

of the entire group was 47%. The 5 year survival of patients with (n = 148) or without (n = 31) CMV/EBV reactivation was respectively: 50% vs 28% (p = 0.02). This was due to a lower risk of relapse related death (RRD) in patients with reactivation 19% vs 42% (p = 0.005) and similar transplant related mortality (TRM), 28% vs 27% (p = 0.8). In multivariate analysis disease phase (\leq CR2) predicted survival (RR 0.33) relapse related death (RR 0.50) and TRM (RR 0.72); CMV/EBV reactivation predicted survival (RR 0.69); and RRD (RR 0.61); RIC transplants were associated with increased RRD (RR 4.56).

In conclusion: reactivation of CMV/EBV is a common event in our transplant program. Possibly because of an early diagnosis and pre-emptive therapy, TRM is comparable in patients with and without infection. Viral reactivation seems to promote the graft versus leukemia effect of the transplant.

371

CO-TRANSPLANTATION OF HUMAN Tregs AND CD34+ CELLS: A PRE-CLINICAL STUDY

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Co-transplantation of purified CD34+ cells and regulatory T cells (Tregs) might favor the immune reconstitution and limit the incidence of graft-versus-host disease and graft rejection in an HLA mismatched transplant setting. In this study we immunomagnetically selected human CD4+CD25+ Tregs, which resulted >80% FoxP3 positive by flow cytometry, and CB CD34+ cells. Then we tested if Tregs would affect CB CD34+ cell clonogenic activity in-vitro an in-vivo, and if co-incubation of Tregs and CD34+ cells may modify the phenotype and function of Tregs. A colony-forming cells (CFU-C) assay performed with CD34+ cells mixed with allogeneic Tregs at 1:2 ratio resulted in comparable numbers of Granulocyte-Macrophage CFU (CFU-GM), burst-forming unit-erythroid (BFU-E) and CFU-Mix as compared to cultures with CD34+ cells alone (p = 0.2, p = 0.5 and p = 0.5, respectively)(n = 3 expts). Human CD34+ cells were then co-transplanted with human CD4+CD25+ allogeneic Tregs into NOD/SCID mice at 1:1 and 1:2 ratio. After 6 weeks mice marrow was harvested and showed a $1.3 \pm 1.1\%$ (n = 3 mice) and $1.6 \pm 0.8\%$ (n = 4 mice) engraftment of huCD45+ cells, respectively, which was comparable to the engraftment observed in control animals transplanted with CD34+ cells alone (1.4 ± 0.4). Among the engrafted huCD45+ cells similar proportion of CD33+ cells, CD14+ monocytes and CD1c+ dendritic cells were observed in the three groups of animals. Mixed lymphocyte culture (MLC) experiments showed that irradiated CD34+ cells stimulated brisk proliferative responses of allogeneic CD4+CD25- cells, but not of Tregs (n = 3 expts). After incubation with CD34+ cells in the presence of IL2, on average >80% CD4+CD25+ cells maintained the intracellular expression of FoxP3 and surface expression of CD62L and CD152 (n = 3 expts). Tregs and CD34+ cells were then isolated from the same CB unit. A primary MLC with irradiated CB CD34+ cells and HLA mismatched PB T cell responders was performed w/wo the addition of CB Tregs at 1:1 ratio with CD34+ cells. A $68 \pm 14\%$ (n = 3 expts) inhibition of T cell alloreactivity to CD34+ cells was observed in MLC with Tregs. Our findings demonstrate that co-transplantation of CD34+ cells and Tregs results in normal stem cell engraftment and potentially reduces T cell alloreactivity. These results will prompt the design of new strategies in transplantation of HLA incompatible stem cells by combining donor-derived CD34+ and CD4+CD25+ cells.

