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Title: Mesenchymal stromal cell therapy for Crohn's disease: from perianal fistulizing disease to experimental colitis
Issue Date: 2016-03-15
CHAPTER 1
INTRODUCTION
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2014 Jun;28(3):505-518
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INTRODUCTION

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CROHN’S DISEASE

Crohn’s disease (CD) is a chronic disease characterized by idiopathic inflammation of the gastrointestinal tract. An inappropriate immune response to extracellular pathogens in the gut in a genetically predisposed host, e.g., NOD2/CARD15 gene-associated, is thought to be the cause of CD.\textsuperscript{1, 2} However, the exact etiology remains unclear. The incidence of CD has classically been higher in developed countries; however, urbanization and modernization of undeveloped countries seem to increase the incidence of CD to a similar level as the developed countries.\textsuperscript{3} The peak age of onset for CD is 15 – 30 years, with a second peak between the ages of 50 to 80.\textsuperscript{4} There is no gender specificity. A systematic review of population-based cohorts estimated the prevalence of CD to be 26.0 to 198.5 cases per 100,000 persons and the incidence to be 3.1 to 14.6 cases per 100,000 persons/years in North America.\textsuperscript{5} In Europe the incidence of CD is 6.0 to 15.0 cases per 100,000 persons/years and the prevalence 50.0 to 200.0 cases per 100,000 persons.\textsuperscript{6}

CD can affect any part of the intestine, from mouth to anus, but preferentially involves the ileum and colon. Characteristic for CD are skip lesions; inflamed parts of the intestine interspersed by apparent normal healthy tissue. Patients with CD can suffer from diarrhea (with blood or mucus), abdominal pain, fever, weight loss, nausea, vomiting and fatigue.\textsuperscript{7} The diagnosis of CD is established by the clinical features confirmed by endoscopy. Biopsy specimens from inflamed gut mucosa typically show transmural inflammation, including submucosal oedema, ulcerations and fibrosis.

PERIANAL FISTULAS

Frequent manifestations of CD are the development of abscesses, stricture formation, intestinal obstruction, and fistulas.\textsuperscript{13-7, 8} Fistulas are abnormal connective passages from the epithelial lining of the intestines to another organ or to the skin caused by inflammation. Fistulas in CD are a major problem which can result in considerable morbidity. Approximately 35\% of all CD patients have at least one fistula episode. A quarter of the fistulas is between two parts of intestine, 9\% is rectovaginal and 13\% is different, including fistulas between the intestine and bladder and around a stoma.\textsuperscript{9} In case of an enterovesical fistula there may be recurrent polymicrobial urinary tract infections, pneumaturia and fecaluria. When a rectovaginal fistula develops, dyspareunia, malodorous vaginal discharge and recurring episodes of vaginitis can occur. Perianal fistulas are the most common type of fistulas in fistulizing CD. The cumulative incidence of perianal fistulas was estimated at 23\%-26\% after 20 years of CD.\textsuperscript{9, 10} Patients with perianal fistulas can present with symptoms such as
constant anal pain or pain after defecation, (painful) swelling around the anus, continuous (malodourous) discharge of pus and/or blood from the external opening with skin irritation around the anus, fever and even incontinence. In 20-45% of the CD patients a perianal fistula developed before or at the time of diagnosis CD. Patients with colonic and active rectal disease have more frequently perianal fistulas compared to patients with isolated ileal or ileocolonic disease. Male gender, age at diagnosis of CD and smoking are other risk factors, although data are conflicting. The formation of perianal fistulas in CD is based on the presence of a penetrating ulcer in the rectal or anal mucosa resulting in an abnormal granulating connection between the epithelial lining of the rectum or anal canal and the perianal skin. However, most perianal fistulas are cryptoglandular fistulas (90%) and are not associated with CD. They originate from the intersphincteric anal glands due to a local infection with abscess formation. Normally the internal sphincter is a barrier for bacterial overgrowth, only chronic infection, CD inflammation or local trauma can cause abscesses beyond this barrier in the intersphincteric space. When such an abscess increases it will usually drain in two ways: it can either drain through the intersphincteric space downwards to form a transsphincteric fistula erupting into the perianal skin or it can overcome the external sphincteric plane into the ischiorectal fossa resulting in a transssphincteric perianal fistula. In CD these abscesses are often a complex delta of channels and patients might present with large or multiple abscesses.

