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Stem cell therapy for inflammatory bowel disease
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CHAPTER 6

Summary, discussion, and future perspectives

SUMMARY

Hematopoietic stem cell transplantation and mesenchymal stromal (MSC) cell therapy are currently under investigation as novel therapies for inflammatory bowel diseases (IBD). Hematopoietic stem cells are thought to repopulate the immune system and reset the immunological response to luminal antigens. MSCs have the capacity to differentiate into a wide variety of distinct cell lineages and to suppress immune responses in vitro and in vivo. Recent results from animal models and early human experience in graft-versus-host disease (GvHD) and also Crohn's disease (CD) suggest that ex vivo expanded MSCs may have clinically useful immunomodulatory effects. The main goal of this thesis was to study the safety, feasibility, and applicability of stem cell therapy in IBD.

Chapter 1 gives an overview on inflammatory bowel diseases and stem cell therapy. This introduction highlights the present knowledge on hematopoietic stem cells and mesenchymal stromal cells in IBD treatment. The focus is on the immunomodulatory characteristics of stem cells and application of these cells in experimental colitis models. Furthermore, an overview on clinical trials with stem cells in IBD is provided.

In **Chapter 2** the long-term (4 to 6 years) outcome of hematopoietic stem cell therapy (HSCT) is presented. Three patients (two male, one female) with active severe Crohn's disease intolerant or refractory to conventional therapies, including anti-TNF-**α** antibodies were planned to undergo autologous HSCT. All three patients successfully completed stem cell mobilization and two of them subsequently underwent conditioning and autologous HSCT with CD34+ cell selection. Treatment was well tolerated, with acceptable toxicity. Five and six years post-transplantation, these patients are in remission under treatment. The third patient went into remission after mobilization and therefore she decided not to undergo conditioning and HSCT transplantation. After a successful pregnancy she relapsed two years later. Since then, she suffers from refractory Crohn's disease for which we are now reconsidering conditioning and transplantation. These observations suggest that autologous HSCT appears to be safe and can be an alternative strategy for Crohn's disease patients with severe and therapy resistant disease.

The aim of the clinical trial in **Chapter 3** was to determine the safety and feasibility of autologous bone marrow derived MSC therapy in patients with refractory Crohn's disease. To this extent ten adult patients with refractory Crohn's disease (eight females/two males) underwent bone marrow aspiration under local anesthesia. Bone marrow MSCs were isolated and expanded and MSCs were tested for phenotype and functionality in vitro. Nine patients received 2 doses of $1\text{-}2\times10^6$ cells/kg bodyweight, intravenously, 7 days apart. MSCs isolated from Crohn's disease patients showed similar morphology, phenotype and growth potential compared to MSCs from healthy donors. Importantly,

immunomodulatory capacity was intact, as these MSCs significantly reduced peripheral blood mononuclear cell proliferation in vitro. MSC infusion was without side effects, besides a mild allergic reaction probably due to the cryopreservant DMSO in one patient. Three patients showed clinical response 6 weeks post treatment, conversely three patients required surgery due to disease worsening. Our data imply that MSCs isolated from Crohn's disease patients have similar characteristics compared to MSCs from healthy donors and that administration of autologous bone marrow derived MSCs appears to be safe and feasible in the treatment of refractory Crohn's disease as no serious adverse events were detected during bone marrow harvesting and administration.

Previous studies showed that immunosuppressive drugs can be harmful to hematopoietic stem cells or endothelial progenitor cell proliferation, thereby affecting their functional capacities. Likewise, immunosuppressive agents used in the treatment of IBD might have an effect on MSC function and could, change the outcome of MSC therapy and affect safety. In **Chapter 4,** we therefore investigated the interaction between MSCs and immunosuppressive drugs frequently used in the treatment of IBD and concluded that in vitro, MSC phenotype and function are not affected by therapeutic concentrations of drugs commonly used in the treatment of IBD. These findings are important for the potential clinical use of MSCs in combination with immunomodulating drugs and anti-TNF-α therapy.

Recent data suggest that resting MSCs do not have significant immunomodulatory activity, but that the immunosuppressive function of MSCs has to be elicited by interferon-gamma (IFN-γ). In **Chapter 5,** we assessed the effects of IFN-γ prestimulation of MSCs (IMSCs) on their immunosuppressive properties in vitro and in vivo. To this end, we pretreated MSCs with IFN-γ and assessed their therapeutic effect in two experimental colitis models in mice. We found that mice treated with IMSCs (but not MSCs) showed a significantly attenuated development of induced colitis. IMSC-treated mice displayed an increase in body weight, lower colitis scores and better survival rates compared to untreated mice. In addition, serum amyloid A protein levels and local proinflammatory cytokine levels in colonic tissues were significantly suppressed after administration of IMSC. We also observed that IMSCs showed greater migration potential than unstimulated MSCs to sites within the inflamed intestine. In conclusion, we show that prestimulation of MSCs with IFN-γ enhances their capacity to inhibit inflammatory responses, resulting in diminished mucosal damage in experimental colitis. These data show that IFN-γ activation of MSCs increases their immunosuppresive capacities and importantly, their therapeutic efficacy in vivo.

DISCUSSION

Despite the improvement in medical therapy of inflammatory bowel disease with the introduction of anti-TNF-α compounds, disease control remains hard to achieve in many patients. Adult stem cells are currently under investigation for a variety of inflammatory disorders. HSCT may be an effective treatment for IBD and can be successfully used as a last resort in an attempt to control debilitating disease. However, it is associated with significant morbidity and mortality related to chemotherapy. The use of

MSCs derived from either bone marrow or adipose tissue could be an alternative approach. If effective, the big advantage of the use of MSCs is the fact that this treatment does not involve conditioning chemotherapy. In the limited number of patients treated with MSCs in the last decade, few adverse events have been attributed to MSC administration. Although acute toxicity appears low, little is known about long-term unwanted side effects. Potential hazards include the possibility of malignant transformation, ectopic tissue formation, and the possible xenogenic transmission of disease and antibody formation when fetal bovine serum (FBS) is added to the culture medium. Furthermore, questions remain to be addressed about the mechanism underlying the immunomodulating properties of MSCs and their in vivo survival after exogenous administration. Discrepancies in MSC isolation, source, and culture protocols, as well as experimental conditions and timing of analysis can explain variation in obtained results and possibly will complicate the interpretation of future trial outcomes.

FUTURE PERSPECTIVES

A multicenter, prospective, randomized phase III study has been initiated by the European Crohn's and Colitis Organisation (ECCO) in collaboration with the European Group for Blood and Marrow Transplantation (EBMT) to evaluate the efficacy of HSC mobilization followed by high dose immune ablation and autologous stem cell transplantation versus HSC mobilization only. In the case of MSC therapy, the Royal Perth Hospital, Australia, has just launched a multicenter phase II trial in 20 patients to evaluate the safety and efficacy of weekly intravenous infusion for 4 weeks with allogeneic bmMSCs. The University Hospital La Paz in Madrid is performing a phase I/IIa trial on allogeneic MSCs derived from fat tissue in the local treatment of recto-vaginal fistula in CD. Finally, the LUMC is currently investigating the safety and preliminary efficacy of allogeneic bmMSCs in the induction of response for active fistulizing CD in a dose escalation study.