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

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 methotrexal 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 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 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 735 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

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 retrospectively 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. RiskBMT 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 GVHD. Discuss: 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 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 cytopsins 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+ dural cells: CD14+ CD1a+ IXIIIa+; CD14+ CD1a+ XIXIIa+; CD1a+ CD14+ XIXIIa+. Characterization of these cells in vitro shows that CD14+ cells are more adherent and phagocytic than CD1a+ cells and that the IXIIIa+ 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+ XIXIIa- 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+ XIXIIa+ 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.
In conclusion, we have identified a subset of human dermal APC with protracted survival after HSCT and potential importance in promoting donor T cell reactivity to host antigens.

377 LOW DOSE ANTI-THYMOCYTE GLOBULIN FOR GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS OF MISMATCHED UNRELATED PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA

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Anti-thymocyte globulin (ATG) has been introduced in preventing acute graft-versus-host disease (AGvHD) in several studies. Many of them suggest that ATG at a total dose range of 4.5–15 mg/kg reduces the risk of severe AGvHD but increases the risk of infections. We tried to investigate the role of ATG in HLA-mismatched unrelated hematopoietic stem cell transplantsations (uHSCT), specifically in patients who received G-CSF mobilized peripheral blood stem cells (PBSCs) or from allele(s)/antigen mismatched unrelated donors from worldwide donor registries. Sixty five patients with intermediate or unfavourable risk AML who received HLA-mismatched uHSCT from the available Asian as well as Caucasian donors were enrolled. We compared 2 different groups according to the use of ATG (group 1) or not (group 2). The addition of ATG (thymoglobulin, Genzyme), at a dose of 1.25 mg per kilogram of body weight per day for 2 consecutive days, for recipients who received either PBSCs and/or from mismatched unrelated donors (group 1, N = 55); this was added to prevent the development of AGvHD together with our standard regimen which consisted of methotrexate (10 mg/m2 intravenously intravenously bolus on day +1; and methotretate 5 mg/m2 intravenously bolus, on days +3, +6, +11) and tacrolimus starting at day –1. G-CSF was administered in all patients at a dose of 5 mg/kg subcutaneously per day from D +7 after transplantation until neutrophil recovery. The median age was 38 (range, 16–65) and the median follow-up duration was 24 months (range, 3–72). The majority of patients had intermediate or unfavourable cytogenetic features. The main conditioning regimen consisted in cyclophosphamide plus total body irradiation. The transplanted patients were all successfully engrafted. The median time to neutrophil (<0.5 x 10⁹/kg) and platelet (<20 x 10⁹/kg) recovery was 15 vs 17 days, 14 vs 17 days in the group 1 and 2, respectively. The overall incidence of AGvHD and chronic GVHD was 38% and 41%; 33% and 35%; 37% and 48% for patients with group 1 and group2, respectively. Nine (14%) patients were relapsed so far. The comparison of estimated probability of disease-free survival rate at 2-year for each group was 89% vs 74%, respectively. The estimated probability of event-free survival rate at 2-year was 63% vs 51%, respectively. The overall 2-year non-relapse TRM was 14%. These results suggest that the uHSCT performed with a very low dose of ATG (total 2.5 mg/kg) are feasible with a promising outcome.

378 TACROLIMUS, SIROLIUMUS AND ANTI-THYMOCYTE GLOBULIN (ATG) FOR GRAFT VERSUS HOST DISEASE PROPHYLAXIS FOR UNRELATED DONOR HEMATOPOIETIC CELL TRANSPLANTATION


The effect of adding ATG to Tacrolimus/Sirolimus combination in graft-versus-host disease (GVHD) prophylaxis is largely unknown. We reviewed our records for patients who underwent unrelated transplants and received this combination for GVHD prophylaxis. Nineteen patients were identified who received their transplants between 8/06 and 02/07. Median age at time of trans-plant was 51.1 years (17–61.5). Nine recipients were males and 10 were females. The indications for transplant were as follows: ALL n = 9, AML n = 5, NHL n = 2, MDS n = 2 and CML n = 1. Twelve patients were in remission (CR1 = 10, CR2: n = 2), and seven had refractory disease. Eight patients received reduced-intensity conditioning (fludarabine/melphalan: n = 8) and 11 received full-intensity conditioning (FTBI/VPI-16: n = 8, FTBI/Cyt: toxan: n = 3). All received peripheral blood hematopoietic stem cell products except for two patients who received bone marrow products with the median CD34+ cell dose of 6.82 (1.36–9.46) x10⁹/Kg. Donors were molecularly matched in A, B, C, and DR in 11 patients; the remaining donors were mismatched in class I (n = 5) or both class I and II (n = 1). After a median follow-up of 9 months 16 patients are alive. Two deaths were related to infections within the first hundred days and not associated with acute GVHD. One patient died of relapsed AML. The probabilities of one-year overall survival, disease-free survival, relapse, and non-relapse mortality were 78%, 80%, 11%, and 11%, respectively. Ten patients developed acute GVHD with median onset of 24 days (7–35 days) after transplantation including five (26%) grade II and two (11%) grade III. We observed no grade IV GVHD in this cohort. Two patients developed chronic GVHD (limited: n = 1, extensive: n = 1). Patients tolerated rATG treatment well except for one who developed atrial fibrillation. Three patients developed thrombotic microangiopathy that required discontinuation of tacrolimus and sirolimus. Six of 15 CMV seropositive recipients developed reactivation of CMV including one with CMV pneumonia. None had reactivation of EBV. Eight patients had at least one documented bacterial infection.

Conclusion: In conclusion, the combination of Tacrolimus/sirolimus+rATG appears to be well tolerated, with a low rate of acute GVHD in this high risk group of patients without increased risk of early relapse or CMV/EBV reactivation. A prospective trial of this new combination in unrelated donor transplants is underway at our institution.

379 INTRA-ARTERIAL CATHETER DIRECTED IMMUNOSUPPRESSIVE THERAPY FOR STEROID RESISTANT OR DEPENDENT GRAFT VS HOST DISEASE (GVHD)

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Introduction: Prolonged treatment of graft vs. host disease (GVHD) is extremely immunosuppressive. Local therapy with intra-arterial (IA) injection of steroids may induce remission with lower extent of systemic immune suppression. Here, we present our experience with IA treatment of gastrointestinal (GI) and/or hepatic steroid resistant/dependent GVHD with 2 consecutive protocols. Methods: Thirty five patients (37 GVHD events (hepatic, n = 15), (GI, n = 16), (combined, n = 6)) were treated with 53 IA sessions. Results: We found that IA steroid therapy was associated with partial and complete remission among patients with steroid resistant/dependent hepatic or GI GVHD. Hepatic partial response was observed in 14 (66.6%) patients among whom 7 (33.3%) reached complete response. GI partial response was observed in 19 (86.4%) patients among whom 12 (54.4%) reached complete response. Most side effects were minor. An early administration of the local therapy, female gender, myeloid basic disease, and a non-active status of the basic disease at the day of transplantation were found related for predicting a better response for the intra-arterial treatment. The use of high dose steroids in the hepatic IA protocol from was at least as good as intermediate dose steroids with methotrexate and may be safer. Conclusion: Intra-arterial catheter guided steroid therapy can be safe and effective in steroid resistant/dependent GVHD. Hepatic artery treatment with methotrexate can be safely substituted with high dose IA methylprednisolone. Further research is warranted characterizing the patients benefit most.