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