into lethally irradiated BALB/c mice along with 1×10^7 T cell depleted bone marrow cells and 1×10^5 purified BCL1 cells (a BALB/c-origin leukemia/lymphoma cell line). As a negative control, T cell depleted bone marrow alone rescued all lethally irradiated recipients and all survived more than 100 days after transplantation without GVHD. All the recipients of T cell depleted bone marrow and BCL1 cells developed tumor and died within 21 days post transplantation. Addition of 1×10^3 T cells in the graft protected one out of five animals from developing tumor. Addition of 1×10^4 T cells protected all recipients from developing tumor and all survived more than 100 days after transplantation without GVHD. Addition of both 1×10^5 and 1×10^6 protected all animals from developing tumor. However, all animals in these two groups developed GVHD and the majority died before 50 days post transplantation. These data directly demonstrate that unmanipulated T cells alone can mediate GVL effect without causing GVHD in a given animal model using a carefully titrated dose. Based on these data, one can envision that separation of GVL from GVHD may be achievable in a selected model by any approach that is able to inhibit T cell function to a certain degree, specifically or non-specifically. Knowledge of this information is important in all animal models testing different approaches for the prevention of GVHD without loss of GVL. Unfortunately, separation of GVL from GVHD is very difficult to achieve in humans by using unmanipulated T cells alone due to unknown minimum dose required for induction of GVHD in individual patients. Therefore, in order to translate the findings into human setting, it will be important to determine not only whether separation of GVL from GVHD is achievable but also how wide the therapeutic window is. The therapeutic window will help to determine whether the approach is translatable in humans or not.

Mature T Cells Alone Separate GVL From GVHD

	Presence of Tumor	Body Weight (gram)		100-day Survival
		Day +21	Day +98	
TCD BM alone	—	18.6 ± 0.9	19.1 ± 2.1	5/5
TCDBM$^+$ BCL1	5/5	14.7 ± 0.8*	—	0/5
1×10^3	4/5	16.7 ± 2.1*	15.0 ± 0.0	1/5
1×10^4	0/5	18.5 ± 1.1*	18.7 ± 2.0	5/5
1×10^5	0/5	15.7 ± 1.6*	14.7 ± 3.2*	2/5
1×10^6	0/5	14.9 ± 0.9*	—	0/5

*$P < 0.05$, compared with TCD BM alone.

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CC CHEMOKINE RECEPTOR 2 IS INVOLVED IN INTESTINAL HOMING OF ALLOREACTIVE DONOR CD8$^+$ T CELLS DURING GVHD

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Chemokines and chemokine receptors play an important role in T cell homing and T cell mediated diseases, such as graft-versus-host disease (GVHD). In a series of experiments using DNA microarrays and ELISA analysis we found that monocyte chemoattractant protein-1 (MCP-1) is upregulated in GVHD target organs in the first two weeks after bone marrow transplantation (BMT). CC Chemokine Receptor 2 (CCR2) is the primary receptor for MCP-1 and is expressed on a variety of hematopoietic cells including monocytes, macrophages and memory T cells. The relevance of CCR2 for homing of monocytes and macrophages has been demonstrated in a variety of disease models but data on the relevance of CCR2 for T cell homing are limited. Therefore, we analyzed the effects of CCR2-deficiency on the homing capacity of donor T cells in clinically relevant murine GVHD models. We first demonstrated that proliferation, alloactivation, IFN-γ production and cytotoxicity of B6.CCR2$^{-/-}$ T cells were intact. However, preliminary studies with a donor T cell inoculum consisting of 50% B6.WT and 50% B6.CCR2$^{-/-}$ T cells showed a reduced infiltration of B6.CCR2$^{-/-}$ donor CD8$^+$ T cells into the intestinal epithelium of C3FeB6F1 recipients. Homing to other organs and homing of CD4$^+$ T cells was not impaired. In accordance with these findings we could demonstrate a reduction of GVHD morbidity and mortality in recipients of B6.CCR2$^{-/-}$ CD8$^+$ T cells while preliminary data showed no major difference for CD4$^+$ T cells. Recipients of B6.CCR2$^{-/-}$ CD8$^+$ T cells had a significant reduction in large intestine histopathology scores and showed a trend towards lower scores for the small intestine. Moreover, the graft-versus-tumor (GVT) activity of B6.CCR2$^{-/-}$ CD8$^+$ T cells against P815 mastocytoma in B6D2F1 recipients was intact, as demonstrated by in vivo bioluminescent imaging. Again GVHD mortality was reduced for B6D2F1 recipients of B6.CCR2$^{-/-}$ CD8$^+$ T cells. In conclusion, our data suggest that the MCP-1-CCR2 pathway is involved in intestinal homing of alloreactive T cells during GVHD but is not essential for GVT activity.

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RISK FACTORS FOR THE DEVELOPMENT OF ACUTE AND CHRONIC GVHD AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN AND ADOLESCENTS

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Children and adolescents undergoing hematopoietic SCT have to be protected from the deteriorating side effects of severe acute and chronic GvHD which impairs quality of life and causes severe long lasting acute and late effects. To analyse which risk factors are of importance for the development of GvHD in young patients we analysed 297 pediatric recipients of allogeneic stem cell transplants at a single centre between 1993 and 2003. We evaluated the influence of patient/donor sex, age, CMV status, disease and disease status, donor type, graft manipulation, use of monoclonal antibodies or ATG, conditioning regimen, HLA-matching techniques, year of transplantation and the number and source of transplanted stem cells. The results showed an association of disease status (advanced malignancies vs. early stages), donor type (unrelated vs. sibling), year of SCT (before vs. after 1997) and HLA-typing methods (high resolution techniques vs. serological typing) with an increased relative risk (RR) of aGvHD. Increased risk of cGvHD was associated with non T-cell depleted (TCD) PBSCT from UD, patient's age >15 years, transplantation before 1997 and evidence of aGvHD. No significant influence for the development of aGvHD/cGvHD was found for TBI vs non TBI-containing regimen, use of ATG, or myeloablative vs. non myeloablative conditioning regimen, respectively. Furthermore, donor's age, gender, underlying disease (malignant vs. non malignant disorders) and number of transplanted stem cells did not significantly influence the severity of aGVHD/cGVHD. However, in a Cox Regression analysis with time dependent covariates treatment related mortality was higher in patients with malignancies when transplanted at an advanced disease status or in CMV- IgG pos pts receiving stem cells from a CMV-negative donor. Interestingly, the overall and disease free survival in pts with malignant diseases was similar with no, mild or severe aGvHD (5-yrs pSU: 0.67, 0.74 and 0.65) Our data support the preferential selection of CMV-pos donors for CMV-pos recipients and the consideration of HLA-typing with high resolution techniques to apply an appropriate GvHD-prophylaxis according to the identified HLA disparities. Furthermore, patients with high risk leukemia might benefit from allogeneic SCT at an early remission stage. Children with non-malignant disorders can be treated with reduced intensity conditioning without significant higher risk of acute or chronic GvHD, respectively.