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Autologous and Allogeneic Stem Cell Transplantation for Treatment of Crohn's Fistulae

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Abstract

Up to 20% of patients with Crohn's disease (CD) may have perianal fistula disease. Classically, surgery has played an important role; in recent years, medical treatment has taken a leading role. Immunosuppressants and biological trea tments have proven beneficial in many patients, but still, the percentage of patients who do not respond remains significant. In this scenario, cell therapy is envisaged as an effective alternative to surgery. The promising preclinical and clinical data that we review below suggest that cell therapy could represent a major advance in the clinical management of this difficult problem.

Keywords: stem cells, allogenic, autologous, transplantation, Crohn, fistulas

1. Introduction

Up to 20% of patients with Crohn's disease (CD) may have perianal fistula disease, which is frequently associated with perianal collections [1–3]. Classically, surgery has played an important role, by the placement of drains or setons creation of ostomies, and in severe cases, even proctectomy [4]. However, in recent years, medical treatment with or without the temporary placement of drains has taken a leading role. Immunosuppressants such as azathioprine, 6-mercaptopurine, methotrexate and cyclosporine have proven beneficial in many patients. In more complicated cases where these drugs are ineffective, biological treatments based on monoclonal antibodies have been shown to have some success for the

induction and maintenance of remission of perianal fistula disease and associated proctitis [5–11]. Still, the percentage of patients who do not respond or do so only partially remains significant. Furthermore, the existence of serious complications associated with treatment should not be overlooked [9, 12, 13].

It is as a result of these inadequacies in current treatment strategies that cell therapy has arisen as a complementary option [14]. The promising results published in recent years, both with autologous and in allogeneic cells, highlight a need for greater understanding of the basic principles of this new route and for clarification of the current state of the topic.

2. Basic concepts of cell therapy

Stem cells have both the capacity for self-renewal or self-replication and for production of daughter cells that proceed along specific developmental pathways that will eventually lead to differentiation into specialised cell types [15].

Embryonic stem cells are obtained from the inner cell mass of the embryo at the blastocyst stage. They are able to generate cell lines derived from any of the three embryonic germ layers (ectoderm, mesoderm and endoderm), giving them great therapeutic potential. In mature adult tissues, we find adult multipotent stem cells, which are generally only able to renew and regenerate tissues from the embryonic layer of which they come. However, based on the so-called phenomenon of cellular plasticity, in some instances, they can differentiate into cell populations different to those of their embryonic origin, providing many therapeutic options [16].

Finally, we have the so-called induced pluripotent stem cells (iPS), which are somatic cells that have been subjected to a process of nuclear reprogramming by ectopic expression of specific transcription factors. These acquire molecular and functional characteristics of pluripotency that make them akin to embryonic stem cells. They also display similar characteristics to these in terms of morphology, proliferation, gene expression, epigenetic status of pluripotent genes and their ability to differentiate *in vivo* and *in vitro* [17].

Although embryonic stem cells and iPS have great potential for cell-based therapies, there are several limitations to their use, including regulatory, ethical and genetic engineering considerations. As a result, there are currently no clinical trials evaluating their use [18].

On the other hand, adult stem cells can be obtained using much simpler methods and have no restrictions or ethical considerations. Furthermore, because of their autologous origin, they are not immunoreactive. Early studies using adult stem cells have focused on mesenchymal stem cells (MSCs). These can be found in the stroma of virtually every organ, for example, in subcutaneous adipose tissue and bone marrow. Being fibroblastoid cells, they are the precursors of all types of non-haematopoietic connective tissues (bone, fat, cartilage, etc.). MSCs are generally obtained by selection through adherence to tissue culture plastic, as they are able to adhere and grow in conditions where other cell types do not usually proliferate [19]. They are required to meet minimal criteria defined by the International Society for Cellular Therapy,

namely, more than 95% of cells must express CD105, CD73 and CD90, as measured by flow cytometry; and <2% must be positive for CD45, CD34, CD14, CD11b, CD79a or CD19 and human leukocyte antigen (HLA) Class II. Moreover, they should be able to differentiate into osteoblasts, chondroblasts and adipocytes under standard *in vitro* differentiation conditions [20].

