REVIEW ARTICLE

Mesenchymal stromal cells and their derivatives – putative therapeutics in the management of autoimmune pancreatitis

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Autoimmune pancreatitis, a derivative of chronic pancreatitis, frequently causes acute episodes with clinical symptoms parallel to those of acute pancreatitis. Corticosteroids are effective in the treatment of 90% of autoimmune pancreatitis cases, but for the remaining 10%, options are limited. Due to their significant immunomodulatory capabilities, mesenchymal stromal cells (MSCs) have been proposed as a novel treatment strategy for various immune and inflammatory pathologies including those with autoimmune origins. Here, we not only highlight the most recent MSC live-cell experiments to address acute pancreatitis, but also discuss the opportunities afforded by the emergence of the newly identified field of MSC necrobiology. We conclude that the putative employment of MSC derivatives provides a newer and simpler therapeutic approach that could have significant advantages over the use of cells themselves.

Autoimmune pancreatitis is a form of chronic pancreatitis that frequently causes acute episodes with clinical symptoms parallel to those of acute pancreatitis. If these acute inflammatory episodes are not properly managed, they can cause pancreatic fibrosis which can lead to the development of pancreatic cancer as well as the loss of exocrine and endocrine functions [1]. The disease is currently most commonly dealt with through the use of corticosteroids [2]. In around 90% of cases, corticosteroids are effective in the treatment of autoimmune pancreatitis [2]. However, for the other 10% of patients whose conditions do not respond favorably to corticosteroids, there is a lack of options. Of importance to note is that corticosteroids have, in some cases, been shown to provoke side effects including osteoporosis, hypertension, diabetes, weight gain, increased vulnerability to infection, cataracts, and

glaucoma, thinning of the skin, and muscle weakness [3]. In terms of alternatives, the immunomodulator, thiopurine, and an anti-CD20 monoclonal antibody, rituximab, are occasionally used to treat autoimmune pancreatitis; however, their success rates are variable [2]. For these reasons, there is considerable demand for a novel and superior strategy to manage the symptoms of autoimmune pancreatitis.

We recently (2019) summarized the available scientific literature pertaining to the putative use of mesenchymal stromal cells (MSCs) as a treatment strategy for acute pancreatitis [4]. In brief, we highlighted how MSCs, through various studies, have been proven to abrogate the effects of acute pancreatitis in rodent models. We also discussed the lack of clarity in current experimental designs that fail to take the original cause of acute pancreatitis into account. This is problematic

Abbreviations

aGVHD, acute graft-versus-host disease; IFN-Y, interferon-Y; IL-1β, interleukin-1β; MSC, mesenchymal stromal cell; OVA, ovalbumin; TNF-α, tumor necrosis factor-α; TSG-6, tumor necrosis factor-α-induced gene/protein 6; VEGF, vascular endothelial growth factor.

because MSCs clearly would not be an ideal therapeutic strategy for gallstones or alcohol-induced pancreatitis which already possesses successful treatment regimens. Here, we provide updates on new approaches reported in the field, and also discuss the use of MSC derivatives in the treatment of autoimmune pancreatitis.

Mesenchymal stromal cells were once thought to mitigate various pathologies solely through anti-inflammatory and tissue regenerative pathways. Autologous MSC therapy gained its first regulatory approval, for the treatment of acute myocardial infarction, in 2011 [5] although not without creating some continuing controversy [6]. In fact, MSCs have been shown to exhibit an immunomodulatory phenotype through four distinct mechanisms [7] (Fig. 1). The latter include modulating the proliferation and differentiation of dendritic, B, and T cells and mediating the polarization of monocytes from an inflammatory M1 phenotype to an anti-inflammatory M2 phenotype. In addition, MSCs can reduce the production of reactive oxygen species such as superoxide anions, which inhibit the apoptosis of neutrophils. Finally, MSCs can diminish endothelial cell responses to pro-inflammatory cytokines such as TNF-α, IL-1, and IFN-γ [7].