372

LEUKEMIC BLASTS ACTING AS HOST ANTIGEN PRESENTING CELLS TRIGGER A COMBINED CD4 AND CD8 ALLO-IMMUNE RESPONSE DIRECTED AGAINST MISMATCHED HLA CLASS I

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Single locus HLA mismatched stem cell transplantation (SCT) is applied in patients with hematological malignancies who may benefit from allogeneic transplantation but lack an HLA-matched donor. Donor lymphocyte infusion (DLI), applied in patients with mismatched SCT, have a high likelihood of inducing graft versus host disease (GVHD), however not all patients develop GVHD. In view of the high frequency of allo-HLA reactive T-cells, and almost all nucleated cells express HLA class I, one would expect all single HLA class I mismatched transplanted patients to develop severe GVHD. We hypothesized therefore that the presentation of the HLA class I mismatched allele on cells of the patient is not sufficient to elicit an effective allo-immune response. We characterized the allo-immune response in a patient with acute myeloid leukemia who was treated with a T-cell depleted SCT from a sibling donor who was HLA identical except for HLA-A2. Six months after SCT, DLI was given for mixed chimerism. No clinical response and no GVHD developed. 12 months after SCT the AML relapsed with 9% blasts in bone marrow for which a second DLI was given. Five weeks after the DLI the patient died of grade IV GVHD. During the GVHD conversion to donor chimerism developed. In peripheral blood of the patient 90% of CD8 and 40% of CD4 donor T-cells were activated as determined by HLA-DR expression. To analyze the nature of the immune response, the activated CD8 and CD4 donor T-cells were single cell sorted, expanded and tested for alloreactivity. Most CD8 T-cell clones were alloreactive and restricted to the allo-HLA-A2 molecule. 26% of the CD4 clones were alloreactive, and these clones recognized epitope 103-120 of the hypervariable region of HLA-A2 in the context of HLA-DR1. Both T cell responses were highly polyclonal. Prior to the first DLI, only HLA class I expressing T-cells and non-hematopoietic patient derived cells were present, these cells were isolated and were shown to activate the CD8 clones but not the CD4 clones. Leukemic blasts isolated at the time of the second DLI, expressed HLA-DR, and activated the CD4 as well as the CD8 clones. We hypothesize that the HLA class II expression on hematopoietic cells of the patient at the time of the relapse was essential for the development of GVHD. These results indicate a role for patient leukemic blasts acting as host APCs in initiating the GVHD by activating both a CD4 and CD8 T-cell response in an HLA class I mismatched setting.

373

WHAT IS THE ROLE FOR REGULATORY T-CELLS AFTER NONMYELOABLATIVE CONDITIONING?

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Purpose: We investigated the association between regulatory T-cell (T-reg) levels and chronic graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation following nonmyeloablative conditioning. **Methods:** Data from 34 patients given nonmyeloablative conditioning as treatment for hematological malignancies were analyzed. Conditioning regimens consisted of low-dose TBI with or without fludarabine (n = 28), or cyclophosphamide plus fludarabine (n = 6). T-reg (CD4⁺FoxP3⁺) levels on day 100 were determined by flow cytometry. Chimerism levels among total white blood cells, CD3⁺ T-cells and CD4⁺CD25⁺CD127^{dim/neg} regulatory T-cells were determined by multiplex STR PCR or X-Y FISH. Thymic function was determined by assessing sjTREC levels. **Results:** Median T-reg frequency (i.e. % of lymphocytes that were CD4⁺FoxP3⁺), T-reg level and T-reg chimerism on day 100 were 1.7% (0.3-9%), 14 cells/ μ L (3-127 cells/ μ L), and 90% (46-100%) respectively. Chimerism levels among CD4⁺CD25⁺CD127^{dim/neg} regulatory T-cells did not correlate with chimerism levels among total white blood cells (r = 0.29, P = 0.31), nor CD3⁺ T-cells (r = 0.38, P = 0.18). Fifteen patients develop chronic GVHD, and 19 did not. Analyzing data as median, there was not any correlation between low T-reg frequency, T-reg levels or T-reg chimerism levels on day 100 and high incidence of chronic GVHD (HR = 1.7, P = 0.25; HR = 1.2, P = 0.70; and HR = 0.5, P = 0.32, respectively). Finally, T-reg levels correlated weakly with sjTREC levels both assessed on day 100 after transplantation (r = 0.37, P = 0.04).