MSCs have a high capacity for proliferation and differentiation. Furthermore, under certain experimental conditions, they have displayed the ability to differentiate into non-connective cell lineages, such as neuronal and endothelial. Finally, as a particularly interesting property for the use at hand, they are capable, both *in vitro* and *in vivo*, of inhibiting immune response. This ability to immunoregulate includes inhibition of the activation of T, B and NKcells, the maturation of dendritic cells, as well as protecting against inflammatory and/or autoimmune pathologies, including transplant rejection [21].

3. Mesenchymal stem cells as therapies

Early studies with adult stem cells focused on MSCs isolated from bone marrow stroma, which have demonstrated adipogenic, osteogenic, chondrogenic, myogenic and neurogenic potential *in vitro*. However, obtaining stem cells from this source is painful for the patient and only provides a small number of cells [22]. Recently, methods of harvesting adult stem cells from adipose tissue by simple liposuction have been developed. Adipose tissue is rich in such cells, and their preparation is easier than that from bone marrow. Although there is some debate about whether stem cells originate in the fat tissue itself, or if perhaps they are mesenchymal or even peripheral blood stem cells passing through the fat, it is clear that adipose tissue represents a valuable source of potentially useful stem cells. These adipose-derived stem cells (ASCs) have been shown to have an inherent ability to self-renew, proliferate and differentiate into mature tissues, depending on the microenvironment that surrounds them. Such characteristics, intrinsic to all stem cells, make them highly attractive for use in cell therapy and regenerative medicine [23].

Interest in multipotent ASCs is increasing, owing to the ability to harvest large quantities of tissue under local anaesthesia via the liposuction process. Indeed, from just 1 g of adipose tissue, 5×10^3 stem cells can be obtained, which is much greater than the amount that can be acquired from bone marrow. Furthermore, compared to bone marrow MSCs, in the early stages, ASCs express CD34 to a greater extent (100–500 times higher) [24].

The terms adipose tissue-derived stromal cell (ADSC), adipose stromal–vascular cell fraction (SVF) and adipose-derived regenerative cells (ADRC) all correspond to cells obtained immediately after digestion of adipose tissue by collagenase. On the other hand, the terms processed lipoaspirate cells (PLA) and plastic-adherent adipose-derived stem cells (ASCS) describe those that are obtained after culturing those produced by the digestion process. As a unifying term, we refer to these cell types as adipose-derived stem cells (ASC), in accordance with the International Fat Applied Technology Society Consensus [25].

4. Utilisation of MSCs in the treatment of perianal fistula disease

The precise mechanism of the therapeutic action of MSCs is not fully understood, but is likely to reflect their inherent characteristics, in particular their differentiation potential [26, 27]. MSCs have the ability to migrate to the site of a lesion or inflammatory process, stimulate the proliferation and differentiation of resident stem cells through the secretion of growth factors, remodel the matrix and exert an immunomodulatory and anti-inflammatory effect. Together, these properties aid help the healing of tissues [28–31]. It has also been demonstrated that MSCs can induce an increase in epithelialisation and angiogenesis through a process of differentiation and paracrine interaction with skin cells [32–34].

Today, we know that Crohn's disease delays T-cell apoptosis [35, 36], and a mechanism of action of ASCs when injected into the inflammation site in the fistula tract has been postulated. Initially, the cells recognise proinflammatory cytokines such as IFN-γ, followed by activation of the indoleamine 2,3-dioxygenase (IDO) enzyme, which is ultimately responsible for creating a microenvironment—lymphocyte freezing by inhibition of phosphorylation. This results in a reduction in the release of proinflammatory mediators (TNF-α, IL-6, etc.) and an increase in that of anti-inflammatory species such as IL-10 [37].

5. Treatment protocol for anal fistulae

The protocol for stem cell treatment of anal fistulae inevitably starts with the harvesting of the MSCs, either from the patient's bone marrow or their fat (autologous), or from a healthy donor (allogeneic). Bone marrow cells are harvested by aspiration, and then, the MSCs are expanded *ex vivo* for subsequent use in the fistula tract [38, 39]. Although there are various protocols for expansion and differentiation of cells obtained from adipose tissue (with a consequent variation in results), ASCs are normally used after digestion with collagenase under constant stirring. The obtained solution is then centrifuged at low speed, and the resultant is filtered through a nylon mesh of 40–200 μm. The new solution is then centrifuged again, and the cells are re-suspended in fresh expansion medium. It is important to stress that this procedure must be carried out in extremely sterile conditions [40].

