possess immunosuppressive properties by selectively halting immune cells at the G0-G1 phase of the cell cycle but only partially affecting their effector function (split anergy). For these reasons MSC have been used to manipulate graft-versus-host disease (GvHD). We tested the therapeutic potentials of human MSC to prevent and/or treat GvHD in a xenogeneic model. Sublethally irradiated NOD/SCID mice were transplanted with CFSE-labelled human PBMC obtained from normal buffy coats. In a group of mice MSC were given in a single infusion at the time of PBMC infusion whilst in another group MSC were administered at weekly intervals. Recipient mice were evaluated at serial intervals for human T cells proliferation as measured by CFSE staining and number of CD45+/CD3+ cells; clinical signs of GvHD (wasting, ruffled hair, hunched back) were also monitored. At the end of the experiment lymphoid and non lymphoid tissues were examined by histological analysis. In control mice, the proliferation of human T cells was already evident in the peripheral blood 3 weeks after infusion and progressed thereafter. The mice started to develop signs of GvHD after 8-10 weeks and the disease was then confirmed by histology. Lymphoid infiltrates were evident in lymphoid tissues as well as in liver, kidney, spleen, lung, and peritoneal washing. The mice injected with a single dose of MSC at the time of PBMC infusion did not behave differently form the controls. However, when MSC were given at weekly intervals, there was a marked decrease in human T cell engraftment and none of the mice developed GvHD. If MSC were administered when GvHD had already developed, T cell expansion and the course of the disease were comparable to controls. MSC were tracked 10 days after infusion by using cells which had been transduced with eGFP. PCR analysis showed their presence only in the BM in mice receiving only PBMC, but were distributed also in the lungs, liver and peritoneal washing in the mice in which MSC were administered with PBMC. Our study shows that the frequent administrations of MSC prevent the development of GVHD but fails to treat the disease when established. These findings are consistent with the notion that the immunosuppressive effect of MSC resembles split anergy, thus supporting the use of MSC as a prophylactic rather than a therapeutic agent for GvHD.

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MULTIPOTENTIAL MESENCHYMAL STROMAL CELLS (MMSC) ABROGATE ACUTE GRAFT-VERSUS-HOST DISEASE IN A MURINE MODEL

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Acute Graft-versus-Host Disease (GVHD) remains a major complication after allogeneic hematopoietic cell transplantation (HCT). Several publications show a potential beneficial effect of human MMSC for the treatment of refractory GVHD. The mechanism of action remains to be determined. We set out to develop an animal model that can be used to further study the effect of MMSC on GVHD. MMSC were obtained from female C57Bl/6J (H2b) mice by standard culture technique. ISCT criteria were used to confirm development of bona fide MMSC by flow cytometry and by in-vitro differentiation experiments. GVHD was induced by transplantation of C57Bl/6J donor bone marrow cells (5 × 10E6) and spleen cells (6 × 10E6) into lethally irradiated DBA (H2d) recipient mice. This model resulted in acute GVHD starting at transplant day +10 with near-complete lethality by transplant day +35. MMSC were infused at various doses on transplant day +10. Abrogation of GVHD was noted at all dose levels studied, with complete rescue from lethality in the group receiving 1 × 10E5 MMSC. Source of MMSC (donor, recipient, third party) did not affect the beneficial effect on GVHD. An MMSC cell line was equally effective in providing abrogation of acute GVHD. We conclude that a mouse model can be used to study the effect of MMSC on acute GVHD.

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NAIVE AND MEMORY T REGULATORY CELLS RESPOND TO MESENCHYMAL CELLS REGULATION

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In T cell replete bone marrow transplantation GvHD remains a major problem despite prophylaxis with immune suppressive agents. In animal models CD4(+)CD25(+)FoxP3(+) T regulatory (T reg) cells protected from rejection and GvHD after bone marrow transplantation. 70% of T reg are memory/effector cells with a CD45RO+ phenotype. The others are naive CD45RA+ T cell. When sorted and/or purified both subpopulations inhibit mixed lymphocyte cultures. T cells from healthy subjects were enriched by immnuselection to provide populations of CD45RA+ cells (95 % ± 2.9) and CD45RO+ cells (97 % ± 0.25). Naive and memory cells were cultured in presence of human mesenchymal cells (hMSC) (ratio 5:1). After 7 days' culture, in the naive population the T reg starting fraction of 0.05 % ± 0.01 of CD4/CD25 positive cells, rose to 0.2 % ± 0.14 in presence of MSC. In the memory population the T reg starting fraction of 0.3 % ± 0.05 of CD4/CD25 positive cells, rose to 1.5 % ± 0.9 in the presence of MSC. The naive T reg starting fraction expressed 3 % ± 1.2 CD127 which was down-regulated to 0.29 % ± 0.2 with MSC. Memory T reg cells expressed CD127 in 15% ± 1.2 of the starting fraction which was down-regulated to 1.32 % ± 0.34 with MSC. FoxP3 expression was measured by real time quantitative PCR in sort-purified subsets of peripheral blood, identified by staining with a combination of CD4, CD25, CD45RA or CD45RO. FoxP3 expression increased 1.15 fold in the presence of MSC in naive T reg and 1.14 fold in memory T reg. Observing that naive and memory T regulatory cells respond to MSC regulation opens new perspectives for clinical use. Post transplant infusion of MSC and/or donor regulatory cells after co-culture with MSC might be a new option for GvHD treatment.