The exact etiology of perianal fistulizing CD remains unclear. However, genetic, microbiological and immunological factors seem to play important roles. The risk haplotype of the carnitine/organic cation transporter (OCTN) on the IBD5 locus (5q31) is associated with penetrating and perianal CD due to altered bacteria killing resulting in inflammation. Furthermore, diminished clearance of intracellular pathogens by autophagy caused by a specific polymorphism in the immunity-related GTPase family M (IRGM) gives an increased risk of penetrating and perianal CD. It is hypothesized that microbiota also contribute to the development of perianal fistulas as fecal diversion leads to long-term improvement. Especially gram-positive microorganisms are present in CD perianal fistulas.

**Diagnosis of perianal fistulas**

Knowledge about the exact route and internal opening of the fistula, its relation to the sphincters and the presence of abscesses, is crucial in the management of perianal fistulizing CD. Inspection of the perianal area is the first step in this process. The fistula disease activity can be quantified with the Perianal Disease Activity Index (PDAI) and comprises five categories: discharge, pain/restriction activities, restriction of sexual activity, type of
perianal disease and the degree of induration (all 0 – 4 points). Pelvic magnetic resonance imaging (MRI; figure 1) is accurate in determining the exact route of the fistula, differentiating between a fibrotic and septic fistula and locating abscesses.\textsuperscript{26-29} Introducing an endoanal coil receiver results in more detailed images of the location of the internal opening, the extent of the fistula tract and its relation with the sphincters.\textsuperscript{30, 31} Anorectal endoscopic ultrasound (EUS) requires expertise, but can be equivalent to pelvic MRI and is less expensive and time consuming.\textsuperscript{32, 33} Examination under anaesthesia (EUA) has the advantage of the possibility of concomitant drainage of abscesses and placement of non-cutting setons. The presence of related abnormalities are not detectable with digital examination (e.g., high abscesses and sinuses). In addition, MRI predicts patient outcome better than solely EUA.\textsuperscript{34, 35} The random combination of two of three methods (MRI, EUS and EUA) resulted in a 100\% correct classification of perianal fistulizing CD.\textsuperscript{36} Independent of the diagnostic method used, proctosigmoidoscopy should be performed to assess whether the rectum and/or sigmoid is inflamed since active inflammation influences therapy choice and outcome. Nowadays, the relative simple Parks classification\textsuperscript{37} identifying four types of

\textbf{Figure 1} Pelvic magnetic resonance imaging (MRI) of a perianal fistula. Left: Transversal image of a perianal fistula (arrow) after introduction of an endoanal coil receiver. Right: A coronal image of a perianal fistula (arrows). The fistula tract shows a high signal intensity indicating active disease.
perianal fistulas [intersphincteric, transsphincteric (figure 2A), suprasphincteric and extrasphincteric (figure 2B)] is often used to describe a perianal fistula. This Parks classification is also useful for CD, although more (complicated) tracts can occur in a CD patient. Furthermore, this system does not include other perianal manifestations of CD (e.g. abscesses or strictures). In clinical practice the ‘simple’ or ‘complex’ classification combining physical inspection of the perianal area and endoscopy to determine rectal inflammation, is mostly used. A simple fistula has its origin low in the anal canal (superficial, low intersphincteric or low transsphincteric) and has a single external opening without evidence of a perianal abscess, a recto-vaginal fistula.

