Mesenchymal stem cell therapy of Crohn’s disease: are the far-away hills getting closer?

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Persistence of unmet therapeutic needs in patients with Crohn’s disease with luminal or perianal refractory disease has fostered the interest in innovative cellular immunoregulatory and regenerative medicines. Apart from autologous haematopoietic stem cell transplant, in which efficacy has been suggested in small series of patients and is now being evaluated in controlled trials, various groups have undertaken initiatives to determine the potential of mesenchymal stromal cell (MSC)-based therapy in Crohn’s disease.

Bone marrow MSCs were first identified by Friedenstein, who described an adherent fibroblast-like population able to differentiate into bone. He referred to these cells as osteogenic precursor cells.1 Subsequent studies demonstrated that these cells have the ability to differentiate into various other mesodermal cell lineages, including chondrocytes, tenocytes and myoblasts. Based on this multilineage differentiation capacity, the term ‘mesenchymal stem cells’ was introduced.2 Although MSCs at a population level fulfill stem-cell criteria (i.e., self-renewal and the capacity for multilineage differentiation), it remains questionable whether the qualification ‘stem cell’ is legitimate for MSCs at the single-cell level. It was therefore recently proposed that the term ‘multipotent mesenchymal stromal cells’ (with the abbreviation MSCs) should be used to describe fibroblast-like plastic-adherent cells.3

Although, originally, MSCs were isolated from bone marrow, similar populations have been isolated from other tissues, including adipose tissue, placenta, amniotic fluid and fetal tissues such as fetal lung and blood.2 To date, the isolation of MSCs still relies on their adherence to plastic, resulting in a heterogeneous population of adherent cells. No specific marker or combination of markers has been identified that specifically defines MSCs. Phenotypically, ex vivo expanded MSCs express a number of non-specific markers, including CD105 (SH2 or endoglin), CD73 (SH3 or SH4), CD90, CD166, and CD29, and are devoid of haematopoietic and endothelial markers, such as CD11b, CD14, CD31 and CD45.4 MSCs possess the ability to expand in vitro under normal culture conditions. Extensive expansion of a subpopulation of MSCs, in the order of 104-fold in 6 weeks has been obtained by culturing cells at a low density (1.5 or 3 cells/cm2).5 The clinical feasibility of culture-expanded MSCs has been validated by a number of studies.2 Thus, a small amount of source tissue aspirate is sufficient for the generation of a large number of cells needed for cell therapies following in vitro expansion. A growing body of evidence indicates that MSCs possess immunomodulatory properties and may play specific roles as immunomodulators in the maintenance of results of a randomised controlled trial. Gut 2011;60:780–7.

peripheral tolerance, transplantation tolerance, autoimmunity and tumour evasion, as well as in fetal—maternal tolerance. In vitro experiments have shown the ability of MSCs to (i) suppress T lymphocyte activation and proliferation, (ii) interfere with dendritic cell differentiation, maturation and function, (iii) modulate B cell function, and (iv) suppress NK cell proliferation and interferon γ production. The immunomodulatory effects of MSCs have been examined in a variety of animal models related to immune-mediated diseases, including IBD, and in human studies.

The most impressive clinical effect of MSCs in humans has been observed in the treatment of acute graft-versus-host disease developing after allogeneic haematopoietic stem cell transplant. The benefit deriving from the infusion of MSCs in patients with steroid-resistant acute graft-versus-host disease has been shown in a study reporting 55 patients, both adults and children. Infusion of MSCs appeared to be safe, and no major toxicities were observed. Treatment with MSCs resulted in a response in the majority of patients, with a significant difference in survival between complete responders and partial and non-responding patients. Two phase I studies on autologous bone marrow-derived MSCs for the treatment of active refractory Crohn’s disease are now published in Gut, one study using systemic administration in patients with luminal Crohn’s disease appeared recently, and a second study assessing the effects of local injection of MSCs for the treatment of fistulising disease appears in this issue (see page 788). The first study treated nine patients with two doses of 1–2×10⁶ cells/kg body weight intravenously 7 days apart. All patients had previously failed corticosteroids, at least two anti-tumour necrosis factor (TNF) drugs, and the majority (9/10) also two immunosuppressants (a thiopurine and methotrexate). In this study no clear signal of efficacy was observed; remission was not achieved in any patient, three patients had a reduction of at least 70 points in Crohn’s disease activity index (CDAI), but the disease worsened significantly in four patients requiring surgery (three cases) or rescue medication (one case) within 14 weeks after cell treatment. Endoscopy improved in two cases but no significant changes in C-reactive protein levels were seen. In contrast with these findings, the second study performing local injection of MSCs treated nine patients with perianal and one patient with enterocutaneous fistulas with injections of a median of 20×10⁶ cells (range 15–50) every 4 weeks until a response was obtained or ‘no more cells were available.’ Complete fistula closure sustained for 1 year was obtained in seven and a response (reduction of at least 50% of fistula tracts) in three. Furthermore, all nine patients with perianal fistulas had active disease in the rectum at baseline, and healing of rectal lesions was observed in the seven patients who underwent endoscopy at month 12 of follow-up. Thus, the latter study suggests a considerable therapeutic benefit of local injection of MSCs in fistulising lesions.

