

Stromal cells in inflammatory bowel disease : perspectives of local mesenchymal stromal cell therapy

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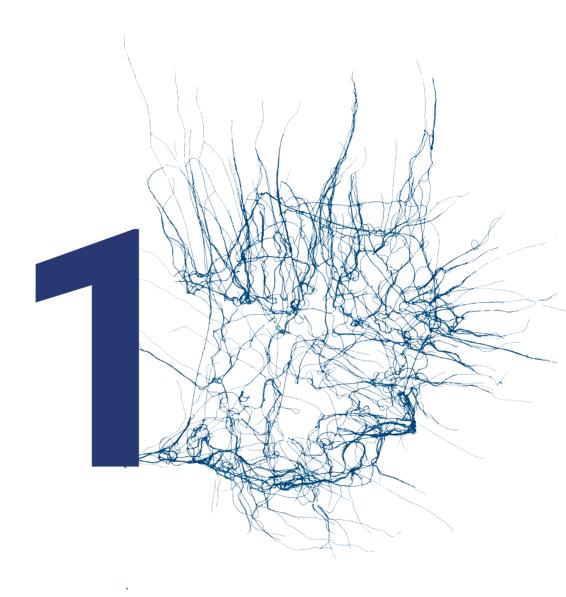


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GENERAL INTRODUCTION

INFLAMMATORY BOWEL DISEASE

Clinical presentation and pathogenesis

Inflammatory bowel disease (IBD) is a chronic disease, characterized by relapsing inflammation of the intestines. Approximately 87,000 patients suffer from IBD in the Netherlands¹ and worldwide the number of patients is increasing due to accelerating frequency in newly industrialized countries². Most patients are diagnosed in the second and third decade of their life and consequently the disease strongly impacts the patients' personal and professional lives. Patients commonly present with abdominal pain, (bloody) diarrhea and fatigue. Although gastrointestinal symptoms are most pronounced in IBD, extra-intestinal manifestations of the disease are present in up to 50% of the patients and include inflammation of the skin, eyes or joints³. Two different forms of IBD are distinguished; ulcerative colitis (UC) and Crohn's disease (CD). Whereas UC is characterized by inflammation of the mucosal layer of the colon, CD is known for its transmural inflammation that may occur in a patchy pattern along the whole gastrointestinal tract⁴⁻⁶. Inflammation in UC is usually seen in the distal colon. The Montreal classification defines UC into ulcerative proctitis (E1; limited to the rectum), left-sided colitis (E2; up to the splenic flexure) and extensive colitis (E3; beyond the splenic flexure)⁷. In CD most often the terminal ileum and colon are affected. For CD the Montreal classification distinguishes ileal, colonic, ileocolonic or upper-isolated disease⁷, with or without perianal disease. Complications of the inflammation in the gastrointestinal tract are common and result in stenosis. abscesses, fistulas or even colitis-associated colorectal cancer.

The exact pathogenesis of IBD is unknown, but it is generally accepted that IBD is the result of an aberrant immune response against the intestinal microbiota in a genetically susceptible person⁸. Genetic polymorphisms in loci encoding molecules involved in cytokine signaling, antimicrobial peptide processing, autophagy and epithelial barrier defense have been shown to increase the susceptibility for IBD^{9,10}. Mutations in interleukin (IL)-10 or its receptor are also associated with early onset IBD¹¹. In addition, environmental factors, such as smoking and a Western-type diet influence the risk of developing IBD^{12,13}. The impaired epithelial barrier, due to defects in tight junctions, autophagy or disturbed mucin production, leads to the influx of pathogens and subsequent disproportional immune responses in IBD patients⁶. Innate immune cells, such as macrophages and neutrophils, take up and respond to exogenous antigens, by producing chemokines and cytokines attracting other immune cells. Antigen presenting cells in the intestines, for example dendritic cells, then present processed exogenous antigens to lymphocytes, mainly T and B cells, in the lymph nodes, lamina propria or Peyer's patches and thereby activate them. So both innate mucosal cells (neutrophils, natural killer cells, innate lymphoid cells, dendritic cells and macrophages) and adaptive immune cells (T and B cells) play an important role in the pathogenesis of IBD. Among T cells various subsets are known of which helper (Th) CD4+ and cytotoxic CD8+ T cells are the most common. CD4+ Th cells can further differentiate into Th1, Th2, Th17, Th9, Th22, T follicular helper and regulatory T (Treg) cells, based on their specific production of cytokines^{14,15}. CD is recognized as a Th1 and Th17 driven disease, whereas in UC mainly Th2 and Th17 cells are found¹⁶. Also IL-9 producing Th9 cells, that suppress the proliferation of epithelial cells, are frequently found in the inflamed mucosa of UC patients¹⁷. Besides more pro-inflammatory immune cells, patients with IBD seem to have fewer anti-inflammatory cells, such as IL-10 producing Th cells¹⁸.

