(HSCT) patient. The degree of immune suppression appears to be the most important risk factor for development of serious adenoviral disease. While withdrawal of immune suppression, and the use of cidofovir may have some effect in reducing viral load and hastening immune recovery, these approaches have appreciable side effects and are not always effective. Little is known about the reconstitution of immunity to adenovirus after HSCT. Therefore, in this current study we have prospectively monitored the recovery of adenovirus cellular immunity in pediatric HSCT patients receiving matched-related, mismatched related or unrelated donor grafts. 23 pediatric HSCT patients (age range 14 months-20 years) were enrolled on this study which aims to monitor viral load and recovery of cellular immunity to adenovirus post HSCT. We have developed and validated a real-time PCR (Q-PCR) to obtain a quantitative measure of viral load. Q-PCR is more sensitive than semi-quantitative PCR and shows that adenovirus can be detected (despite the absence of symptoms) in the blood, stool or urine at rates of up to 70%. 22 patients have >30 days follow-up (mean 147 days, range 60–240 days). 18/22 (81%) have had detectable levels of adenoviral DNA in stool (14/18) or blood (13/18). Positivity in the stool usually preceded adenovirus detection in the blood. 3 patients were exclusively positive in the blood on one occasion with low viral copy number (range 650 to 1200 copies/ml). 8 patients have had multiple blood samples positive for adenovirus DNA ranging in peak viral copy concentration from 1200 to 92500 cp/ml (mean = 31056 cp/ml). Peak copy number in the stool ranged from 1×10^3 to 9×10^9 cp/gram stool. In a preliminary analysis rising levels appear to correlate with risk of infection. ELISPOT assay and tetramer analysis for known HLA-restricted adenovirus epitopes will be used to monitor recovery of adenovirus specific T-cells in these patients. The results from this study will, therefore provide critical information for our forthcoming clinical trial where we will evaluate if immunity to adenovirus can be improved by adoptive transfer of adenovirus specific CTLs generated by culture with monocytes transduced with an Ad5/35 adenoviral vector (Leen et al, Blood 2004;104:2432–40).

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THE EFFECTS OF CYTOTOXIC AND IMMUNOSUPPRESSIVE AGENTS ON THYMIC RECONSTITUTION

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One key component of long-term outcome after stem cell transplant (SCT) is successful reconstitution of the immune system. In this regard, effective reconstitution of antigen-specific immunity requires de novo T cell generation. Bone marrow derived progenitors seed the thymus and undergo a complex process involving lineage commitment, proliferation and selection. Coordinated interaction of marrow-derived lymphoid progenitors with thymic stromal cells is required for successful T lymphopoiesis in the post-natal thymus. Disruption of the thymic microenvironment can result in disrupted T cell lymphopoiesis. One cause of prolonged defects in generating functional T lymphocytes after SCT is damage to the thymic microenvironment by radiation or cytotoxic therapy. Specific damage to the thymic microenvironment by the individual agents used in both myeloablative and non-myeloablative regimens has not been fully evaluated. We have developed a model system using immunodeficient mice as a platform on which to assess thymic reconstitution. The thymus of mice deficient for the alpha chain of the IL-7 receptor ($IL7R^{-/-}$) can be reconstituted by the injection of low doses of wild type bone marrow. The ability to achieve this reconstitution appears to depend on absolute numbers of early intra-thymic precursors, rather than on total thymic cellularity. Exploiting this model, the adequacy of thymic reconstitution following the administration of cytotoxic and immunosuppressive agents can be compared to the thymic reconstitution that occurs in the absence of agents potentially toxic to the thymic stroma. Our model system allows the evaluation of thymic reconstitution after single agent regimens insufficient to allow donor cell engraftment in wild type mice. The effects of several agents on thymic reconstitution will be presented. In addition we have identified morphologic consequences of specific ablative agents and a phenotype of abnormally developing T lymphocytes that is specific to injury from busulfan. It is anticipated that this information will lead to strategies to both minimize delayed immune reconstitution and to augment T cell lymphopoiesis post-transplant. In addition, further evaluation of impaired thymic reconstitution will augment the understanding of lymphostromal interactions crucial to normal T cell lymphopoiesis.

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FACTORS UNDERLYING EARLY LYMPHOCYTE RECOVERY AND ASSOCIATED SURVIVAL BENEFIT FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANT FOR LYMPHOPROLIFERATIVE DISORDERS

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Increasing data now exists to substantiate early absolute lymphocyte count (ALC) recovery following both allogeneic and autologous stem cell transplant as a strong prognostic indicator for survival, particularly with lymphoproliferative disorders (Myeloma and Lymphoma). Yet, there is still a paucity of insight into specific factors affecting the ALC. Furthermore, the level and time of optimal lymphocyte recovery reported has varied, ranging between 500–1000 cells/mcl at days 15–25 after transplant. Based on the above, we performed a retrospective analysis of 59 patients with Myeloma and Lymphoma (HD and NHL) who had undergone high-dose chemotherapy and autologous stem cell transplant at Dartmouth-Hitchcock Medical Center during 2002–2004 to assess potential factors that might correlate with the ALC. For this analysis, ALC thresholds of >500 on day +15 (ALC-15) and >1000 on day +25 (ALC-25) post-transplant were included, and correlated with the following four parameters: 1) age; 2) number of pre-transplant regimens; 3) dose of $CD34^+$ cells/kg recipient infused; 4) ± IL-2 post-transplant. By both univariate and multivariate analysis, no statistically significant correlation was found between the above four parameters and attainment of ALC-15 >500 or ALC-25 >1000. A trend toward statistical significance was noted for patients receiving $\geq 5 \times 10^6$ $CD34^+$ cells/kg or for those receiving post-transplant IL-2. Given the limitations of a retrospective analysis, a follow-up prospective assessment of ALC determinants (both clinical and laboratory) during immune reconstitution after transplant is planned. Hopefully, this will help to clarify optimal transplant conditions for patients with lymphoproliferative disorders as well as to elucidate specific immunologic events involved in this early lymphocyte recovery that provide the basis for a post-transplant survival benefit, presumably via improved immune surveillance against infection and minimal residual disease.

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THYMIC ACTIVITY OF HUMAN GROWTH HORMONE ON MURINE THYMOCYTES: POTENTIAL ROLE IN PROMOTING THYMOPOIESIS FOLLOWING BONE MARROW TRANSPLANT

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Thus far, very few agents have been demonstrated to positively affect thymic function. Many cytokines have been shown to promote peripheral T cell function but only a select few (i.e. IL-7 and SCF) have been shown to promote thymopoiesis or T progenitor cell survival. Most cytokines were meant to exert local effects and it is often difficult to generate the levels needed after systemic administration to yield sustained biological effects. Hormones, by their very nature, are well suited for systemic administration. Neuroendocrine hormones such as growth hormone (GH) and prolactin (PRL) have long been associated with effects on immune cell function. We have shown that administration of recombinant human GH (rhGH) results in the enhanced recovery of thymopoiesis following murine syngeneic bone marrow transplants. To explore the mechanism of action, we investigated the effects of GH on