**IMMUNE RECONSTITUTION**

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**EFFECTS OF PREPARATIVE REGIMENS ON HOST DENDRITIC CELLS**

**PRE-TRANSPLANT IN ALLOGENIC STEM CELL TRANSPLANTATION**

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Background: Host dendritic cells (DCs) present antigens to donor T-cells, thereby initiating graft-vs-host disease (GVHD) in allogeneic stem cell transplantation. The effects of preparative regimens on host DCs remain unknown. We prospectively studied changes in host DC populations pre-transplant. Methods: 17 patients, median age 47 years, with various hematologic malignancies underwent a conventional preparative regimen of cyclophosphamide 120mg/kg and 1200Gy total body irradiation (TBI) (n=8) or a reduced intensity preparative regimen of extracorporeal photopheresis (ECP) x 2 days, pentostatin 8mg/m2 by continuous iv infusion over 48 hours, and 600-Gy TBI (n=9), followed by allogeneic stem cell transplantation from fully matched related (n=7), fully mismatched unrelated (n=9), or 7/8 DR-mismatched unrelated (n=1) donors. The Blood Dendritic Cell Enumeration Kit (Miltenyi Biotec, Inc., Auburn, CA) was used to identify the percentages of myeloid DC1 (MDC1), plasmacytoid DC (PDC), and myeloid DC2 (MDC2) subpopulations within the non-B-cell, non-monocytic, non-granulocytic gate in peripheral blood obtained before initiation and after completion of the preparative regimen. Results: The median percentages of MDC1, PDC, and MDC2 cells were as follows: at initiation: 0.26%, 0.11%, and 0.07% respectively, following ECP: 0.38%, 0.07%, and 0.01% respectively, and pre-transplant, 0.07%, 0%, and 0% respectively. Following ECP, the median change in MDC1, PDC, and MDC2 cells were 0%, -58%, and -65% respectively. On Day 0, the median change in MDC1, PDC, and MDC2 cells were -49%, -96%, and -73% respectively among ECP-based regimens, and -98%, -100%, -100% respectively among conventional regimens (p=0.73, p=0.30, p=0.09 respectively). The median ratios of MDC1 to PDC cells were 2.5 pre-transplant, 3.6 following ECP, and 0.56 on Day 0. ECP decreased at least 1 DC lineage by 100% in 50% of patients, but decreased all 3 DC lineages by 100% in only 10% of patients. By Day 0, 76% of patients had at least 1 DC lineage decreased by 100%, and 24% of patients had all 3 DC lineages decrease by 100%. Conclusions: Preparative regimens can decrease all 3 subtypes of host DCs pre-transplant. The effects of ECP on host DCs may be delayed since the decrease in DC percentages and the shift from a high, pro-inflammatory, DC1/DC2 ratio to a low, pro-tolerogenic DC1/DC2 ratio did not occur until day 0. The effects of changes in pre-transplant host DC populations on GVHD outcomes require further study.

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**RADIOPROTECTION BY GROWTH HORMONE**

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Hematopoietic and immune systems are sensitive to ionizing irradiation and the recovery is slow. Very few effective therapeutic agents are currently available for this condition. In this study, we investigated the effects of growth hormone on hematopoietic and immune recovery post irradiation and its ability to protect against lethal irradiation. The studies were performed using BALB/c mice. Recombinant human growth hormone (rhGH) was given at the dose of 20 μg/kg/day, i.p. or i.v. once or twice a day, starting within one hour after irradiation. The hematologic and immune recovery was monitored weekly post irradiation. In a sublethally irradiated (5 Gy) model, treatment with rhGH for 30 days significantly accelerated the recovery of platelets in peripheral blood (9087±13860 vs. 42825±7425 on day +8, P=0.01). Similar trends were also observed in absolute blood cell counts and spleen weight (T, B, NK cells). Similar results were obtained when higher dose of radiation (7.5 Gy) was used. These data suggest that growth hormone can promote hematopoietic and immune recovery after irradiation. We next tested whether growth hormone can rescue animals from lethal irradiation. BALB/c mice were irradiated with 7.5 Gy and treated with rhGH for 35 days. As demonstrated in the table, 13 out of 20 mice in the growth hormone treated group survived more than 60 days after irradiation, whereas only 3 out of 20 mice survived more than 25 days in the saline control group. The radioprotective effect was still observed when higher dose of radiation (8.5 Gy) was used. These findings demonstrate that growth hormone has significant radioprotective effects even when given after total body irradiation.