As for the route of administration, there is a single study in which allogeneic bone marrow MSCs were given intravenously, with the closure of fistulas being a secondary objective of the study [41]; all other published studies have employed the intralesional route [38, 39, 42–50].

Before intralesional injection of the isolated MSCs, the lesion site must be prepared with similarly intensive curettage, avoiding the use of cytolytic substances (hydrogen peroxide). The inner fistula orifice can then be sealed with an absorbable suture. At this point, half of the cell preparation is administered to the tissue around the inner hole, making small submucosal wheals. The other half is applied along the walls of the fistula tract, if possible along its whole length, while taking care not to go deeper than a few millimetres, again in small wheals (**Figure 1**). Several studies have investigated the use of fibrin glue as an adjuvant or scaffold, in order to enhance the attachment of cells in the fistula tract [43, 45–47]. The dose of cells

required for optimum results remains to be determined; in published studies, this ranges from 3.5×10^6 to 40×10^6 cells [39–50].
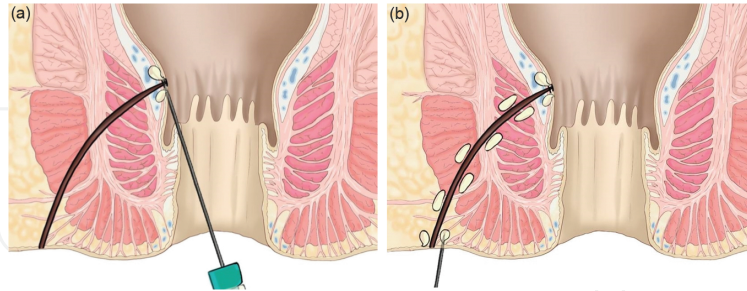
Figure 1. Implant points. (a) Wheal in the internal fistula orifice; (b) injection in the fistula tract at a depth of no more than 2 mm (courtesy of Tigenix).

Most studies have used ASCs, but there are also some that have evaluated the use of bone marrow cells. As for the cell source, the advantages of an allogeneic source (from healthy donors) are innumerable in comparison with those of an autologous source, especially in terms of greater accessibility, easy expandability and good stability. Their use is possible because of their low immunogenicity and limited persistence, which reduce the chances of provoking an adverse effect in the host [51].

6. Safety and efficacy of MSCs in the treatment of anal fistulae

The first experience with stem cells in the treatment of anal fistulae was reported by García-Olmo et al. [52]. Several studies have since been published, the majority of which are from Spanish groups. The MSCs used have mainly originated from adipose tissue, with only two studies using bone marrow MSCs. In these latter cases, both allogeneic and autologous cells have been used. In all studies, administration was intralesional, with fibrin glue often used [38, 39].

Today, any questions as to the feasibility and safety of such treatment seem to have been resolved, at least within the range of doses used. A retrospective study evaluating whether MSC treatment has any influence on fertility, course of pregnancy, birthweight or physical status was recently published [53]. Five patients with fistula associated with Crohn's disease treated with ASCs, and who indicated their intention to have children after completion of treatment, were tracked. Fertility and pregnancy course were not found to be affected by this therapy. Furthermore, no treatment-related malformations in newborns were observed. Therefore, it was concluded that in the patients analysed in the study, local injection of ASCs was not associated with adverse effects on the ability to conceive, pregnancy course or the newborn's condition.

In the published literature, there are differences in cure rate depending on the follow-up, but in general, it is estimated to be between 50 and 70% (**Table 1**).