**Figure 2** Parks classification of perianal fistulas. (A): An intersphincteric fistula situated between the internal and external sphincter and a transsphincteric fistula passing the internal and external sphincter into the ischiorectal fossa. (B): A suprasphincteric fistula passing through the external sphincter above the puborectalis muscle into the ischiorectal fossa and an extrasphincteric fistula with its origin above the puborectalis draining through the pelvic floor into the ischiorectal fossa without passing the internal or external sphincter. Figure reproduced with permission from Schouten et al.
or an anorectal stricture. A complex fistula is high intersphincteric, high trans-sphincteric, extrasphincteric or suprasphincteric and may have multiple external openings. They may be associated with a rectovaginal fistula, an anorectal stricture, active rectal disease at endoscopy and pain or fluctuation suggesting a perianal abscess.

**Treatment of perianal fistulas**
Treatment of perianal fistula is often indicated. Apart from the inconvenience of discharge from an untreated fistula, development of abscesses, sepsis, incontinence and carcinoma have been described. Both medical and surgical treatments are available.

**Medical treatment**
*Antibiotics*
Antibiotics such as ciprofloxacin and metronidazole are broadly used as first-line treatment of perianal fistulas; however, only one randomized, double-blind, placebo-controlled pilot study was published to evaluate the safety and efficacy of ciprofloxacin and metronidazole as a treatment for active perianal fistulizing CD. No significant differences were found after 10 weeks of treatment. In addition, re-exacerbations were common after discontinuation of these treatments. Monotherapy with antibiotics is therefore not considered to induce complete healing of perianal fistulas.

*Immunosuppressants*
An open-label study from 2003 showed that perianal fistulas treated with the immunosuppressant azathioprine in combination with 8 weeks of antibiotics responded significantly more often than fistulas treated with antibiotics alone. In addition, immunosuppression with azathioprine and 6-mercaptopurine solely has been shown to be effective in the treatment of perianal fistulas (54% response in comparison to 21% in placebo group). In patients with previous failure or intolerance to 6-mercaptopurine methotrexate seemed an effective option with a 44 - 56% response rate. However, the recurrence rate was high when the dose of methotrexate was tapered or changed to oral administration.

*Anti-TNFα agents*
Several trials clearly demonstrated the benefit of the anti-tumor necrosis factor alpha (anti-TNFα) agents infliximab, adalimumab and certolizumab pegol for the induction and maintenance of remission in perianal fistulizing disease. However, a meta-analysis reported no statistically significant difference in the relative risk of fistulas remaining unhealed with anti-TNFα agents versus placebo. On exclusion of short-term follow-up
results, the effect of anti-TNFα on fistula healing became significant. Unfortunately, in a retrospective analysis the 5-year probability of recurrence of a perianal fistula that was initially healed with infliximab therapy, was estimated at 40.1%. When infliximab was combined with ciprofloxacin a trend to higher response compared to infliximab alone was observed. In the ADADI trial patients with perianal fistulizing CD were treated with adalimumab monotherapy or adalimumab plus ciprofloxacin. After 12 weeks, the treatment with ciprofloxacin was stopped. At that point, a significant higher response and remission rate was reported in the combination group. However, at week 24 no significant difference in fistula healing between the two treatment groups was found. Another possibility is to inject anti-TNFα locally in the fistula tract. In three studies a small number of patients were open-label treated with multiple local injections of infliximab without any reported adverse events of the treatment. The remission rate varied among the studies with a sustained remission between 36.4% and 87.5% after approximately 1 year. However, in the latter study, local injection of infliximab was combined with fistulectomy. Local injections with adalimumab as a treatment for perianal fistulizing CD also appeared to be safe with remission rates of 75 – 77.8%. No relapse was observed after a mean follow-up time of 17.5 months in one study. Because the results of locally injected anti-TNFα agents are encouraging, it would be worth it to set up randomized controlled studies to evaluate the efficacy of these local treatments for perianal fistulizing CD.

Vedolizumab
Recently Sandborn et al. reported the results of the first induction and maintenance trials of vedolizumab, an α4β7-integrin antibody. Although the number of patients with fistulas at baseline in this trial was quite low (165/1115; 14.8%) and the number of patients available for evaluation at week 52 even lower (n = 57), vedolizumab every 8 weeks resulted in a significant higher closure rate (41.2%) compared to placebo (11.1%) (p = 0.03).