Conclusions: Kinetics of T-reg and T-cells engraftment were independent, underlying the need for assessing chimerism levels among each T-cells and T-regs in patients given nonmyeloablative conditioning. Our data did not show any significant correlation between T-reg levels and occurrence of chronic GVHD thus far. Data including higher number of patients will be presented.

374

PUVA THERAPY FOR ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD) OF THE SKIN

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Glucocorticoids remain the standard for initial treatment of acute GVHD. However, toxicities and immunosuppression are severe and steroid-sparing strategies would be desirable. Between 5/96 and 4/07 we treated 55 patients with isolated skin GVHD with methoxsalen plus ultraviolet-A light therapy (PUVA), with the objective of avoiding systemic immunosuppressive therapy. Patients were treated with PUVA three times/week initially at doses of 0.25 J/m², with exposure increased 0.25 J/m² per treatment as clinically indicated. The median patient age was 48 (range 4–71) years. Twenty-six received a calcineurin inhibitor plus mycophenolate mofetil for GVHD prophylaxis, 24 received a calcineurin inhibitor plus methotrexate, and 5 received other regimens. Sixteen had related donors (1 HLA-mismatched), and 39 had unrelated donors (15 HLA-mismatched). The median onset of GVHD was 26 days after transplant, and the median start time of PUVA was 43 days. Forty-five patients received PUVA as initial therapy for acute GVHD, and 10 patients received PUVA for recurrent GVHD after discontinuation of prednisone administration. At the start of PUVA therapy, 31 patients (56%) had rash involving > 50% body surface area (BSA), 19 (35%) had rash 26–50% BSA and 5 (9%) had rash ≤ 25% BSA. The median number of PUVA treatments was 13 (range 2–26). Sixteen patients (29%) had complete responses after a median of 14 (range 8–26) PUVA treatments and required no subsequent systemic immunosuppressive therapy for treatment of acute GVHD. Twelve patients required systemic therapy after starting PUVA for treatment of isolated gastrointestinal GVHD, although 8 of these patients had cleared or improved skin rash before starting systemic therapy. Twenty-four patients (44%) required systemic immunosuppressive therapy after starting PUVA for treatment of skin GVHD (18) or skin plus gastrointestinal GVHD (6). Three patients had evidence of skin GVHD when PUVA was discontinued early due to readmission to the hospital or discharge home. Only four patients required secondary systemic therapy for treatment of acute GVHD. Thirty-one of 52 patients who could be evaluated developed chronic GVHD. Thirty-seven patients remain alive at a median of 753 days after transplant. Overall, 24 of 55 patients (44%) responded to PUVA with resolution or improvement of rash. These results suggest that PUVA can be effective in treating skin GVHD and in reducing the need for systemic immunosuppressive treatment.

375

THE ANALYSIS OF CHRONIC GVHD AFTER CORD BLOOD TRANSPLANTATION IN COMPARISON WITH BONE MARROW TRANSPLANTATION

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Backgrounds: Umbilical cord blood can be an alternative stem cell source for the patients with hematological malignancies requiring allogeneic stem cell transplantation. However, little is known about graft versus leukemia/lymphoma (GVL) effect in cord blood transplantation (CBT). Here, we analyzed chronic GVHD (cGVHD) in CBT compared with that in BMT and evaluated the relevance between cGVHD and GVL. **Patients/Methods:** We ret-

