extracellular and transmembrane domains of human CD34 were fused in frame to the entire coding region of HSV-tk (CD34-tk). In this study, we developed a new model for xenogenic GvHD using immunodeficient NOD SCID β2M null mice (β2) and evaluated the ability of ganciclovir (GCV) to prevent GvHD after infusion of CD34-tk-modified human T cells (Td). Sublethally irradiated (250 cGy) β2 mice injected retro-orbitally (ro) with human PBMCs (10^7T cells; n=11) or human T cells (HuT; 10^7; n=14) had 19% HuT engraftment after 2–3 weeks with an OS of 75% and 25%, respectively, at 5 weeks (p=0.006). These mice lost >20% of their body weight and had extensive HuT infiltration in the spleen, liver, lung and kidney. On day 10 and before the start of any clinical signs of GvHD, mice that went on to develop lethal GvHD had a 10–15 fold increase in the expression of the HuT activation markers CD25, CD30, and CD69 in blood (p<0.0005) and tissues, and a 128-fold increase in serum levels of IFNγ (5150 pg/ml vs 40 pg/ml) compared to mice that did not develop GVHD. To generate CD34-tk-modified human T cells, PBMCs were activated with anti-CD3 and anti-CD28 mAbs immobilized on magnetic beads (Xcyte™ Dynabeads®) in the presence of IL-2 (50 U/mL). After 2 days, cells were incubated with 293 GPG-derived VSV-G pseudotyped CD34-tk oncoretroviral supernatants for 6 h at 37C. Td cells were then expanded for 2 more days and isolated by MACS (Miltineyi Biotech; Td >60%). Td cells were purified to >94% by CD34 immunomagnetic selection using a VarioMACS magnetic cell separator. Naive T cells (n=2), activated non-Td T cells (Act; n=3) and Td and selected T cells (n=6) were then injected ro into 250 cGy conditioned β2 mice. Animals receiving Td cells were then either left untreated or treated with GCV (1 mg/day, ip) from days 1–7. Mice that received naive T cells died of lethal GvHD on day 15. Mice that received Act or Td HuT had an average engraftment of 16% and 34%, respectively, at 3 weeks post-infusion but did not develop GvHD. We were able to demonstrate that Td T cells could be efficiently eliminated *in vivo* by treatment with GCV. In conclusion, this xenograft model provides a unique opportunity for preclinical testing of the CD34-tk/GCV suicide gene system as well as other methods of GvHD control.

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HUMAN LANGERHANS CELLS IN TRANSPLANTATION: RECIPIENT CELLS SURVIVE CONDITIONING BUT DONOR CELLS PREDOMINATE AT DAY 100

Collin, M.P.[1]; Booth, T.[1]; Middleton, P.G.[1]; Jackson, G.H.[1]; Mackinnon, S.[2]; Cook, G.[3]; Cavet, J.[4]; Wang, X.N.[1]; Dickinson, A.M.[1] 1. University of Newcastle, Newcastle-upon-Tyne, United Kingdom; 2. Royal Free Hospital and University College, London, United Kingdom; 3. St. James's University Hospital, Leeds, United Kingdom; 4. Christie Hospital, Manchester, United Kingdom.

Graft versus host disease (GvHD) is mediated by donor lymphocytes responding to recipient antigens. Recent murine studies have highlighted the role of recipient antigen-presenting cells (APC) in triggering acute GvHD. In humans, the fate of recipient APC after transplantation is largely unknown but persistent recipient cells are thought to increase the toxicity of early donor lymphocyte infusion (DLI) and contribute to delayed acute GvHD after immunosuppression withdrawal following reduced intensity conditioning. We have studied Langerhans Cells (LC) in human transplantation using confocal microscopy to measure cell density in the epidermis. We have also determined the origin of LC migrating *in vitro* from epidermal sheets derived from clinical skin biopsies taken at day 100 post-transplant. In sex-mismatched donor-recipient pairs, combined Giemsa-FISH X/Y allows precise cell identification and quantitation, unlike *in situ* techniques. We have studied 144 biopsies from over 50 patients conditioned with myeloablative or reduced intensity regimens and transplanted from related or unrelated donors from pretransplant to 1 year post transplant. LC genotype has been examined at day 100 in 12 patients. Pretransplant, LC density is not significantly different to normal skin (645 ± 273 cells/mm^2 vs 705 ± 94 cells/mm^2). At Day 0, LC density falls to 593 ± 143 cells/mm^2 ($p < 0.05$) but there is no difference between myeloablative and reduced intensity conditioning. Alemtuzumab conditioning has no significant impact. LC nadir is at 3 weeks, 1 week after neutrophil nadir and 2 weeks after monocyte nadir. Normal LC density is recovered by day 100 and exceeds pretransplant density (822 ± 332 cells/mm^2; $p < 0.05$). LC recovered from patient biopsies at day 100 show donor predominance (>95%) if the patient is fully engrafted (>95% donor in whole blood; 10/12 patients). In contrast to murine experiments, the absence of GvHD after T cell depletion (4/12 patients) does not confer an increased number of recipient cells. Recipient cells are only preserved when there is significant partial donor multilineage chimaerism (2/12 patients). These results support a role for recipient APC in early acute GvHD as more than two-thirds of recipient cells survive conditioning. However, recipient APC do not persist for long periods after transplantation unless there is significant partial chimaerism overall. The role of recipient APC late GvHD effects must therefore be questioned.