Authors, year	Study design	Source of cells	Results
Garcia-Olmo et al., 2005 (Spain) [42]	Phase I clinical study ($n = 4$)	ASCs (autologous)	Complete closure: 50% of patients; 75% fistulas
Garcia-Olmo et al., 2009 (Spain) [42]	Open-label, multicenter, phase II study ($n = 14$)	ASCs (autologous); fibrin glue	Fistula healing: 71 vs 14%
Ciccocioppo et al., 2011 (Italy) [38]	Prospective study ($n = 10$)	MSCs (autologous)	Reduction in CDAI, PDAI and pain/discharge PDAI scores
Guadalajara et al., 2012 (Spain) [43]	Retrospective follow-up of Garcia-Olmophase II study ($n = 5$)	ASCs (autologous); fibrin glue	58% sustained fistula closure at end of follow-up by mean 3 years No safety problem
Cho et al., 2013 (Korea) [47]	Open-label, multicentre, dose escalationphase I study ($n = 10$)	ASCs (autologous); fibrin glue	Healing in 50% in the group with 2×10^7 cells
Lee et al., 2013 (Korea) [45]	Open-label, multicentre, phase II study ($n = 42$; 33 completed follow-up)	ASCs (autologous); fibrin glue	Fistula closure in 79%, recidive 11%
de la Portilla et al., 2013 (Spain) [48]	Open-label pilot study ($n = 24$)	ASCs (allogeneic)	Complete closure: 56.3% at 24 weeks
Ciccocioppo et al., 2015 (Italy) [44]	5-year follow-up of 2011 study ($n = 10$)	MSCs (autologous)	37% fistula relapse-free 4 years later
Cho et al., 2013 (Korea) [46]	Retrospective, 1-year follow-up from 2013 study	ASCs (autologous); fibrin glue	Complete closure maintained in 75% at 2 years ITT analysis; 80% PP analysis
Garcia-Olmo et al., 2015 (Spain) [49]	Retrospective, open-label ($n = 3$ with CD)	ASCs (allogeneic andautologous)	Healing in 2/3 CD fistula patients
Molendijk et al., 2015 (The Netherlands) [39]	Double-blind, placebo-controlledphase II study ($n = 21$)	MSCs (allogeneic)	Healing up to 85%
Park et al., 2015 (Korea) [50]	Multicentre, open-label, dose escalation pilot study ($n = 6$)	ASCs (allogeneic); fibrin glue	Group 1 (1×10^7 cells/ml); healing 100% Group 2 (3×10^7 cells/ml); healing 100%

ASCs, adipose-derived stem cells; CD, Crohn's disease; CDAI, Crohn's disease activity index; ITT, intention to treat; IV, intravenous; MSCs, mesenchymal stem cells/mesenchymal stromal cells; PDAI, Pouchitis disease activity index; PP, per protocol; SC, stem cells.

Table 1. Published studies using MSCs to treat Crohn's disease patients with perianal fistulas.

Ciccocioppo et al. evaluated the long-term safety and efficacy of the use of bone-marrow-derived MSCs. In their study, 8 patients were followed prospectively for 72 months. These patients were part of a phase I/II trial previously conducted, in which a cure rate of 70% per year was reported, with improvement observed in the remaining 30% [44]. Patients received serialised injections of MSCs (4 on average) at intervals of 4 weeks. Secondary endpoints were the time patients remained without fistula and the time they were free of medical or surgical treatment. The Chrohn's Disease Activity Index (CDAI) increased over the first 2 years, followed by a gradual decline in the third year, and stabilisation at the end of follow-up at figures similar to those of the first year. The probability of remaining without fistula was 88% for the first year, 50% at 2 years and 37% over the next 4 years. The probability of patients being free from surgery was 100% for the first year, 75% for years 2–4 and 63% at years 5 and 6. Finally, the probability of patients being free from medical treatment was 88% for the first year, 25% at years 2–4 and 25% at years 5 and 6. No adverse effects related to treatment in these follow-up periods were recorded. The authors conclude that the fact that the activity indices increase again in the second year might suggest that this therapy is not curative, but that it does improve the remission rate in patients with refractory disease. Moreover, almost all patients required the reintroduction of biological or immunosuppressive therapy after the second year [44].

We are currently awaiting the publication of the results of a phase III, randomised, placebo, double-blind, multicentre, and international clinical trial employing Cx601, a preparation of allogeneic ASCs. It has recently been reported that, after 24 weeks, Cx601 was statistically superior to placebo in achieving the combined response (clinical and imaging) of complex perianal fistulas in Crohn's disease patients whose response to previous treatment, including anti-TNFs, had been inadequate.

7. Future perspectives

There is no doubt that a new avenue has opened for the treatment of Crohn's disease patients suffering from fistulae refractory to conventional therapy. Since the first description of the treatment, interest in this therapy has grown, so that in addition to the 11 studies published to date, at the time we write this chapter, there are more than a dozen clinical trials in recruitment or in the results publication phase.

While the safety of ASC therapy seems to have been well established, the optimal dosage, route of administration (intravenous versus intralesional), administration technique (alone or together with fibrin glue), among other matters, are yet to be adequately determined. However, these should be investigated and resolved in the coming years.

Acknowledgements

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