**Growth hormone protects against lethal irradiation**

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<tr>
<th>Groups</th>
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<th>Survival</th>
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<tbody>
<tr>
<td>Saline control</td>
<td>20</td>
<td>3/20</td>
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<td>Growth hormone</td>
<td>16/20*</td>
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**KIR2DS1 AND 2DS2 INDUCE NK CELL CYTOKINE RESPONSE AGAINST ALLOGENIC B-LYMPHOBLASTOID CELL LINES**

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NK cells reconstitute the host immune system early following hematopoietic stem cell transplant (HSCT), and may act to prevent relapse in patients receiving HSCT for malignant diseases. NK cell effector mechanisms include both cytotoxicity and cytokine secretion, and these functions result from different signaling pathways following receptor-ligand interactions. We have previously shown KIR2DS1 and 2DS2 regulate cytokoty against B-lymphoblastoid cell lines (BLCL) positive for HLA-Cw C2 or C1 group antigens, respectively. Alloreactivity was only displayed by NK cells from donors not expressing these same HLA antigens in cis. In this study, we analyzed cytokine production by NK cells from donors possessing the genes for 2DS1 and 2DS2, and homozygous for C1 or C2 group, respectively. IL2 propagated, polyclonal NK cells from 2DS1-positive, respectively, IL2 propagated, polyclonal NK cells from 2DS1-positive, C1 donors revealed significantly higher levels of interferon-gamma (IFN-g) following incubation with C2 group BLCL compared to C1 group BLCL. Intracellular FACS staining revealed that the NK subset expressing the 2DL1, S1 receptor was the IFN-g producing population. NK clones obtained from these same donors demonstrated increased production of IFN-g, TNF-alpha, and GM-CSF after 2DS1 receptor cross-linking. Intracellular FACS using freshly isolated NK cells from donors expressing the genes for 2DS1 and 2DS2, and homozygous for C1 or C2 group, respectively. IL2 propagated, polyclonal NK cells from 2DS1-positive, respectively, IL2 propagated, polyclonal NK cells from 2DS1-positive, C1 donors again revealed increased IFN-g production after incubation with C2 group BLCL compared to C1 group BLCL. Intracellular FACS staining revealed that the NK subset expressing the 2DL1, S1 receptor was the IFN-g producing population. NK clones obtained from these same donors demonstrated increased production of IFN-g, TNF-alpha, and GM-CSF after 2DS1 receptor cross-linking. Intracellular FACS using freshly isolated NK cells from donors expressing the genes for 2DS1 and 2DS2, and homozygous for C1 or C2 group, respectively. IL2 propagated, polyclonal NK cells from 2DS1-positive, respectively, IL2 propagated, polyclonal NK cells from 2DS1-positive, C1 donors again revealed increased IFN-g production after incubation with C2 group BLCL compared to C1 group BLCL. Intracellular FACS staining revealed that the NK subset expressing the 2DL1, S1 receptor was the IFN-g producing population. NK clones obtained from these same donors demonstrated increased production of IFN-g, TNF-alpha, and GM-CSF after 2DS1 receptor cross-linking. Intracellular FACS using freshly isolated NK cells from donors expressing the genes for 2DS1 and 2DS2, and homozygous for C1 or C2 group, respectively. IL2 propagated, polyclonal NK cells from 2DS1-positive, respectively, IL2 propagated, polyclonal NK cells from 2DS1-positive, C1 donors again revealed increased IFN-g production after incubation with C2 group BLCL compared to C1 group BLCL.