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THE CONTRIBUTION OF CCR1 EXPRESSION ON DONOR LEUKOCYTES TO THE DEVELOPMENT OF EXPERIMENTAL IDIOPATHIC PNEUMONIA SYNDROME

Hildebrandt, G.C.[1,2]; Choi, S.[2]; Olkiewicz, K.M.[2]; Corrion, L.A.[2]; Chensue, S.W.[3]; Liu, C.[4]; Cooke, K.R.[2] 1. Department of Hematology and Oncology, BMT Program, University of Regensburg, Regensburg, Germany; 2. Department of Pediatrics, University of Michigan, Ann Arbor, MI; 3. Department of Veterans Affairs Medical Center, Ann Arbor, MI; 4. Department of Pathology, University of Florida College of Medicine, Gainesville, FL.

Idiopathic Pneumonia Syndrome (IPS) is a severe complication of allogeneic (allo) bone marrow transplantation (BMT). IPS is characterized by cellular infiltration into the lung that results in altered pulmonary function and death in the majority of patients. Leukocyte recruitment during the development of IPS is mediated by chemokines and their receptors. Specifically, we have recently shown an important role for CCL5 (RANTES) in the development of lung injury following BMT. Receptors for CCL5 include both CCR5 and CCR1. Although the absence of CCR5 expression has been shown to worsen GVHD and IPS in some models, the role of CCR1 in each context remains to be determined. CCR1 is expressed on a variety of cell types including Th1 lymphocytes, monocytes and macrophages and contributes to lung injury in a variety of non-BMT animal models. Using an established mouse BMT system, we investigated the contribution of CCR1 to experimental IPS. Lethally irradiated B6D2F1 mice received BM and T cells from syngeneic B6D2F1 or allo B6 CCR1$^{+/+}$ donors. Lung injury developed between 2 and 6 weeks after allo BMT and was associated with increases in whole lung CCR1 and CCL5 mRNA expression. Transplantation of CCR1$^{-/-}$ donor cells resulted in a significant reduction in IPS severity 6 weeks after BMT as measured by lung pathology and BAL fluid cellularity, protein content and TNFα levels (Table 1) when compared to allo CCR1$^{+/+}$ recipients. Similar differences between groups were also noted at day 30 (data not shown). CCR1$^{-/-}$ BMT also resulted in reduced systemic GVHD as assessed by survival (92% vs. 50%), clinical score (1.0 ± 0.3 vs. 3.0 ± 0.5) and intestinal injury (index at day 14: 15.8 ± 1.8 vs. 23.8 ± 1.0) compared to allo controls. In addition, both splenic T cell expansion (3.7 ± 0.4 vs. 9.6 ± 0.9 × 10^6 cells) and serum IFNγ levels (4561 ± 559 vs. 10028 ± 681 pg/ml) were significantly lower at day 7 following allo BMT with CCR1$^{-/-}$ donor cells. In conclusion, our data demonstrate a heretofore unknown role for CCR1 on donor leukocytes in the development of IPS and GVHD. Improvement in lung injury observed after CCR1$^{-/-}$ BMT may be attributed to 1) alterations in leukocyte recruitment to the lung and 2) modulation of donor T cell responses to host allo-antigens. These data support our previous findings that blocking specific chemokine receptor:ligand interactions may represent novel, non-cross reactive approaches to reduce deleterious graft-versus host reactions following allo BMT.