rapamycin, and TGF- β on human Treg cell generation ex vivo. Normal donor CD4 T cells were isolated by CD4 positive selection and CD4+CD127- cells were isolated by CD127 negative selection and CD4 positive selection. Relative to total CD4 T cells, CD4+CD127- cells were enriched for the Treg transcription factor, Foxp3 (n = 8 experiments; P = 0.042); and also had reduced secretion of IL-2 (P = 0.05) and IFN-γ (P = 0.03). Both subsets were expanded using anti-CD3, anti-CD28 co-stimulation and IL-2 ± rapamycin and ± TGF-β. Relative to culture input of total CD4 cells, input of CD4+CD127- cells were potent suppressors at day 12, as measured by inhibition of responder T cell proliferation in response to allogeneic dendritic cell (DC) stimulation. The enhanced capacity of CD127 negative selection to generate Tregs occurred whether expansion was performed in rapamycin (P = 0.02), TGF-β (P = 0.02), or combination of both (P = 0.01). CD127 negative selection alone (without rapamycin or TGF-β) was not sufficient to generate Treg cells, as the resultant product secreted high levels of IL-2 and IFN-γ. In contrast, addition of rapamycin or TGF-β, and in particular the combination, resulted in a Treg phenotype as defined by: (1) high Foxp3 expression (~50% CD4+Foxp3+); (2) reduced Th1 cytokine secretion; and (3) suppressor function in the allo-MLR. Transwell showed that expanded Treg cell suppression was contact dependent. We hypothesized that such suppression was mediated via modulation of DC function; to address this, Treg cells and DC were co-cultured, and purified "conditioned" DC were utilized as the APC source in allo-MLR. Indeed, DC conditioned by Treg cells generated from CD4+CD127- enriched cells and expanded in rapamycin and TGF-β had: (1) significantly reduced secretion of IL-6 and TNF-α; (2) increased expression of the inhibitory molecule PDL1; and (3) greatly reduced allostimulatory function; importantly, antibody blockade of DC expression of PDL1 partially reversed the suppressive DC phenotype. In conclusion, CD127- selction combined with ex vivo expansion in rapamycin and TGF-β generates human Treg cells that inhibit alloreactivity by modulating DC function. Adotive transfer of such Tregs has implications in preventing GVHD after haematopoietic stem cell transplantation.

337

THE PREDICTIVE VALUE OF GENE EXPRESSION PROFILES FOR ACUTE GRAFT-VERSUS-HOST DISEASE AFTER HEMATOPOIETIC CELL TRANSPLANTATION WITH NONMYELOABLATIVE CONDITIONING FOR HEMATOLOGICAL MALIGNANCY

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Purpose: To test the hypothesis that global gene expression profiles of peripheral blood mononuclear cells (PBMNC) day +14 after hematopoietic cell transplantation with nonmyeloablative conditioning could predict the later occurrence of acute graft-versus-host disease (GVHD) grade II-IV. Material: Between March 2000 and Marts 2006, 100 patients with hematological malignancies received peripheral blood stem cells from an human leukocyte antigen identical sibling/mother donor or from a matched unrelated donor following nonmyeloablative conditioning with low dose fludarabine and 2 Gy of total body irradiation. Post-transplant immunosuppression consisted of cyclosporine and mycophenolate mofetil. Only patients with sustained engraftment, who did not experience late-onset acute GVHD after day +100 were included; eight patients were excluded due to graft rejection, three patients due to suboptimal RNA or lacking PBMNC samples, and further 15 patients due to late-onset acute GVHD. Seventy-four patients were then eligible for microarray analysis. **Methods:** RNA was precipitated from frozen PBMNC from day +14 post-transplant and gene profiling analyses were performed using Human Genome U133 Plus 2.0 GeneChip Array. The array data were normalized, RMA modeled and asinh transformed in R. The differentially regulated gene expression between the group of patients developing acute GVHD before day +40, +56 and +84 post-transplant compared to the patients never experiencing acute GVHD was identified and formed the basis for the subsequent principal component analysis (PCA) and classifying models. No patients experienced acute GVHD between day +85 and +100 post-transplant. **Results:** The patients experiencing acute GVHD by different time points were separated from the patients never experiencing acute GVHD by the PCA plot. Furthermore, the best classifying models could separate the groups correctly in up to 98% of cases. In addition, differentially regulated genes between the two groups were identified. **Conclusion:** These data suggest that the pattern of gene expression profiles early post-transplant is able to predict patients with a high risk of later occurrence of acute GVHD from those never experiencing acute GVHD. This knowledge could be exploited to increase the immunosupression and thus prevent acute GVHD in patients at risk. Furthermore, candidate genes of interest for the pathogenesis of acute GVHD have been identified.

338

T CELL RECEPTOR GENE TRANSFER TO VIRUS-SPECIFIC T CELLS FOR CELLULAR ANTI-TUMOR IMMUNOTHERAPY

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T cell receptor (TCR) transfer to engineer tumor specific T cells may be an alternative strategy for adoptive immunotherapy. For complete eradication of leukemic cells and to achieve long-term protection, potent effector T cell function and long-term T cell persistence are necessary. Therefore, we propose to use virus specific T cells for TCR transfer since such engineered dual specific T cells can be triggered via their endogenous TCR by latent presence of viral antigens, improving their long-term persistence. We previously demonstrated that transfer of the minor histocompatibility antigen HA-1 and HA-2 TCRs to CMV specific T cells led to the generation of anti-leukemic T cells with dual specificity. Based on these results, we developed a clinical grade method for generation of TCR transferred virus T cells for cellular immunotherapy. Pentamers combined with clinical grade available anti-biotin magnetic beads were used to isolate CMV and EBV specific T cells. At day 3 after isolation, T cells were transduced with multi-cistronic retroviral vectors encoding the alpha and beta chains of the HA-2 TCR linked by an IRES or 2A-like sequence. Isolation by pentamer-coated beads led to the generation of T cell lines with high frequencies (>80%) of virus-specific T cells with 20–40% transduced cells. The T cells exhibited dual specificity, and tetramer staining correlated with lysis of target cells expressing endogenously-processed HA-2. We determined whether these cells preserve their dual specificity upon repetitive stimulation. When frequent encounter of viral antigens leads to selective survival of TCR transferred T cells predominantly expressing the CMV TCR incapable of proliferating via the introduced TCR, persistence in vivo of TCR transferred CMV T cells capable of controlling minimal residual disease may fall short. We analyzed the TCR expression and functional activity of the TCR transferred T cells after repetitive stimulation with CMV or HA-2. TCR-transferred T cells repetitively stimulated skewed to T cells predominantly expressing one TCR. However, this skewing was not due to selective outgrowth, but to change of TCR make up. This TCR make up appeared to be reversible, and HA-2 TCR transferred CMV T cells remained their functional activity via both TCRs after repetitive stimulation. In a clinical phase I/II study, the safety, clinical and immunological efficacy of TCR-transduced virus T cells as cellular anti-tumor immunotherapy will be investigated.

339

INFLIXIMAB COMBINED WITH DACLIZUMAB RESULTS IN A HIGH COMPLETE RESPONSE RATE WHEN USED TO TREAT ACUTE STEROID REFRACTORY GRAFT-VERSUS-HOST DISEASE (SR-GVHD)

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