The apparent efficacy of the local injection of bone marrow-derived MSCs for treatment of fistulising lesions in Crohn’s disease is in keeping with previous evidence generated using adipose tissue-derived MSCs. In an initial phase I study, nine complex fistulas (enterocutaneous, rectovaginal or perianal) in four Crohn’s disease patients were treated by a single injection of 5–50×10⁶ cells. At week 8, the external openings of six of the nine lesions were closed. In a second multicentre randomised study 50 patients with perianal fistulising disease were included, but only 14 of these had Crohn’s disease. Patients were randomised to treatment with fibrin glue or fibrin glue plus 20×10⁶ MSCs. Fistula healing was evaluated at 8 weeks and 1 year. If healing was not seen at 8 weeks, a second dose of fibrin glue or fibrin glue plus 40×10⁶ MSCs was administered. Closure of fistulas was observed in 5/7 (71%) of those receiving injection of MSCs compared to 1/7 (14%) of those treated with fibrin glue alone. Although the sample size is small and differences were not statistically significant, the proportion of Crohn’s disease patients responding in each treatment arm was similar to the overall population included in the study. At this stage, considerations on the reasons for the apparent discrepancies between efficacy of local injection of MSCs for treatment of fistulas compared to systemic administration for treatment of luminal Crohn’s disease are speculative, but might help to gain some insights for the design of future trials. The two most obvious differences are dosing and route of administration. As for the latter, MSCs have been reported to home to sites of injury and disease following intravenous infusion and contribute to the repair process. The expression of adhesion molecules and chemokine receptors on MSCs may be responsible for their ability to migrate selectively to sites of inflammation, through a ICAM1- and VCAM1-dependent interaction with endothelial cells. In an experimental model of colitis, it has been demonstrated that systemically injected MSCs are detected in the mesenteric lymph nodes and spleen of the recipient colitic mice 1–3 days post-injection. Interestingly, labelled MSCs were recruited by the inflamed colon, but not by non-inflamed intestine. However, the proportion of cells recruited to inflamed or damaged organs and the survival of cells at sites of inflammatory lesions remains to be clarified for optimising a potential systemic treatment.

Another obvious difference between systemic and local injections is cell density achieved at sites of inflammatory lesions. In studies showing efficacy of local injections, 30–60×10⁶ MSCs are injected in a single fistulous tract, and these injections are generally repeated. In the study using systemic injection for treatment of luminal disease, a total amount of 100–400×10⁶ MSCs were injected (depending on the patient’s weight). Considering the extension of the inflamed intestine, and that only a proportion of MSCs will reach the inflamed organ, cell density at sites of luminal inflammation would be considerably lower than that achieved in fistula tracts by local injection. Ongoing trials are testing fourfold higher systemic doses. There are still other basic issues that should be considered before planning clinical studies. Because the true identity of MSCs in vivo remains elusive, current approaches used for their isolation have resulted in heterogeneous subpopulations exhibiting MSC-like characteristics. Therefore, identification of MSC-specific markers for isolation of a homogenous population of cells directly from tissue is necessary. This would accelerate the pace of research on MSCs as a comparison of results among laboratories would then be feasible.

Another potential hurdle in the applicability of these therapies is the requirement for a large number of cells. For example, bone marrow transplant requires on average 5×10⁶ cells/kg body weight. Although MSCs are easy to isolate and undergo in vitro proliferation, extended expansion was observed to alter their properties. Stem cells must exhibit indefinite self-renewal as per definition, but human MSCs have been shown to undergo senescence and exhibit reduced differentiation potential from the 6th
passage onwards, which is in agreement with other studies showing that about 13–25 population doublings result in complete senescence. Senescence-associated changes in cellular morphology, expression of surface markers and global gene profile have been observed with increasing number of passages beginning from the first passage itself. A precise definition of the production process, along with a phenotypic and functional characterisation of the cells produced in a particular process are key to understanding the biology of MSCs and assess the influence of production variables on their therapeutic effect.

Use of allogeneic MSCs may be another approach to obtain the required cell number of cells for treatment of human disease. MSCs are considered to be hypo-immunogenic, displaying low expression levels of human leucocyte antigen major histocompatibility complex class I and, importantly, no expression of co-stimulatory molecules, and in vivo studies demonstrated that MSCs avoid normal alloresponses. These characteristics support the possibility of using universal donor MSCs for therapeutic applications, and on-going trials in Crohn’s disease are exploiting this approach. Potential rejection of MSCs and its clinical consequences should be carefully considered in these studies.

Little is known regarding the in vivo survival of MSCs, and there are no clinical studies reporting whether MSCs remain present after transplantation. The lack of adverse events uniformly reported in published studies could be due to the limited survival of MSCs. Furthermore, no neoplastic transformation of human MSCs has been documented, and normal karyotyping has been reported in all studies assessing genetic stability after cell expansion, including one of the recent studies in Crohn’s disease.

The real efficacy of MSC therapy in the management of patients with Crohn’s disease remains to be proved in adequately powered randomised trials comparing this innovative treatment with more conventional approaches. Likewise, MSC cell origin (allogeneic versus autologous), the number of administrations to be performed, the optimal dose of MSCs to be delivered for each administration, the route of administration (local injection being also possible for luminal disease), and the possible synergism of MSCs with other therapies demonstrated to be active in patients with active Crohn’s disease, all remain to be defined.

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