MSCs for the treatment of immune pathologies

Due to their significant immunomodulatory capabilities, MSCs have been proposed as a novel treatment strategy for various pathologies wherein the immune system is either compromised or dysfunctional, including those with autoimmune origins (Fig. 2). Indeed, MSCs have shown success in the treatment of systemic lupus erythematosus [8], rheumatoid arthritis [9], type 1 diabetes mellitus [10], multiple sclerosis [11], liver failure associated with hepatitis B virus [12], ulcerative colitis [13], dacryoadenitis [14], Sjögren's syndrome [15], and systemic scleroderma [15]. A very recent example is the co-administration of MSCs with pancreatic islets in immunocompetent type 1 diabetic wild-type mice. Glycemic control was restored, using human mesenchymal cells, and a clear demonstration provided evidence of the suppression of T-cell activation without the need for prior *ex vivo* licensing (stimulation) with the inflammatory cytokines interferon-Y (IFN-Y), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) [16]. The first approval, with government reimbursement, for the treatment of an immune condition with MSCs was that in Japan in 2016, for the treatment of both pediatric disease and adult acute graft-versus-host disease (aGVHD) [17]. Thus, the

acute inflammatory episodes of autoimmune pancreatitis may represent an appropriate therapeutic target for MSCs in cases refractory to the use of corticosteroids.

Recent cellular approaches

One recent approach has pretreated (licensed) umbilical-derived mesenchymal stromal cells with angiotensin II before employing them in the treatment of severe acute pancreatitis in Sprague-Dawley rats [18]. The rationale was to maximize the constitutive angiogenic properties of UCMSCs. The pretreated cells demonstrated an enhanced ability to abrogate pancreatitis compared with cells that had not been licensed. This conclusion was established through the histological assessment of pancreatic sections using measures of necrosis, edema, vacuolization, and inflammation as well as through the observation of myeloperoxidase and serum amylase levels. The treated cells were also shown to increase the paracrine release of vascular endothelial growth factor (VEGF) which has been proven to be an important factor in pancreatic tissue healing [18].

In another study, human adipose tissue-derived mesenchymal stromal cells were transfected with siRNA targeting tumor necrosis factor-α-induced gene/protein 6 (TSG-6) and compared with their untransduced, control counterparts in the treatment of caerulein and lipopolysaccharide-induced severe acute pancreatitis in C57BL/6 mice. Pancreas-to-body weight ratio, tissue edema, necrosis of acinar cells, and inflammatory cell infiltration were all improved in the control group, demonstrating the role played by TSG-6 in ameliorating the disease. Specifically, the pro-inflammatory cytokines, TNF-α, IL 1β, and IL-6 levels, and markers of endoplasmic reticulum stress, Grp78, CHOP, and caspase-12, were decreased, while anti-inflammatory cytokine, IL-10, was increased in the control group [19].

