Stem Cells as Potential Targeted Therapy for Inflammatory Bowel Disease

Maria Gazouli, PhD,* Maria G. Roubelakis, PhD,*† and George E. Theodoropoulos, PhD‡

Abstract: The incidence and prevalence of inflammatory bowel disease is increasing in Western countries. Current therapies, ranging from anti-inflammatory drugs, immunosuppressive regimens to new biological therapies, remain inadequate. Advances in our understanding of the pathophysiological mechanisms underlying the pathogenetic disease process and the recent findings on the regenerative and immunoregulatory potential of stem cells open new opportunities in the therapy of inflammatory bowel disease. Therapeutic modalities, including hematopoietic stem cells, adult mesenchymal stem/stromal cells, and the recently identified amniotic origin stem cells, attracted much attention in the recent years. The current review highlights the recent pivotal findings for stem cell–based approaches to inflammatory bowel disease therapy.

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Inflammatory bowel disease (IBD), which includes Crohn’s disease (CD) and ulcerative colitis, is a lifelong, relapsing and remitting disease, profoundly affecting the quality of life in an enlarging portion of the population. The incidence and prevalence of IBD markedly increased in recent years, and IBD is considered to be one of the most prevalent gastrointestinal diseases.1,2 The pathogenesis of IBD still remains unclear even if considerable advances in this field have been made. IBD is thought to be the result of an aberrant immune response to commensal bacteria and luminal antigens in a susceptible host. Genetic and environmental factors play a role in IBD development, mediated by changes in innate and adaptive immune function, epithelial barrier function, and microbiome composition.3 Current nonsurgical treatments of IBD mainly include the administration of corticosteroids, 5-aminosalicylic acid preparations and immunosuppressive drugs, such as azathioprine. However, only a proportion of patients achieve sustained remission with these drugs, and the treatment may cause many side effects, including the toxicity of corticosteroids and cytopenia caused by azathioprine.4 Recently, biological therapies (anti–tumor necrosis factor [TNF] mainly agents) that target immune pathways have been emerged as an effective therapeutic approach for the treatment of immune dysfunction–mediated diseases. As IBD is an immunological disease, biological therapy targeting excessive cytokines and immune responses in inflamed mucosa should be a highly promising approach for treatment. Although biological therapies have been increasingly used in clinical practice in recent years, their safety has also been emphasized because these therapies have a number of adverse effects.5 Furthermore, failure to respond to the current available therapies, inability to provide surgical management to fistulizing IBD, and the recurrent need for surgeries remain challenges requiring new therapies for IBD.6

Recent advances indicated that the pathogenesis of IBD can be influenced by multiple aspects and the different cell types involved, and therefore, the various treatment strategies, so far, have had limited success. Nowadays, the concept of stem cell (SC)–based therapy has been reported as a promising approach for the treatment of IBD, mainly by the use of hematopoietic SCs (HSCs) and mesenchymal SCs (MSCs). The latter are known to exhibit regenerative, paracrine, and immunoregulatory properties.7,8

It is evident that HSCs and MSCs have already been used in clinical settings. The intestinal environment, with its crypts and niches, supports migrating SCs and allows them to engraft and differentiate.9 These findings implied that in the near future, SC-based therapy may represent a promising alternative to conventional therapy for IBD. In this review, we aim to discuss the recent findings supporting the use of 3 SC types as potential targeted therapeutics for IBD treatment.

HEMATOPOIETIC SCs

HSCs play a fundamental role in controlling chronic inflammation and immune regulation and are capable of regenerating immune cells.10 They are characterized by their ability to self-renew and also to differentiate into diverse types of blood
cells. They were initially characterized as rare cells within the bone marrow (BM) or fetal liver comprising \(1 \times 10^3\) to \(1 \times 10^5\) cells, with the majority belonging to the CD34+ fraction.

HSCs are able to home the damaged tissue and migrate directly to the injury site or differentiate into the epithelial and immunomodulatory components that are unique to the intestinal compartments, leading to tissue recovery and restoration of the normal mucosal system. HSC transplantation has gained much consideration as cell therapy for hematological malignancies and for the treatment of severe autoimmune and inflammatory diseases. In IBD, it is well documented that impairment of the intestinal immune cell function and turnover play a crucial role in the deregulated and extended inflammatory response. A high-dose immune ablation regimen might allow detrimental T lymphocytes to be eliminated, and after HSC transplantation, hematopoiesis might generate naive cells that can restore tolerance. Patients receiving HSCs are in the process of an immune system reboot. The HSC-transplanted patients probably experience an autoreactive response, with the immune system almost completely replaced. Drakos et al were the first to document the case of CD regression after autologous HSC transplantation for hematopoietic malignancy. Gratwohl et al suggested that autologous HSC transplantation is preferred to allogeneic one because of the lower risk of toxicity. Oyama et al reported a phase I study with autologous HSC infusion of 12 patients with active CD refractory compared with conventional therapies. Of them, 11 entered a sustained remission. Also, Cassinoti et al reported similar results with no mortality observed in these studies. Burt et al reported that 91% of the 24 CD patients with severe disease resistant to conventional therapies, including anti-TNFa antibodies, stayed in remission for 1 year after autologous HSC transplantation, and 19% of them stayed in remission after 5 years. More recently, Hommes et al and Clerici et al reported similar outcomes. Of interest is a prospective, randomized, phase III study, the Autologous Stem Cell Transplantation International Crohn’s Disease trial. This study analyzed the effect of autologous HSC transplantation versus SC mobilization alone in refractory CD patients (www.nottingham.ac.uk/icr/astic). Preliminary data suggested a considerable improvement in patients who received HSC transplantation compared with controls; however, adverse events raised concerns about safety. Apart from autologous HSCs transplantation, allogeneic marrow transplantation seems to be useful for the treatment of IBD. It is important to notice that up to now, all trials have focused on severe refractory CD patients, and no trials have been published yet on HSC transplantation in ulcerative colitis patients.