respectively studied 162 patients who had been free from disease progression for more than 100 days after either unrelated BMT (n = 75) or CBT (n = 87) at Toranomon Hospital from January 2002 to December 2006. Median age of the patients was 52 years old (BMT vs CBT: 49 vs 53). Underlying diseases were acute leukemia (n = 88), myelodysplastic syndrome (n = 17), lymphoma (n = 39) and others (n = 18). Conditioning regimens were mainly composed of Fludarabine 125–180 mg/m² with several combinations of Melphalan 80–140 mg/m², Busulfan 8–16 mg/kg and/or total body irradiation (4–8 Gy). **Results:** The Median observation period after the transplantation was 612 days (range, 109–1944). The cumulative incidence of cGVHD was 84% in BMT and 62% in CBT (p = 0.09). The severity of cGVHD was analyzed based on its type; limited or extensive. In CBT, the percentage of the former type was 34% (vs 25% in BMT) and the latter was 23% (vs 48% in BMT). High-risk disease (p = 0.03) and preceding acute GVHD (p = 0.03) are related to the occurrence of cGVHD. RICBT tended to increase cGVHD compared to CBT using BU/CY or CY/TBI regimen. Multivariate analysis showed that cGVHD increased overall survival rate (p < 0.01) and suppressed recurrence of the disease (p < 0.01). During observation period, no patients after CBT were died of cGVHD. **Discussion:** We demonstrated that cGVHD in CBT is tolerable compared with that in BMT and that the occurrence of cGVHD could result in good prognosis. Our analysis also suggested that CBT could have GVL effect as well as BMT.

376

PERSISTENT RECIPIENT ANTIGEN-PRESENTING CELLS IN HUMAN HEMATOPOIETIC STEM CELL TRANSPLANTATION: IDENTIFICATION OF A DERMAL SUBSET THAT OUTLIVES EPIDERMAL LANGERHANS CELLS

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Graft versus host disease (GVHD), a lethal complication of hematopoietic stem cell transplantation (HSCT), is initiated by recipient antigen presenting cells (APC) priming donor T cells. The skin, a commonly affected organ, contains diverse APC including heterogeneous dermal dendritic cells (DC) whose contribution to GVHD is not well understood.

We have obtained 22 pairs of skin biopsies pre and post conditioning (high dose BuCy or CyTBI and reduced intensity Flu/Mel) and 85 biopsies at day 40, 100 and 365 post HSCT from patients with sex-mismatched donors. Pre and post conditioning samples were digested and analysed with CD45, HLA-DR, CD14 and CD1a antibodies in a single step Trucount protocol. Chimerism was determined on cytopins of spontaneously migrated dermal APC and collagenase digested dermal cells using sequential four-colour confocal microscopy and X/Y fluorescence in situ hybridization.

We find 3 populations of CD45+ DR+ dermal cells: CD14+ CD1a- fXIIIa-; CD14+ CD1a- fXIIIa+; CD1a+ CD14- fXIIIa-. Characterization of these cells in vitro shows that CD14+ cells are more adherent and phagocytic than CD1a+ cells and that the fXIIIa+ component is in addition, heavily granulated with ingested melanin. CD14+ APC are resistant to depletion by conditioning (210 pre/213 post; cells per mm²; P 0.86) compared with CD1a+ cells (201 pre/97 post; mean cells per mm²; P 0.03). Both are reduced in HSCT patients compared with controls (706 CD14+ and 468 CD1a+ cells per mm²; both P 0.01).

Chimerism analysis at 40, 100 and 365 days post HSCT reveals two strikingly different patterns of engraftment. CD1a+ and CD14+ fXIIIa- cells, obtained either by migration or digestion, engraft rapidly in all patients, ahead of LC, reaching medians of 99% and 100% donor, respectively, at day 40. In contrast, CD14+ fXIIIa+ cells, which are obtained only from digested dermis, are very slow to engraft in the absence of GHVD, reaching only 63% median donor after high dose and 10% median donor chimerism after reduced regimens, at 1 year. Acute GVHD promotes engraftment with 100% donor chimerism seen in nearly all patients at day 100. Cell cycle analysis indicates that these cells are not actively synthesizing DNA, suggesting a different mode of survival compared with persistent recipient LC.