Next to the importance of the epithelial barrier and the immune system in the pathogenesis of IBD, recently published articles revealed major changes in the stromal compartment of the inflamed gut in IBD¹⁹⁻²². The current knowledge on the contribution of stromal cells in the pathogenesis of IBD is reviewed in **Chapter 2** of this thesis.

Perianal fistulas

A serious and often persistent complication of CD, occurring in nearly one-third of the patients, is the development of perianal fistulas^{23,24}. Perianal fistulas are tracts that connect the intestinal lumen, usually from the anal canal or rectum, with the perianal skin. The pathogenesis of perianal fistulas involves the interaction between microbial factors with persistent inflammation and failure of wound healing responses. The formation of a fistula tract is furthermore supported by epithelial-to-mesenchymal transition, a process where epithelial cells develop a mesenchymal phenotype and are able to migrate and penetrate in adjacent tissues²⁵. Associations between fistulizing CD and specific mutations in the nucleotide-binding oligomerization domain-containing protein 2 and the IBD5 gene locus, both involved in bacterial handling, and the immunity-related GTPase family M protein, that plays a role in autophagy, have been shown^{26,27}. Perianal fistulas can also occur in patients without CD. These so-called cryptoglandular fistulas are thought to have a separate etiology since they arise from the anal gland²⁸.

Differences in tract anatomy distinguish 'simple' and 'complex' perianal fistulas in CD²⁹. Simple fistulas originate low in the anal canal and have a single external opening, without the presence of an abscess, rectovaginal fistula or anorectal stricture. All other fistulas are considered complex fistulas, that are often high intersphincteric or trans-sphincteric fistulas or have an extrasphincteric or suprasphincteric route. Simple fistulas are more likely to heal compared with complex perianal fistulas³⁰. Fistulas are associated with a strongly impaired quality of life, due to fecal incontinence, perianal pain and recurrent infection.

THERAPEUTIC STRATEGIES

Treatment of luminal disease

Currently. IBD cannot be cured by medication and requires sophisticated lifelong therapy and life style changes to prevent progression of the disease. The first goal of medical therapy is to bring the disease in remission, and later on to preserve this remission. Local therapy with topical mesalazine or steroids is used in patients with UC when inflammation is limited to the splenic flexure (E1-2), or as additional therapy in patients with more extensive disease. When the disease is (also) affecting proximal parts of the colon, systemic therapy with mesalazine, steroids, immunosuppressants, biologicals or small molecules is given. Besides anti-tumor necrosis factor (TNF)-a therapy, biological therapy includes anti-a4β7 integrin and anti-p40 therapy³¹. The JAK-inhibitor tofacitinib is the first small molecule approved for the treatment of UC³¹. All these therapies interfere with the immune cascade in order to dampen the aberrant immune response. These lifelong therapies have some side-effects, such as an increased risk of opportunistic infections and malignancies. Furthermore, they only induce remission of the disease in a subset of patients. Next to that, most medical therapies for IBD are expensive and thereby place a burden on the health system. When patients are refractory to these medical therapies surgery is needed to remove the inflamed or stenotic intestine