**MESENCHYMAL STEM/STROMAL CELLS**

Recently, interest has been focused on potential therapeutic uses of mesenchymal stem/stromal cells (MSCs) because of their ability to suppress inflammation and to promote tissue healing. First identified by Friedenstein et al in BM, MSC is a multipotent, mesoderm-derived cell type that can generate at least osteogenic, adipogenic and chondrogenic cells, while exhibiting immunomodulatory properties. The possible mechanisms of the potential immunomodulatory effects of MSCs are characterized by the release of soluble factors (cytokines, chemokines, growth factors and others), the induction of cell cycle arrest in proinflammatory lymphocytes or by the induction of T-cell apoptosis. However, the exact mechanism by which MSCs contribute remains unclear. Recent data support that MSCs can induce tissue healing by their immunomodulatory function, differentiation or paracrine effects. Nevertheless, the immunomodulatory and tissue healing effects of MSCs provide the basis for investigation of their therapeutic value in IBD. MSCs can be isolated from several tissues, with differences in yield and in differentiation capacities. To date, the best-characterized MSC population is the one found in BM (BM-MSC). Alternatively, adipose tissue-derived MSCs (AT-MSC) can be isolated from liposuction aspirates and exhibit very similar immunophenotype and immunosuppressive capabilities compared with BM-MSCs. Regarding IBD, both autologous and allogeneic transplantation of BM-MSCs or AT-MSCs have been tested mainly in fistulizing and luminal CD clinical trials. However, up to now, the results are inconsistent. Dujivestine et al used autologous BM-MSCs for luminal refractory CD. Conventional treatments and anti-TNF therapy failed to all patients participated in the study. Unfortunately, no clear evidence of therapy had been observed. Conversely, Ciccocioppo et al evaluated the effect of BM-MSCs on fistulizing CD with promising results. Recently, preliminary results of a phase II clinical trial reported by Forbes et al indicated that the use of allogeneic BM-MSCs for refractory luminal CD resulted in encouraging results. Improvement was noticed in 12 patients, remission in 8, and endoscopic improvement in 7, respectively. Interestingly, a phase III, prospective, randomized, placebo-controlled study of prochymal (allogeneic BM-MSCs) in CD was initiated by Osiris Therapeutics Inc (http://clinicaltrials.gov/ct2/show/NCT00294112). Two hundred seventy CD patients with active moderate-to-severe disease who had previously failed treatment with steroids, immunosuppressants, and anti-TNF agents were enrolled to the study. The patients received 2 infusions of 600 million cells, or 1200 million cells, or placebo. After 28 days, the patients were evaluated for disease remission and clinical response. The results were encouraging, and Osiris received orphan drug designation from the Food and Drug Administration and the European Medicine Agency for Prochymal.

AT-MSCs have been also reported to retain promising potential for ulceration healing mainly in perianal disease. Garcia-Olmo et al, using autologous AT-MSCs in combination with fibrin glue for direct injection in perianal complicated CD, reported fistula healing in a considerable number of patients who received AT-MSCs plus fibrin glue compared with those who received fibrin glue alone. More recently, the same scientists reported that the combination AT-MSCs/fibrin glue was also more effective than fibrin glue alone in IBD patients with suprasphincteric fistulous tract. MSC-based therapeutic approaches for IBS seem promising. However, it is important to notice that MSCs represent a heterogeneous population, and different subpopulations of cells are exhibiting a variety of functional potentials.
However, to improve efficacy, robust priming of MSCs, to isolate and use those with enhanced immunosuppressive capabilities, is of basic importance. Furthermore, improving the targeting and engraftment of MSCs is of the ultimate importance for their potential use in cellular therapy and for the progression of MSC-based therapies to the clinic because their efficiency of delivery into injury sites is quite low especially when delivered systemically.\textsuperscript{38} An extensively investigated approach is the regulation of the expression of cell surface antigens by forcing the expression of appropriate receptors to the desired site of injury. Lately, Ko et al\textsuperscript{39} using an experimental mice model for acute colitis, developed a new method by coating murine MSCs with antibodies against addressins to enhance their specificity to the inflamed colon and thereby increase their therapeutic efficiency in IBD in vivo. More recently, Levy et al\textsuperscript{40} used messenger RNA transfection to generate BM-MSCs that simultaneously express functional rolling machinery (P-selectin glycoprotein ligand-1 and sialyl-Lewisx) to rapidly target inflamed tissues and that express the potent immunosuppressive cytokine interleukin-10 (IL-10), which is not inherently produced by MSCs. Their findings seem promising because the triple-transfected P-selectin glycoprotein ligand-1/sialyl-Lewisx/IL-10 MSCs transiently increase the levels of IL-10 in the inflamed area and showed a superior anti-inflammatory effect in vivo by significantly reducing local inflammation following systemic administration. This was dependent on the rapid homing of MSCs into the inflamed site. These results suggested that a similar approach can be also applied for IBD treatment.

