by day 4 as efficiently as wt B6 T cells, with a similar reduction in pEC viability, from 93 ± 6.7 to 43 ± 6.4 in the presence of gld T cells and 44 ± 8.6 in the presence of wt B6 T cells ($p > 0.5$). However, upregulation of Fas on pEC co-cultured with gld T cells was delayed and seen only by day 4 rather than day 2. The capacity of gld T cells to modulate the epithelial environment appears intact, since they induced the upregulation of $H2K^b$ by 10-fold and IA^b by 3-fold on the pEC, similar to the effect induced by wt B6 T cells. We also found higher interferon gamma (IFNg) in the supernatants of co-cultures with gld T cells (141.2 ± 17.8), as compared to wt B6 T cells (100.3 ± 20.1, $p < 0.004$). However, in contrast to the expression of $H2K^b$ and IA^b which were up-regulated 5.5-fold and 2.8-fold by IFNg added to pEC cultures, Fas expression was not altered by this cytokine even after 7 days and at high doses (50 and 500 ng/ml). In summary, T cells increased the expression of Fas on miHA-mismatched pEC. This event was of no evident consequence, since FasL expression on "donor" T cells was not required to kill "host" pEC in the 4-day period studied. FasL-deficient T cells also modulated the expression of epithelial MHC molecules and might be associated with the high levels of IFNg, a key cytokine during GVHD, detected in supernatants of co-cultures. However, IFNg did not regulate Fas on pEC, indicating that other cytokines/mechanisms are responsible for this effect. Altogether, our results suggest that the Fas-FasL pathway may have a minor role in the early pathogenesis of cutaneous GVHD.

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DACLIZUMAB AND INFLIXIMAB FOR TREATMENT OF STEROID RESISTANT ACUTE GRAFT VERSUS HOST DISEASE (aGVHD) IN PEDIATRICS

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Patients with steroid resistant aGVHD have a poor prognosis. Daclizumab (anti-IL2 receptor alpha chain), and infliximab (anti-TNF) have shown activity in early clinical trials in adults. We are reporting our experience using daclizumab and infliximab in 3 pediatric patients. All received daclizumab (1 mg/kg/dose IV on days 1, 4, 8, 15 and 22 and infliximab (10 mg/kg/dose IV on days 1, 8, 15 and 22). Steroid tapering was at the discretion of the attending physician based on clinical condition and response to treatment.

UPN 377 was a 19 month old girl with Hemophagocytic Lymphohistiocytosis who received a related bone marrow graft matched for 8/8 alleles. She received a preparative therapy consisting of busulfan, VP16 and cytoxan and methotrexate (mtx) and cyclosporine (csa) for GVHD prophylaxis. She developed stage 3 grade 2 aGVHD of the skin on d + 32 post transplant. After 19 days of steroids (2 mg/kg/day), daclizumab and infliximab were started. GVHD improved by d + 36. The patient developed extensive chronic GVHD and is presently 522 days post transplant with a Lansky score of 100%.

UPN 394 was an 8 yr old male with ALL in CR2 who received a related bone marrow graft matched for 5/8 alleles. He received TBI, VP16 and cytoxan and mtx and csa for GVHD prophylaxis. He developed stage 3 grade 2 aGVHD of the skin on d + 24 post transplant. After 2 days of steroids (4 mg/kg/day), he was treated with daclizumab and infliximab. GVHD improved by d + 31. The patient has extensive chronic GVHD. He is 210 days post transplant and has a Lansky score of 100%.

UPN 395 was a 13 month old male with ALL in CR1 who received an unrelated bone marrow graft matched for 8/8 alleles. He received TBI, VP16 and cytoxan and csa and mtx for GVHD prophylaxis. He developed stage 3 grade 2 aGVHD of the skin on d + 19 post transplant. After 11 days of steroids (2 mg/kg/day), he was treated with daclizumab and infliximab. GVHD improved by d + 33. The patient relapsed at d + 189 post transplant and has no signs of chronic GVHD.