Treatment of perianal fistulas

Perianal fistulas in CD are treated with a combined medical and surgical approach. The cornerstone of surgical treatment is placement of a non-cutting seton to drain the fistula and prevent abscess formation. Unfortunately, this will also prevent the fistula from closure. Surgical fistula closure is only possible when rectal inflammation is limited and can be performed by a fistulotomy³², creation of a mucosal advancement flap that covers the internal opening³³ or by ligation of the fistula between the internal and external sphincter³⁴. The optimal medical approach is the use of anti-TNF- α therapy, that shows significantly improved fistula closure rates in CD in randomized controlled trials³⁵⁻³⁷. However, in the end, only 37% of the patients with complex perianal fistulas show fistula closure after a median follow-up of 10 years using combined medico-surgical therapies³⁰. Fecal diversion, by the creation of a colostomy, is considered one of the last treatment options for patients that do not respond to standard therapy³⁸. For both luminal IBD and CD-associated perianal fistulas new therapeutic strategies are being developed, including local transplantation of mesenchymal stromal cells (MSCs).

MESENCHYMAL STROMAL CELL THERAPY

MSCs are generally defined as plastic-adherent stromal cells, that express a distinct set of surface markers, such as CD73, CD90 and CD105, while lacking immune and endothelial cell markers such as CD45, CD11b and CD31³⁹. Furthermore, MSCs have ability to differentiate in vitro into osteogenic, adipogenic and chondrogenic lineages³⁹. Despite these definition criteria, the term 'MSCs' still covers a heterogenous group of cells. Initially, MSCs were isolated from the bone marrow, but currently they can be isolated from a wide variety of tissues including adipose tissue, umbilical cord, placenta tissue and many others. Both autologous and allogeneic MSCs, derived from either the patient self or a donor respectively, have been used in clinical trials. Almost twenty years ago the first patients were treated with cultured autologous bone marrow-derived MSCs to support autologous bone marrow transplantations⁴⁰, since MSCs were found to support the hematopoietic stem cell niche. Nowadays, the interest in these cells results from their unique immunomodulatory properties and tissue regenerative capacities⁴¹. Therefore, in vitro expanded MSCs have been studied in several immune-mediated diseases, such as graft-versus-host disease, rejection after kidney transplantation, systemic lupus erythematosus and IBD. The number of diseases in which MSC-therapy is tested is not in proportion to the current knowledge about the working mechanism of the 'MSC-product'. MSCs were initially described as 'immuno-privileged', indicating that they will not induce a host immune response. Later results showed, however, that major histocompatibility complex-mismatched MSCs can be detected by the host immune system, leading to donor-specific antibodies after allogeneic MSC-therapy^{42,43}, although transfusion reactions have not been reported in clinical trials^{44,45}.

Mesenchymal stromal cells in inflammatory bowel disease

Exploration of MSCs as a novel therapy for IBD started in murine models for colitis. *In vivo* studies showed that systemic application of MSCs, both through intravenous or intraperitoneal injections was able to alleviate experimental colitis⁴⁶⁻⁴⁸. Although both *in vitro* and *in vivo* studies showed promising results, systemic MSC-therapy is not being used in today's clinical practice. Several clinical phase I/II trials have been conducted in IBD, showing that systemic MSC-therapy is safe, but without conclusive results in terms of clinical efficacy⁴⁹⁻⁵². For other diseases, systemic MSC-therapy did not meet its expectations as well. In steroid-refractory graft-versus-host disease a clinical phase III study with the commercial MSC-product Prochymal® failed to demonstrate increased remission rates after intravenous MSC-therapy. Due to disappointing clinical response rates after systemic administration, new administration routes for MSCs were explored for IBD. In 2015, our group published the first placebo-controlled randomized clinical trial on the treatment of CD-associated perianal fistulas with locally injected, allogeneic bone marrow-derived MSCs⁵³. Higher rates of fistula closure were found in patients treated with

MSCs compared with placebo. These promising results were confirmed in a multicenter trial using local injections with Cx601/darvadstrocel, a commercial product consisting of allogeneic adipose tissue-derived MSCs^{42,54}. In 2017, the European Medicines Agency approved darvadstrocel for the treatment of CD associated perianal fistulas, a breakthrough in MSC research.