\textbf{AMNIOTIC FLUID SCs}

Recently, fetal SCs, such as second-trimester amniotic fluid SC (AFSCs) and AF mesenchymal SCs (AF-MSCs) are reported to have increased proliferative potential and multipotentiality and longer telomere lengths than those sourced postnatally.\textsuperscript{41–43} The AF samples were obtained after informed consent, during routine amniocentesis for prenatal diagnosis, usually from the excess volume of sample that is normally discarded.\textsuperscript{41–43} Kaviani et al\textsuperscript{44} were the first who described the presence of a population of AF cells with mesenchymal characteristics. In't Anker et al\textsuperscript{41} demonstrated that AF-MSCs exhibit a phenotype and a multilineage differentiation potential similar to that of BM-MSCs. Our group\textsuperscript{45,46} has identified and enriched for 2 subsets of human AF-MSCs obtained at the time of amniocentesis. These cells have distinctive morphologies, phenotypic differences and abilities to differentiate into multiple cell types.\textsuperscript{45} Prusa et al\textsuperscript{46} described the presence of a distinct population of proliferating AF cells with pluripotent SC characteristics, and De Coppi et al\textsuperscript{42} documented the presence of a cell population able to generate clonal cell lines capable of differentiation into lineages representative of all 3 embryonic germ layers. These cells were named AFSCs and characterized by the expression of the surface antigen c-kit (CD117). Recently, Zani et al\textsuperscript{48} showed that AFSCs were more effective than BM-MSCs in the amelioration of some aspects of experimental necrotizing enterocolitis. AFSCs were found to have prolonged survival, were able to improve clinical status, and the macroscopic and microscopic appearance of the gut. The authors suggested that the protective role of AFSCs was accompanied by decreased inflammatory markers and improved enterocyte proliferation and migration in vivo. The apparent “homing” of AFSCs to the damaged intestine suggested that they can potentially be used for developing IBD therapeutic approaches.

\textbf{SAFETY OF SC THERAPEUTIC APPROACHES}

Several potential risks should be taken into account before the clinical use of HSCs, MSCs, or fetal SCs for the IBD treatment, with the most important issues being the immunogenicity of the cells, the safety of the culture media, and the risk of ectopic tissue formation and the potential in vitro transformation of the cells during expansion. Concerning the immunogenicity, it is encouraging that the majority of the clinical reports on MSC therapeutic application have reported a decreased immunogenicity of human MSCs.\textsuperscript{47} Because the expansion of several types of SCs is based on the presence of fetal calf serum, a risk of zoonoses transmission and possible immune reactions in the host as a result of bovine proteins exists. For this reason, several animal-free additives, such as platelet lysate/platelet-rich plasma and growth factors, have been used. However, clinical trials conducted up to now mainly used fetal calf serum–expanded cells, whereas in vivo data on platelet lysate/platelet-rich plasma cells are still limited.\textsuperscript{48} Another potential risk of MSC treatment involves the formation of mesenchymal tissues at ectopic sites. So far, no ectopic tissue or tumor formation in vivo has been reported in the respective clinical trials. However, a long-term follow-up of patients treated with MSCs is required to monitor the potential formation of mesenchymal ectopic tissues. Regarding the risk of malignant transformation, it has been reported in few studies that long-term manipulation in vitro of BM-MSCs and AT-MSCs may lead to the accumulation of genetic alterations and malignant transformation.\textsuperscript{49} In contrary, other studies supported that there is not a danger of development chromosomal aberrations after long-term cultures of MSCs.\textsuperscript{50} Nevertheless, a phenotypic, functional, and genetic assay, although known to have limited sensitivity, should be routinely performed on MSCs before in vivo use, in particular for patient-derived MSCs.

\textbf{CONCLUSIONS}

The currently available experimental preclinical and clinical data indicated that HSC-based and MSC-based therapeutic approaches for IBD are very promising. However, the determination of the best SC type and administration route and also the optimal cell dose needed at the lesions to guarantee safe and effective therapy are the basic challenges that still remain ahead.

\textbf{REFERENCES}


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