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CO-TRANSPLANATION OF HAPLOIDENTICAL MESENCHYMAL STEM CELLS TO OVERCOME GRAFT DYSFUNCTION ASSOCIATED WITH PARENTAL HAPLOIDENTICAL CD34 POSTIVE SELECTED PERIPHERAL STEM CELL GRAFTS

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Transplantation with haploidentical peripheral blood stem cells from a parent are an acceptable form of treatment for children who require stem cell transplantation but lack a conventional donor. However, the risk of graft dysfunction increases due to the intense T cell depletion of CD34 positive stem cell selection. Mesenchymal stem cells (MSCs) isolated from bone marrow can improve hematological recovery but their role in overcoming graft dysfunction post transplant is unknown.

We conducted an ethically committee approved prospective study of co-transplantation of MSCs in children undergoing haploidentical PBSCT.

MSCs, isolated from haploidentical parental donor marrow were expanded under GMP conditions 4-5 weeks before transplantation. MSCs 1-2 × 10^6/kg recipient weight (fresh or cryopreserved) were administered i.v. 4 hours before same donor G-CSF mobilized PBSCs. To date, 10 children have been treated in two centers. Conditioning depended on underlying disease. No additional GvHD prophylaxis was given. Engraftment and immune recovery was compared to historical controls (n=48).

Characteristics and results are summarized in Table 1. No failure of donor MSC expansion was seen. No acute toxicities were observed during the infusion of MSC's. There was no difference for gender, age, donor type, CD34+ or CD3+ cells infused. Compared to controls (23% graft dysfunction) all patients had 100% documented, sustained engraftment. Hematological recovery of leucocytes was faster ([p=0,008] lymphocyte > neutrophil recovery). Viral reactivations commonly occurred in both patients and controls. Acute GvHD was 12.5% in the patient group compared

to 39.5% of eligible controls. Three patients died, (1 relapse, 2 infection) compared to 17 controls (9 relapse, 5 infection, 3 GvHD). Longer follow-up and immune recovery data are continuing.

MSC use is feasible and safe without infusional toxicities and, as our study seems to suggest, stabilize engraftment. Based on our findings, where graft dysfunction, delayed hematological (immune) recovery compromise the success of stem cell transplant outcome, clinical randomized studies of MSCs are warranted.

Table 1 *Patient characteristics and summary of results*

	PATIENTS (n=10)	CONTROLS (n=48)
Male:Female	**6 (60%): 4 (40%)**	**29 (60%): 19 (40%)**
Age (range)	**8 years (2 – 15)**	**8 years (6 mo –18)**
Malignant	**7 (70%)**	**34 (70%)**
Immune	**2 (25%)**	**6 (13%)**
Benign	**1 (13%)**	**8 (17%)**
Donor Male:Female	**5:3**	**24:24**
CD34 infused	**31.8 (SD 3.1)**	**20 (SD 6.8). NS**
CD3 infused	**0.3 (SD 0.30)**	**0.68 (SD 0.7). NS**
	7/10 (70%)	**31/48 (64%)**
Overall survival	**FU 3-48 mo**	**FU 2-6y**
Days to Leukocyte >1.0 × 109/l	**12 (SD 1.9)**	**18 (SD 3.3) p=0.0005**
Days to Neutrophil >0.5 × 109/l	**13 (SD 1.5)**	**15 (SD 3.6) p=0.14**
Days to Platelets >20 × 109/l	**13 (SD 2.7)**	**21 (SD 14.7) p=0.08**
Days to Reticulocytes >20	**15 (SD 4.9)**	**35 (SD14.9) p=0.04**

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CO-TRANSPLANTATION OF MESENCHYMAL STEM CELLS AND HEMATOPOIETIC STEM CELLS

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Seven patients, 3 leukemics, 2 patients with severe aplastic anemia (SAA) and 2 SCID patients, underwent co-transplantation with mesenchymal stem cells (MSC) and hematopoietic stem cells (HSC). MSC were given in a pilot study to 4 patients and for graft failure and retransplantation to 3 patients. HSCT donors were 3 HLA-identical siblings and 4 unrelated (2 matched and 1 major mismatched unrelated and 1 mismatched unrelated cord blood). Conditioning was myeloablative in 4 patients and RIC in 3 patients. MSC donors were HLA-identical siblings in 3 cases and haploidentical in 4 cases. Passage 2-3 MSC were given at a dose of 1×10^6/kg patient weight within 4 hours of the HSCT graft.