In all patients, there were no instances of opportunistic viral or fungal infections. This small series suggests that the combination of daclizumab and infliximab is well tolerated and an effective therapy for steroid resistant GVHD in pediatrics. Therefore, we have revised our algorithm for the management of aGVHD (to be displayed at the meeting).

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MONOCYTE BLOOD COUNT AND ACUTE GVHD: FLOW CYTOMETRY ANALYSIS RECOMMENDED IN PROSPECTIVE STUDIES

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Patients with a high absolute number of peripheral blood CD14+ monocytes prior to starting the conditioning regimen have been shown to have a greater risk of developing acute graft-versus-host disease (aGVHD) (Arpinati et al. BBMT 2007) after allogeneic hematopoietic stem cell transplantation (HSCT). In this study, we initially pooled the data of 154 consecutive patients with hematologic malignancies who received an allogeneic peripheral blood stem cell transplant at three different institutions, utilizing myeloablative (n = 36) or reduced intensity conditioning (n = 118) regimens not including ATG. The blood absolute monocyte count (AMC) was assessed before starting the conditioning regimen and it was calculated by dividing the absolute white cell count by the percentage of monocytes obtained with an automated CBC. The median value of AMC in the study was 0.5×10^9/L (range:0.0–11.8). Of 154 patients, 73 had < 0.5 and 81 had ≥0.5 AMC $\times 10^9$/L prior to starting the conditioning regimen. All the patients received an 8/8 HLA antigen matched graft from related (n = 114) or unrelated (n = 40) donors. In the group of patients with lower AMC the median age was 51 (range:19–71) and donors were unrelated in 27% and sex mismatched in 41% of the cases, whereas in the group of patients with higher AMC median age was 50 (range:18–63) and donors were unrelated in 25% and sex mismatched in 36% of the cases. The rate of acute GVHD grade II-IV in patients with low or high AMC was 44% and 38%, respectively (p=ns). To test whether the AMC obtained by means of automated CBC was comparable to the absolute count of CD14+ monocytes detected by flow cytometry, blood samples from 43 patients were analyzed utilizing both techniques. In this series of patients, mean values of AMC and CD14+ blood cells were 11.9 ± 11.9 and 7.3 ± 6.9 $\times 10^9$/L and median values were 9.6 (range 95% CI:8.2–15.6) and 6.0 (range 95% CI:5.2–9.5) $\times 10^9$/L, respectively, ($p = 0.0004$). In this study, the analysis of monocyte absolute count by automated CBC did not confirm the correlation between high levels of blood CD14+ cells analyzed by flow cytometry and development of aGVHD. Since we demonstrate here that the absolute number of monocytes obtained by cytofluorimetric expression of CD14 is significantly different as compared to that obtained by automated CBC, future prospective studies addressing the prognostic role of monocyte blood count in allogeneic HSCT should be performed utilizing a flow cytometry-based method.

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SIROLIMUS IN COMBINATION WITH CYCLOSPORINE OR TACROLIMUS PLUS METHOTREXATE FOR GRAFT-VERSUS-HOST DISEASE (GVHD) PROPHYLAXIS AFTER HEMATOPOIETIC CELL TRANSPLANTATION FROM UNRELATED DONORS

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Sirolimus (SIR) is reported to be effective in preventing GVHD when combined with tacrolimus (TAC) and methotrexate (MTX) after related and unrelated allogeneic hematopoietic cell transplantation (HCT). In two consecutive clinical trials, we evaluated the efficacy of SIR plus cyclosporine (CSP Group) or TAC (TAC Group) and MTX after unrelated HCT. SIR was administered as a 12 mg PO loading dose on day -1 (CSP Group) or -3 (TAC Group) followed by 4 mg PO QD. SIR levels were targeted at 4–14 ng/mL in the CSP Group and 3–12 ng/mL in the TAC Group. The dose of CSP was 1.5 mg/kg Q12h IV starting on day -1, and the dose of TAC was 0.02 mg/kg/day IV starting on day -3. CSP was targeted at 150–450 ng/mL and TAC at 5–10 ng/mL. All patients received MTX 5 mg/m^2 IV on days 1, 3, 6 and 11. Nine patients were