Surgical treatment
Before elaborating on the optimal surgical treatment technique, it has to be stated that a conservative surgical approach is warranted in most cases: aggressive surgery may result in outcomes that are worse than the CD itself. Fecal incontinence is a feared complication and may occur even after partial sphincter division. Concomitant proctitis has to be taken into account, and therefore, optimal medical therapy to control disease activity is paramount before embarking on surgery.
Fistulotomy of a superficial perianal fistula. (A): The fistula is explored with a probe to find both openings and (B): is opened to heal by granulation. Figure reproduced with permission from Bemelman from van Koperen et al.62

Non-cutting seton in a perianal fistula to assure drainage and to promote fibrosis of the fistula tract.

Mucosal advancement flap (MAF)

When rectal inflammation is limited, the creation of a MAF to cover the internal opening is a good option. This technique can also be applied in case of a rectovaginal fistula with success rates of 54% to 71% in two retrospective series.63, 64 The mucosa and submucosa and even sometimes the muscle is mobilized and then advanced over the internal opening. This technically demanding procedure is known be successful in experienced hands in even up to 71% of CD patients. 65 The majority of the MAF- reports are, however, limited to patients with cryptoglandular disease, obscuring its clinical value in CD patients. Incontinence may occur in 13% and 9%, respectively.66

Fibrin glue

The success rates of sealing the fistula tract with a mix of both fibrin and thrombin are varying. Fibrin can be retrieved from autologous blood but commercial fibrin adhesives are available as well. Optimistic reports on patients with cryptoglandular disease disclose success rates from 68% to 85% at one year. 67, 68 Results in CD are less favourable: a randomised trial including 77 patients with moderate disease were randomised between observation after seton removal and fibrin glue administration.69 After 8 weeks, 38% of the glue patients experienced disease remission whereas only 16% did in the observation group.

Fistula plug

Fistula plugs consist of inert porcine intestinal submucosa that is known to avoid inflammatory reaction after implantation due to its inert nature. After 3 months the plug is populated with patient’s endogenous cells.70, 71 In patients with CD healing rates were 54%72 without affecting fecal continence.73 A prospective study was conducted on 73 patients with...
**Fistulotomy and non-cutting setons**

The most simple and classical surgical treatment for perianal fistulas is to open the fistula tract widely by fistulotomy and to let the wound heal by granulation (figure 3). Fistulotomy in case of simple superficial fistula is successful in up to 80-100% of the cases.\(^9\) Fistulotomy is not preferred in case of a trans- and extrasphincteric fistula as a part of the sphincters are cut during surgery. For these fistulas a non-cutting seton for initial drainage can best be placed (figure 4). This seton will drain the fistula tract and reduce the local inflammation. If the inflammation has diminished, the seton can be removed. However, the majority of the patients need additional surgical therapy, especially when optimal medical treatment does not appear to prevent disease recurrence. Therefore, seton drainage is often a bridge to a more definite surgical treatment.

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anorectal fistulas of differing etiologies. Only 8 CD patients were included of which 4 patients (50%) were successfully managed by plug insertion. Fistula plugs were compared with MAF in two randomized studies, however no CD patients were included. One trial reported poor healing rates in patients with plug insertion (29%) being not statistically different from patients who received an advancement flap (48%). The other trial was stopped early due dramatic performance of the plug (only 20% success), being much worse than the advancement flap group (88%). Plug protrusion shortly after surgery is the predominant cause of treatment failure.

**Ligation of the intersphincteric fistula tract (LIFT)**
This rather new technique was launched by Rojanasakul in 2009 and consists of dissection between the internal and external sphincter up to the level of the fistula tract. There, the fistula is ligated and the rest of the external tract is curetted. The (theoretical) advantage of LIFT over MAF is the complete preservation of sphincter function. LIFT is an emerging technique that has proved to be effective in 57 to 83% of patients with cryptoglandular disease who had previous unsuccessful treatment. There is no randomized comparison with other techniques yet. A small series of 15 CD patients was recently published showing a LIFT healing rate of 67% at 12 months of follow-up without any development of fecal incontinence.

