ($p < 0.05$) (ASH 2009) This effect was sustained out to 4 days after the last dose of peg-mGM-CSF. Importantly, the function of these peg-mGM-CSF Tregs, as measured in a MLR was found to be equivalent to Tregs from PBS treated control mice (n = 3, p = 0.27) (ASH 2009)

To assess the in vivo impact of peg-mGM-CSF on graft versus host disease, we used the C57/Bl6 → Balb/c acute GvHD model.