Mechanisms of action by MSCs

MSCs are known for their immunomodulatory and tissue regenerative properties. MSCs are able to inhibit T, natural killer and B cell proliferation, to stimulate the differentiation of Treg cells and to inhibit the differentiation of monocytes into dendritic cells *in vitro*⁴¹. The secretion of paracrine factors (such as cytokines and chemokines) is recognized as the primary mechanism by which MSCs regulate immune cell function and tissue healing. Additionally, cell-cell contact, the release of exosomes and microRNAs are thought to be involved in the therapeutic effects of MSCs. It is known that MSCs gain many of their immunomodulatory properties through contact with pro-inflammatory cytokines. For example, stimulation with interferon-γ induces 2,3-idoleamine (IDO) production in MSCs, that increases MSCs' ability to inhibit T cell proliferation and subsequently experimental colitis^{46,55}. Furthermore, there is evidence that intravenously injected MSCs undergo apoptosis *in vivo*, resulting in an immunosuppressive phenotype of the phagocytes that removed them⁵⁶⁻⁵⁸. Whether the tissue regenerative and anti-inflammatory features of viable MSCs or the immunomodulating effects of 'apoptotic' MSCs are most important for the clinical effects of MSC-therapy is not clear yet⁵⁹.

THESIS OUTLINE

The main goal of this thesis was to gain insight into the role of stromal cells in IBD and to assess the therapeutic potential of local MSC-therapy. In **Chapter 2**, the role of stromal cells in the pathogenesis of IBD was reviewed. We discussed the recent insights in the function of stromal cells, mainly fibroblasts, in the healthy and inflamed gut and focused on their effects on epithelial and immune homeostasis. Furthermore, the therapeutic strategies to target or replace the pathogenic stromal cell population in the inflamed intestine were reviewed. **Chapter 3** addressed the characteristics of fibroblasts found in perianal fistulas in patients with CD, with a focus on their role in immunoregulation. In this chapter, fibroblasts derived from fistulas were also compared with MSCs, as this is a new stromal therapy for CD-associated perianal fistulas. In **Chapter 4**, the long-term safety and efficacy data of our phase II clinical trial on local MSC therapy for the treatment of perianal fistulas was described. One of the patients included in this trial developed an Epstein-Bar virus-positive lymphoproliferative disease in the rectum during the follow-up time. Its potential relation

with MSC-therapy was studied as described in Chapter 5. To further explore application of local MSC-therapy in IBD, we investigated different routes of administration of MSCs in a dextran sodium sulfate (DSS) mouse model for colitis. In Chapter 6, administration of MSCs through an enema in DSS-induced colitis was evaluated and in **Chapter 7** the effects of direct local injection of MSCs in the inflamed colon during endoscopy were described. The efficacy of the two administration routes and potential working mechanisms, including their influence on local immune responses and epithelial repair, were revealed. Furthermore, we also looked for the optimization of local MSC-therapy by the aggregation of MSCs into spheroids. To elucidate the working mechanism of local MSC-therapy further, the effects of MSC-derived exosomes, small vesicles produced by MSCs, were studied on epithelial regeneration, as described in Chapter 8. Since the therapeutic effects of MSCs are hypothesized to be highly dependent on the local pro-inflammatory environment they encounter, the phenotype and function of human MSCs were assessed after stimulation with various cytokine mixtures as described in Chapter 9. Finally, in Chapter 10, the summarizing Discussion, the overall potential of the findings described in this thesis was discussed, also in the light of our ongoing phase II clinical trial on the safety and feasibility of endoscopically injected MSCs in patients with proctitis.

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General introduction