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LONG-TERM FOLLOW UP OF T CELL DEPLETED TRANSPLANTS FROM UNRELATED DONORS IN PEDIATRIC PATIENTS

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Graft-versus-host disease (GVHD) remains a major cause of mortality and long term morbidity in recipients of hematopoietic stem cell transplant from unrelated donors. From 1993-2000 we used partial T cell depletion to reduce this risk and transplanted 116 patients with T cell depleted unrelated donor stem cells on two successive IRB-approved protocols at St Jude Children's Research Hospital (1993-1997 n = 75) and Baylor College of Medicine (1998-2000 n= 41). All patients were transplanted for hematologic malignancy and were stratified into standard risk (Acute leukemia or lymphoma in 1st or 2nd remission or CML in 1st chronic phase) or high risk (Acute leukemia or lymphoma in relapse or ≥ CR3, CML beyond 1st chronic phase, myelodysplasia or secondary AML). Patients received marrow from 5/6 or 6/6 matched unrelated donors depleted of T cells by incubation with CD6 and CD8 antibodies and baby rabbit complement, which produced a median T cell depletion of 96%. Conditioning was with Cyclophosphamide 45mg/kg x 2, Ara-C 3g/m^2 x 6, ATG 30mg/kg x 3 and TBI 1200-1400cGy. In 1997 we reported initial outcome data in the first 51 patients (Hongeng et al Lancet 1997, 350: 767-71) with a 2-year disease-free survival estimate for standard-risk recipients of 73±12.1% and for high risk recipients of 32 ± 15.1%. We now report long term follow up on all 116 patients with follow up ranging between 6.7 and 13.5 years. The 5 and 10 year Kaplan Meier survival estimate is 60% for 49 standard risk patients and 37% for 67 high risk patients. 4 of the 49 standard risk patients and 26 of the 67 high risk patients relapsed. Three of the patients who relapsed are long term survivors after relapse (> 10 years) after donor lymphocyte infusion. All but one relapse occurred within 2 years of transplant. The incidence of grade 3-4 GVHD was 5% and only 4% of recipients developed extensive chronic GVHD. The 100 day mortality was 21% in high risk recipients and 19% in standard risk recipients but the incidence of late non-relapse mortality was low with only two deaths from causes other than relapse after one year - one death from pulmonary failure at 4.5 years and one at 10 years in a motor vehicle accident. All long term survivors have a good performance status. Partial T cell depletion can therefore reduce the risk of graft versus host disease and long term sequelae from this complication without an increased risk of relapse.

IMMUNE RECONSTITUTION

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INSULIN-LIKE GROWTH FACTOR I POSITIVELY REGULATES THYMIC FUNCTION BY EXPANSION OF THYMOCYTE PRECURSORS AND THYMIC EPITHELIAL CELLS

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Neuroendocrine growth factors help regulate thymic function and serve as potential agents to accelerate thymic T-cell production following hematopoietic stem cell transplantation (HSCT). We present evidence supporting insulin-like growth factor I (IGF-1) as a thymic regulator. Continuous infusion of IGF-1 (100 ug/day) into normal mice resulted in significant increases in all thymocyte populations, including earliest thymocyte precursors (ETP), with subsequent increases in recent thymic emigrants (RTE). Time-sequential enumeration of thymic epithelial cell (TEC) and thymocyte subpopulations and bone marrow and peripheral LSK (Lineage-, Sca-1+, c-kit+) precursor populations showed that expansion of peripheral LSK, occurring on day 4 of IGF-1 administration, preceded quantitative increases in thymocyte and TEC subpopulations by three days. Concomitant with the increase in peripheral LSK numbers, cell cycle entry was increased in bone marrow and peripheral LSK and lineage-CD44+ CD25+ (DN2) thymocytes. IGF-1 administration also affected TEC turnover during this early time period (day 2-7) preceding numeric increases in TEC. The relative proportions of cortical and medullary TEC were not altered throughout the course of IGF-1 administration. Finally, mice lacking IGF-1 receptor (IGF-1R) signaling on T-cells were generated through cre-mediated deletion of the IGF-1R high-affinity binding site (pLCK-cre/loxIGF1R). Compared to wild-type littermates, pLCK-cre-loxIGF1R mice exhibited a decrease in the number of CD4$^+$CD8$^+$ thymocytes, thymic TREC, and splenic naïve T-cells and RTE. IGF-1 treatment, however, restored thymocyte and peripheral subset numbers in these mice. These results demonstrate: 1) IGF-1 expands thymocyte subpopulations and increases thymic output; 2) IGF-1 expands peripheral thymocyte precursor populations leading to their increased availability for entry into the thymus; and 3) IGF-1R signaling is required for the maintenance of normal thymocyte and peripheral T-cell populations, and that presumptive IGF-1 effects on TEC can overcome the absence of IGF-1R signaling in thymocytes. Together, the results support the concept of neuroendocrine growth factors such as IGF-1 in preserving and/or enhancing thymic function recovery following HSCT, and suggest that points of regulation in thymic function by IGF-1 include entry of thymocyte precursors into the thymus and the proportionate expansion of TEC populations that facilitate thymocyte development.

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A SUBPOPULATION OF HUMAN NK CELLS LACKING INHIBITORY RECEPTORS FOR SELF MHC IS DEVELOPMENTALLY IMMATURE RATHER THAN AUTOREACTIVE

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The effector function of human natural killer (NK) cells is down-regulated via ligation of inhibitory receptors (killer immunoglobulin-like receptors [KIR] and NKG2A) that recognize self MHC. In order to study the mechanism producing self-tolerance, which is not yet understood., we developed and validated a quantitative, real-time RT-PCR (Q-RT-PCR) assay to measure mRNA levels from individual *NKG2* and *KIR* genes. Our expression typing assay accurately predicts genotyping by SSOP (sensitivity 0.94. specificity 0.96, PPV 0.97) and gives expression data with a single quantitative readout. We used this assay to investigate NK cells circulating in normal blood. We sorted CD56^{+dim} NK cells into KIR$^+$ and KIR$^-$ subsets by flow cytometry using an antibody cocktail recognizing 6 KIR. Measurements of individual *KIR* gene expression showed that not all CD56^{+dim} cells express KIR. The KIR$^-$ populations were further divided into NKG2A positive and negative cells, defining a novel subpopulation of cells committed to the NK lineage. This KIR$^-$ NKG2A$^-$ subset comprises 19.4 ± 2.8% of CD56^{+dim} NK cells in healthy donors, and expresses the activating NKG2D and NKG2E receptors. Consequently these CD56^{+dim} NKG2A$^-$KIR$^-$ NK cells do not have 'at least one' inhibitory receptor for engaging autologous MHC class I. However, they are not intolerant, autoreactive cells, but instead are immature, already committed NK cells (based on CD56 expression), coexpressing CD7, CD16, and CD18. Functional assays showed this population to be hyporesponsive. Compared to KIR or NKG2A-expressing subsets, they exhibited impaired degranulation (measured by CD107a) and poor cytotoxicity against K562 targets. Furthermore, they produced little IFN-γ after stimulation with IL-12 and IL-18, and compared to that of CD56^{+dim} NK cells that express NKG2A, they showed a diminished capacity to proliferate in response to IL-15. Upon culture on a murine embryonic stromal cell line with IL-15, these CD56^{+dim} NKG2A$^-$KIR$^-$ NK cells