Ongoing challenges

While the above studies demonstrate convincing evidence that mesenchymal stromal cells could, in the future, be used as a novel treatment strategy for the acute episodes of autoimmune pancreatitis, it is important to acknowledge some limitations in the current scientific literature. Firstly, as we have previously discussed, to be clinically relevant, an animal model needs to be designed to specifically reflect the cause of the pancreatitis, such as autoimmune pancreatitis. Also, studies need to be conducted on the use of MSCs in large animal models whose gastroenterological
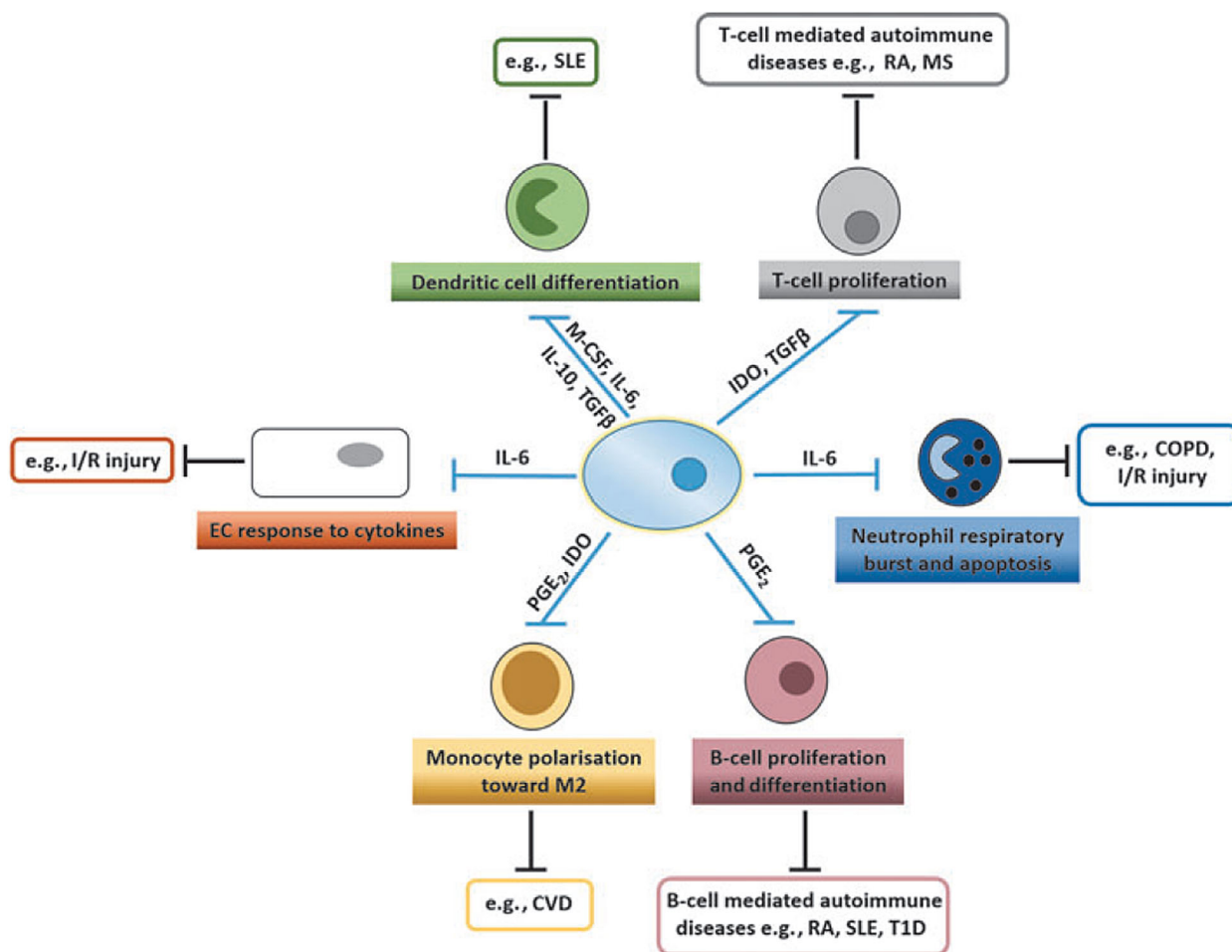
Fig. 1. The immunomodulatory effects of MSCs all have demonstrated proven efficacy in models of chronic inflammatory and autoimmune diseases. Included with permission from Munir and McGettrick [7].

anatomies are more similar to that of humans. Finally, as the cells are being proposed as a novel clinical therapy it is important to recognize barriers that could prove to be problematic as discussed below.

Mesenchymal stromal cells can be isolated from a plethora of human sources including adipose, brain, endometrial, placental, and umbilical cord tissue as well as bone marrow, cord blood, amniotic fluid, various regions of Wharton's jelly, and dental pulp [20,21]. However, it is becoming increasingly clear that the functional phenotype of a particular population of MSCs varies with both the tissue source employed and the conditions under which the cells are culture-expanded [22]. Despite these disparate origins, the overall abundance of MSCs in the human body is still relatively low, for example, only 0.001–0.01% of cells in the bone marrow are MSCs [20]. MSC therapy