ANC $>0.5\times10^9$/L was reached in a median of 12 (range 10-28) days. Platelets $>30\times10^9$/L was achieved in a median of 12 (8-36) days. All patients had 100% donor chimerism for CD3, CD19 and CD33 within 100 days. Acute GvHD grade 0-I was seen in 5 patients, two patients had grade II one proceeded to chronic GvHD. One patient died of aspergillosis, the other patients are alive and well.

One patient with SAA was on Cyclosporin for Henoch-Schönlein purpura since many years. This and graft failure resolved after retransplantation of MSC + HSC.

Conclusion: MSC co-transplantation resulted in fast engraftment of ANC and platelets and 100% donor chimerism, even in 3 patients regrafted for graft failure/rejection.

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LONG TERM ENGRAFTMENT AFTER ALLOGENEIC TRANSPLANTATION FOR BETA-THALASSEMIA MAJOR: EVIDENCE FOR A ROLE OF CD3 CELLS EARLY AFTER TRANSPLANTATION

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Bone marrow or blood stem cell transplantation is the only cure for β-thalassemia major, however, despite myeloablative conditioning regimens, the basic difficulty is a 15-30% graft failure rate in these patients. Our hypothesis is that there may be a T-cell dependent graft-versus-myelon effect that establishes and stabilizes the engraftment of allogeneic blood stem cells in patients with thalassemia major.

In a first attempt, we assessed the CD3 chimerism early after transplantation in a pediatric case series of six transplantations (2 related, 4 unrelated donors) transplanted from 2001 to 2004 with a minimum follow-up period of 2 years after transplantation. The donors were fully matched at HLA-DRB1 (high resolution) and HLA-A and -B (low resolution). Peripheral blood CD3 chimerism was determined at day +30 (median). Chimerism of unfractionated WBC was determined patients beginning day +13 (median) and repeated until a stable engraftment or graft failure was achieved.

Patients (n = 4) with a CD3 donor chimerism of >80% established a long term engraftment and remained free of erythrocyte transfusions (days +133, +775, +815, +2033). Two of these patients developed a low total chimerism (minimum 42% on day +93, and 45% on day +42, resp.) after that a higher total donor chimerism had been achieved (90% on day +17 and 92% on day +20, resp.). We stopped the immunosuppressive therapy in both patients as soon as the result of the CD3 chimerism was available. Both patients increased their total chimerism up to 98%; one developed GvHD II° of the skin that resolved after steroid therapy without impairment of the total chimerism.

Two patients with a CD3 donor chimerism <10% lost their graft completely, despite the fact, that one patient showed a high total chimerism of 89% on day +6. One patient is again on the erythrocyte transfusion programme, the other engrafted after re-transplant from an unrelated donor.

We conclude that early determination of the CD3 chimerism may be helpful for patient management. In addition, monitoring of the CD3 chimerism should be included in transplant protocols for beta-thalassemia major.

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MESENCHYMAL STEM CELLS FOR TREATMENT OF STEROID-RESISTANT GRAFT-VERSUS-HOST DISEASE

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Background

Allogeneic hematopoietic stem cell transplantation is a well established treatment for hematological malignancies and other conditions which can be associated with significant morbidity and mortality, particularly the development of severe acute graft-versus-host disease (GVHD). Severe steroid-refractory acute GVHD continues to be associated with an 80-90% mortality despite the use of many immunosuppressive agents with differing modes of action. Mesenchymal stem cells (MSC) are rare, non-hemopoietic cells found in the bone marrow, capable of differentiation into bone, cartilage, muscle and fat cells. In addition to their potential role in tissue repair, MSC have unique immunomodulatory properties. In vitro studies demonstrate they are both non-immunogenic and immunosuppressive. These property have been exploited in the treatment of severe aGVHD. LeBlanc et al reported the first case of the successful treatment of steroid-refractory GVHD with MSC in 2004 and in a recently reported EBMT study an overall response rate of 69% was seen in 28 patients with steroid refractory aGVHD. We report our experience with the use of MSC for GVHD in 3 patients over the last 7 months.