**Fecal diversion**
Only if other options have proved to be ineffective, the construction of a stoma can ameliorate symptoms related to perianal fistulas. Patients should know that many of these stomas, although often considered as a temporary measure, turn out to be definite.

**Proctectomy**
If all treatments fail, proctectomy may be considered. These patients suffer from debilitating abscess formation, colonic disease, complex high fistulas and/or anal stenosis. Unfortunately, also proctectomy has the risk of bad wound healing and perianal sinus development in almost 50% of the cases.

**Outcomes of treatment**
Although a range of medical and surgical options is available nowadays, the treatment of perianal fistulas is still challenging. Achieving complete closure of the fistula tract is a long process with in many cases multiple relapses. Spontaneous closure of complex fistula in CD is rare, though it has been reported that a simple transsphincteric fistula has a spontaneous
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**Mesenchymal stromal cells**

A new experimental approach to the treatment of perianal fistulas in CD is cellular therapy with mesenchymal stromal cells (MSCs). MSCs are non-hematopoietic precursors of connective tissue cells with immunomodulatory and tissue regenerative properties, making them a potential therapeutic option for inflammatory disorders, including fistulizing CD. Apart from the bone marrow, MSCs have been isolated from several other tissues, such as adipose tissue, peripheral blood, umbilical cord blood and placenta. According to the minimal criteria proposed by the International Society for Cellular Therapy, MSCs should be identified based on their ability to adhere to plastic in standard culture conditions and on their ability to differentiate *in vitro* into bone, fat and cartilage. Human MSCs must express CD73, CD90 and CD105 and may not express CD11b or CD14, CD19, CD45, CD79α and HLA-DR surface molecules.

MSCs are thought to be immunologically relatively inert since they are poor antigen presenting cells (APCs) and do not express MHC class II or co-stimulatory molecules. In accordance, expanded MSCs do not stimulate T cell proliferation in mixed lymphocyte reactions (MLRs) and are also able to downregulate alloreactive T cell responses when added to mixed lymphocyte cultures. These findings suggest that allogeneic MSCs could be used in the clinic and their expansion potential provides the possibility to generate a stock with ‘off the shelf’-treatment potential.

**Immunomodulatory capacities of MSCs**

MSCs have been shown to alter cytokine secretion profiles of dendritic cells (DC), naïve and effector T cells, and natural killer (NK) cells, which is accompanied by the induction of a more anti-inflammatory or tolerant phenotype. Allogeneic human MSCs (hMSCs) have a reversible inhibitory effect on the differentiation of monocytes into dendritic cells (DCs) and are able to down-regulate the expression of the costimulatory molecules CD80 and CD86 in the case of mature DCs. DCs that are cocultured with MSCs before adding them to T cells, show a reduced ability to activate these T cells to proliferate. Furthermore, Aggarwal et al. showed a significant decrease of 50% in TNF-α secretion in response to lipopolysaccharides (LPS) when type 1 DCs are cocultured with hMSCs. Interestingly, when type 2 DCs are cocultured with hMSCs, the percentage of
the anti-inflammatory cytokine interleukin (IL)-10 increases with 140% upon LPS stimulation compared to type 2 DCs cultured without hMSCs. These results suggest that hMSCs cocultured with matured DCs provide a more anti-inflammatory milieu in vitro.