usually requires hundreds of millions of MSCs; in the aforementioned studies, 1 million cells were used in the study conducted on mice and 10 million cells were used on the rats [18–19]. In an ongoing Phase III, human clinical trial being conducted for chronic graft-versus-host disease, a dose of 2 million cells per kilogram was injected six times intravenously [23]. Clearly, due to the scarce number of MSCs in the human body and the large number needed for putative therapy, a barrier might exist wherein the demand for cells needed for therapy cannot be met. *In vitro* cell expansion is the only way to yield such a high volume of cells, but this can take up to 10 weeks [24]. In addition, as described in the aforementioned studies, transfected or pretreated (licensed) cells have been proposed, which are more complicated and costly to produce.
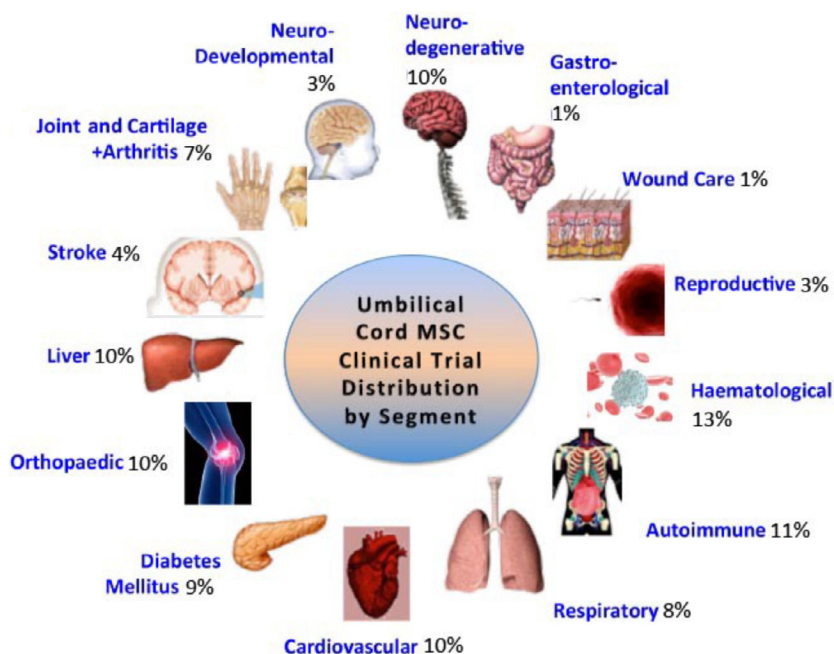
Fig. 2. Pictorial representation of the distinct categories of pathologies for which MSC therapy is being investigated through clinical trials. Data from 954 trials were obtained by searching registered clinical trials on https://clinicaltrials.gov/, using keywords 'mesenchymal stem cell, mesenchymal stromal cells, MSC, mesenchymal progenitor cells, multi stem cells, Pluristem PLXPAD, PDA002/001, adipose derived mesenchymal stem cell, adipose derived mesenchymal stromal cell, adipose derived MSC, ADMSC, adipose derived regenerative cell, CX610 and CX611'. Of importance to note is that this data set includes clinical trials that are recruiting, completed, or abandoned.

Live cells, dead cells, and derivatives

Therapy with live MSCs has a singular advantage over the use of their derivatives: The cells can differentiate to provide the connective tissue stroma of the recipient organ [25]. However, although MSCs have been proposed to assist with the significant stromal tissue damage that occurs in pancreatitis, the cell implantation time is too short for differentiation to occur. The two most common methods of MSC delivery for acute pancreatitis are intravenous and intraperitoneal. Less than 1% of MSCs survive for over a week after intravenous administration [26,27], while when injected intraperitoneally, after 20 min MSCs fail to be detected in peritoneal lavage fluid and begin to form large aggregates [28]. This evidence suggests that the main therapeutic benefit of mesenchymal stromal cells is in their immunomodulatory capacity. Thus, the therapeutic advantage of live MSCs over derivatives does not extend to the cells' immunoregulatory properties.

Recent literature describes the new and exciting field of 'MSC necrobiology', which proposes a novel solution to some of the aforementioned barriers. Necrobiology provides four mechanisms by which derivatives of mesenchymal stromal cells can maintain significant clinical efficacy: apoptosis, autophagy, mitochondrial transfer, and extracellular vesicles [29]. MSC products derived from these pathways have been proven to ameliorate a plethora of pathologies (Table 1). Indeed, MSC derivatives (Fig. 2) have, in some cases, been shown to be as therapeutically beneficial as living

MSCs [30–33], but have the advantage of being able to pass easily through the circulatory system.

Mesenchymal stromal cells that have undergone apoptosis have been shown to reduce inflammatory endpoints in mouse models of allergic airway inflammation induced by ovalbumin (OVA) causing noncytotoxic T helper-type cell inflammation [34]. Apoptotic rat adipose-derived MSCs have also been proven superior in ameliorating the condition of cecal ligation and puncture-induced sepsis in rat models in comparison with living MSCs [35–37]. Autophagic mesenchymal stromal cells have also been proven to have therapeutic benefits. MSCs derived from human bone marrow that have undergone autophagy have been proven to regulate CD4+ T helper cells via TGF-β1 signaling [38]. Similarly, when MSCs underwent autophagy as a result of the administration of rapamycin, their ability to suppress CD4+ T helper cell proliferation was improved [38]. MSCs subjected to mitochondrial transfer also seem to be effective in mitigating the symptoms of asthma [39], chronic obstructive pulmonary disorder [40], cardiomyopathy [41], acute respiratory distress syndrome, and sepsis [42]. There is also evidence that extracellular vesicles derived from MSCs hold clinical efficacy in pathologies of the kidney [43], heart [44], lung [45], and skin [46].

In recent years, exosome therapy for certain inflammatory conditions has garnered significant interest. Almost all cells in the human body can produce exosomes: Extracellular vesicles produced by an

Table 1. Details of published studies investigating the therapeutic efficacy of MSC derivatives originating from four distinct necrobiology pathways.

Authors	Mechanism/ Pathway	Condition(s) of interest	Results
Galleu et al. [34]	Apoptosis	Allergic airway inflammation and Graft-versus-host disease	↓ Eosinophil infiltrate in bronchoalveolar lavage (BAL) and ↓ Inflammatory endpoints ↑ Immunomodulation
Chang et al. [35]	Apoptosis	Sepsis syndrome induced by cecal puncture and ligation	↓ Cecal and kidney injury ↓ TNF-α
Chen et al. [36]	Apoptosis	Kidney injury induced by sepsis	↓ Kidney injury
Sung et al. [37]	Apoptosis	Lung and kidney injury induced by sepsis syndrome	↓ Lung and kidney injury ↓ TNF-α ↓ NF-κB
Gao et al. [38]	Autophagy	This study investigated generally if the activation of autophagy in MSCs ameliorates their immunosuppressive capacity	↑ TGF-β1 secretion ↑ Immunomodulation
Yao et al. [39]	Mitochondrial transfer	Asthma	↓ Asthma inflammation ↓ IL-4, IL-5, IL-13
Li et al. [40]	Mitochondrial transfer	Airway epithelial cell damage induced by cigarette smoke in chronic obstructive pulmonary disorder (COPD)	↓ Lung injury
Zhang et al. [41]	Mitochondrial transfer	Cardiomyopathy	Improved heart function
Morrison et al. [42]	Mitochondrial transfer	Acute respiratory distress syndrome (ARDS)	↑ M2 macrophage expression ↓ TNF-α ↓ IL-8
Collino et al. [43]	Extracellular vesicle derivation	Acute kidney injury	↑ Renal tubular cell proliferation ↓ Renal tubular cell apoptosis
Lai et al. [44]	Extracellular vesicle derivation	Myocardial ischemia/reperfusion injury	↑ Cardioprotective effects
Li et al. [45]	Extracellular vesicle derivation	Acute lung injury	↓ Oxidative stress injury ↓ Inflammatory response
Wu et al. [46]	Extracellular vesicle derivation	Cutaneous injury	↑ Wnt4 ↑14-3-3ζ protein ↑ Wound healing

endosomal pathway are approximately 30–150 nm in size [47]. Exosomes were previously thought of as having the sole responsibility of clearing cellular debris; however, recent scientific literature points to their ability to modulate intracellular environmental conditions. It is believed that only certain exosomes are immunoregulatory. Thus, a subclass of exosomes has been described as 'signalosomes', which, when released from MSCs, have an exceptional capacity for immunoregulation [47].