In the same paper, the interaction between MSCs and T cells is described. Naïve T cells were activated to differentiate into T helper cell type 1 (Th1) or T helper cell type 2 (Th2) in the presence or absence of hMSCs. A significant decrease of 60% in levels of interferon-γ (IFNγ) was observed when hMSCs were present during the differentiation into Th1 cells compared to differentiation without hMSCs. The average increase in the amount of IL-4 in the presence of hMSCs during this differentiation process into Th2 cells was 500%, suggesting that hMSCs provide significant help for naïve T cells to differentiate into Th2 cells. Besides Th1 and Th2 cells, a subset of Th cells that produces high levels of IL-17 exists. These so-called Th17 cells protect against extracellular pathogens at mucosal surfaces and are thought to play an important role in inflammation and tissue damage in autoimmune diseases such as CD. During the differentiation of naïve T cells into Th17 cells, the presence of MSCs inhibits the production of inflammatory cytokines and slightly induces the production of IL-10 and concomitantly strongly enhances the expression of FoxP3 mRNA levels. The induction of FoxP3 mRNA expression gives rise to a functional regulatory T cell (Treg).103

NK cells are cytotoxic effector cells of the innate immune system that play a key role in the elimination of virally infected or transformed cells. Upon stimulation with IL-2, purified NK cells produce IFN-γ and when these stimulated NK cells are subsequently cocultured with hMSCs, the levels of secreted IFN-γ decreases with 80%.99 Although MSCs are able to alter cytokine secretion profiles of different immune cells in order to induce a more anti-inflammatory or tolerant phenotype, the question remains, what are the mechanisms by which MSCs exert this biological activity? Several studies suggest that MSC-derived soluble factors may contribute to this induced immunosuppression.95-98 Prostaglandin E2 (PGE2) is one of the immunosuppressive molecules produced by MSCs when activated by inflammatory cytokines such as IFN-γ and TNF-α.99 Intestinal lamina propria cells constitutively produce COX-2-dependent PGE2, which suggests that the expression of inflammatory mediator COX-2 by lamina propria stromal cells contributes to the low immune response against antigens in the mucosa of the small intestine.104 The ability of MSCs to inhibit Th17 differentiation and to induce a regulatory phenotype is strongly believed to be mediated by the COX-2-dependent soluble factor PGE2.105 In an experimental arthritis model secreted PGE2 was the main factor in reducing the inflammation locally whereas systemical immune suppression by MSCs was mediated by the switch of the inflammatory Th1/Th17 profile towards a more Th2 response.106 In addition, in a TNBS-
induced colitis model PGE₂ produced by MSCs played an important role in suppressing the inflammation by inhibiting Th1 cell proliferation and inducing Tregs.\textsuperscript{107}

Nitric oxide (NO) is an important signaling molecule and is involved in tissue homeostasis and (immuno)regulatory functions. Inducible nitric oxide synthase (iNOS) is expressed by murine MSCs as a result of stimulation with IFN-\(\gamma\) combined with TNF-\(\alpha\), IL-1\(\alpha\) or IL-1\(\beta\).\textsuperscript{108}

Immunosuppression is achieved when MSCs release high levels of NO, but not when MSCs derived from iNOS\(-/\)- or IFN\(\gamma R1^{-/-}\) mice are used. In addition, only wild-type MSCs but not iNOS\(-/\)- or IFN\(\gamma R1^{-/-}\)-MSCs prevented graft-versus-host disease and delayed-type hypersensitivities in mice. Interestingly, iNOS\(-/-\)-MSCs even worsened this delayed-type hypersensitivity.\textsuperscript{108}

Substantiating this theory, a recently published paper on the therapeutic effect of aspirin in TNBS-induced colitis demonstrated that NO-releasing aspirin accelerated colonic healing characterized by a downregulation of COX-2, iNOS, IL-1\(\beta\) and TNF-\(\alpha\) mRNAs.\textsuperscript{109}

The mechanism of MSC-mediated immunosuppression is different in mice and humans. While murine MSCs use iNOS to control immune responses, human MSCs utilize indoleamine 2,3-dioxygenase (IDO), an immunoregulatory enzyme regulating tryptophan levels, and express only very low levels of iNOS.\textsuperscript{110} However, both mouse MSCs and human MSCs need IFN-\(\gamma\), combined with TNF-\(\alpha\), IL-1\(\alpha\) or IL-1\(\beta\), in the induction of the suppression of immune cells such as T cells and NK cells through these enzymes.\textsuperscript{111, 112}