Exosome therapy using 'signalosomes' has been investigated for a plethora of pathologies including diseases of the heart [48], lung [49], kidney [50], brain [51], liver [52], intestines [53], skin [54], and nervous system [55] as well as for sepsis [56] and various cancers [57,58]. In 2014, a clinical trial was conducted using exosome therapy for steroid-refractory graft-vs-host disease and showed positive results [59]. Another study that investigated the use of exosomes as a novel therapeutic strategy for neonatal chronic lung disease compared their efficacy to that of a whole MSC population [60]. The study indicated that exosomes themselves hold an equivalent and sometimes even superior ability to modulate the inflammatory response. The use of MSC derivatives for treatment holds several advantages over the use of a live MSC population. They can be less costly to produce and can last up to 6 months when stored at −20 °C [61]. Derivatives are also a safer treatment option compared with whole MSCs as there are no risks associated with mutagens

or oncogenicity. However, it should be pointed out that there is no documented technique to isolate 'signalosomes' from exosomes derived from other biogenic pathways, which may not possess the same immunomodulatory capacity [47].

Exosomes, in general, can be isolated by several methods. One of the most common methods is ultracentrifugation, although this requires costly apparatus (for industrial scale-up) and the technique can promote vesicle aggregation which is detrimental to the structural and metabolic integrity of the exosomes produced [62]. Milder techniques of isolation have been described, for example, gradient density isolation and size-exclusion chromatography. However, these techniques also have issues as density-based separation may lead to enrichment as opposed to true isolation which may result in the presence of unwanted particles [63,64]. Additionally, this technique does not allow for the large-scale bioprocessing of exosomes, therefore making it an unrealistic technique in the context of therapy [63,64].

Fortunately, there are other MSC-derived products that are disruptive since production technology is far simpler and less costly than producing exosomes. Studies have been conducted indicating that cellular lysates have a similar therapeutic benefit in the treatment of various pathologies as compared to exosomes and whole-cell MSCs themselves. Eleven published studies use cellular lysate derived from mesenchymal stromal cells from different origins to treat diverse conditions. The studies investigate the lysate's effect on aging [65], erectile dysfunction [66], fulminant hepatic failure [67], stroke [68], osteosarcoma and mammary carcinoma [69], epilepsy [70], liver failure [71], wound healing [72], ischemia [73], lymphoma [74], and obesity [75]. All of the aforementioned studies except the study investigating its effects on aging demonstrated that the lysate was able to ameliorate *in vitro* and *in vivo* models.

Recently, MSC derivative therapy has been proven effective in treating pathologies that are autoimmune in nature. Apoptotic MSCs have demonstrated immunosuppressive properties in mouse models of GVHD [38], and extracellular vesicles have improved the symptoms associated with uveitis/uveoretinitis and type I diabetes mellitus [76]. In addition, exosomes have shown success in ameliorating autoimmune conditions including multiple sclerosis [77], Sjögren's syndrome [78], graft versus host disease [59], systemic lupus erythematosus [79], and rheumatoid arthritis [80]. These results suggest the potential use of derivatives for other autoimmune conditions that lack entirely favorable treatment regimens, such as autoimmune pancreatitis.

Conclusion

In conclusion, employing MSCs in the treatment of autoimmune pancreatitis remains a promising putative cell therapy. However, the recent emergence of a variety of methods to produce MSC derivatives provides a newer and simpler approach that could have significant advantages over the use of cells themselves including simpler production, lower regulatory barriers, and easier systemic transport upon intravenous delivery.

Conflict of interest

The authors declare no conflict of interest.

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