Whether or not MSCs need to be in contact with immune cells to release soluble factors and influence those cells is still under debate. Inhibition of T cell proliferation by MSCs was abolished when these cells were separated by transwells.\textsuperscript{110} On the other hand, intraperitoneally injected MSCs alleviated dextran sulphate sodium (DSS)-induced colitis by forming spheroids in the peritoneal cavity that produced the anti-inflammatory protein TNF-stimulated gene 6 (TSG-6). Fewer than 1% of the injected MSCs reached the inflamed colon in this study, thereby contradicting the need of MSCs to migrate to the site of inflammation to attenuate inflammation.\textsuperscript{113}

**CLINICAL TRIALS IN PATIENTS WITH PERIANAL FISTULIZING CD**

In the past years several reports on clinical trials using MSCs derived from bone marrow or adipose tissue as a treatment for perianal fistulizing CD have been published.\textsuperscript{114-117} A phase I trial in which 9 fistulas were injected with autologous MSCs derived from adipose tissue, demonstrated 75% healed fistulas after 8 weeks without the occurrence of serious adverse events.\textsuperscript{114} This trial was followed by a phase II study of the same group\textsuperscript{115} in which they included 49 adult patients with complex cryptoglandular \((n = 35)\) and CD \((n = 14)\) perianal fistulas. Treatment consisted of either local application of fibrin glue or fibrin glue plus 20 million autologous MSCs derived from adipose tissue. Evaluation took place 8 weeks after
the treatment and in case of no healing, a second dose of fibrin glue or fibrin glue plus 40 million MSCs was injected. Although the majority of the patients had perianal fistulas based on cryptoglandular disease, overall healing of the fistulas was observed in 16% of the patients who received only fibrin glue versus 71% of the patients who received fibrin glue with additional MSC treatment. An Italian group treated 10 patients with refractory fistulizing CD every four weeks with a local injection of 20 million autologous MSCs derived from bone marrow as long as the autologous MSCs were available. In 70% of the patients, fistulas closed completely without any serious adverse events. Furthermore, rectal mucosal healing was observed and PDAI was improved. The first paper on the treatment of perianal fistulizing CD with allogeneic MSCs was published last year. Patients were treated locally with 20 million MSCs derived from adipose tissue per fistula. If the fistula was not healed after 12 weeks, another 40 million cells were injected. Although the investigators and patients were not blinded, 69.2% of the patients showed a reduction in the number of draining fistulas after 24 weeks. In more than half of the patients complete closure of the treated fistula was achieved and in 30% of the patients complete healing of all fistulas was observed after 24 weeks. However, all these studies were not powered for efficacy analysis, so double blind randomized controlled trials with a sufficient number of patients are needed in order to prove the actual efficacy of MSCs in the treatment of fistulizing CD.

SAFETY CONCERNS

Until now, only a few MSC-related adverse events have been described in the performed clinical trials. However, not much is known about the long-term effects of MSC administration. One of the safety concerns is the possibility of malignant transformation. No neoplasia were observed after at least 3 years of follow-up in one study. In addition, a recently published meta-analysis reported no de novo tumor formations after MSC administration. However, malignancies did occur in patients with previous malignancies, underlining the importance of screening of patients before MSC administration. In contrast to the general idea that MSCs are not immunogenic, multiple injections of allogeneic MSCs after bone marrow transplantation in sublethally irradiated mice decreased engraftment whereas syngeneic MSCs promoted engraftment. In addition, allogeneic MSCs are capable of inducing a memory T cell response in immunocompetent hosts. Although further studies are needed to elucidate the situations wherein MSCs can be immunogenic, these findings should be taken into account when allogeneic MSCs are used in a clinical setting by, for instance, using MSCs from different donors when multiple infusions are required to avoid a possible memory T cell response and to reduce the possibility of loss of engraftment.
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Chapter 1


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