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Multipotent Mesenchymal Stromal Cells for Autoimmune Diseases

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Key Words
Mesenchymal · Stromal · Stem cell · MSC · Autoimmune disease

Summary
Multipotent mesenchymal stromal cells (MSC) are under consideration for the treatment of autoimmune disease (AD) based on their in vitro antiproliferative properties, efficacy in animal models, apparent low acute toxicity, and the early positive anecdotal outcomes in human acute graft versus host disease. Phase I/II clinical trials are under way in multiple sclerosis and Crohn's disease, and are being planned in systemic lupus erythematosus, systemic sclerosis, systemic vasculitis, and other AD. Open issues include: patient selection, disease stage and activity, MSC source and expansion, and long-term safety. Multidisciplinary groups including EULAR are collaborating to ensure maximal use of available resources to establish the place, if any, of MSC in the treatment of AD.

Schlüsselwörter
Mesenchymal · Stromal · Stammzelle · MSC · Autoimmunkrankheit

Zusammenfassung
Aufgrund ihrer antiproliferativen Eigenschaften in vitro, ihrer Effektivität in Tiermodellen, ihrer scheinbar geringen akuten Toxizität sowie aufgrund einzelner Fallberichte positiver Outcomes bei akuter Graft-versus-Host-Krankheit beim Menschen wird der Einsatz von multipotenten mesenchymalen Stromazellen (MSC) für die Behandlung von Autoimmunkrankheiten diskutiert. Für multiple Sklerose und Morbus Crohn werden derzeit klinische Phase-I/II-Studien durchgeführt, für systemischen Lupus erythematosus, systemische Sklerose und systemische Vaskulitis sowie andere Autimmunkrankheiten sind sie in der Planung. Folgende Fragen sind offen: Patientenselektion, Krankheitsstadium und -aktivität, MSC-Quelle und -Expansion sowie Sicherheit im Langzeitverlauf. Multidisziplinäre Gruppen, einschließlich EULAR, arbeiten zusammen, um bei der Etablierung der Rolle der MSC – wenn sie überhaupt eine spielen – in der Behandlung von Autoimmunkrankheiten die bestmögliche Verwendung vorhandener Ressourcen zu gewährleisten.

Introduction

Multipotent mesenchymal stromal cells (MSC) are also referred to as mesenchymal stem cells, though their true 'stemness', i.e. on division one daughter cell remains stem and the other is able to replenish a whole tissue compartment, has yet to be demonstrated [1]. MSC are capable of differentiating in vitro and in vivo into different MSC lineages, including adipose, bone, cartilage, muscle, and myelosupportive stroma [2–5]. MSC may be isolated from bone marrow, skeletal muscle, adipose tissue, synovial membranes, and other connective tissues of human adults [6–9] as well as cord blood [10] and placental products [11], and are defined by using a combination of phenotypic markers and functional properties. Controversy still exists over the in vivo phenotype of MSC: however, ex vivo-expanded MSC do not express the hematopoietic

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markers CD14, CD34, CD45, and MHC class II. In addition to their multipotentiality, they can be identified as cells that stain positive for CD73, CD90, and CD105 by flow cytometry [12]. In vitro, MSC have vast proliferative potential, can clonally regenerate, and can give rise to differentiated progeny. They also exhibit anti-proliferative and anti-inflammatory properties in vitro and in vivo, making them candidates for treatment of acute inflammatory autoimmune disease (AD) [13]. Regardless of whether or not MSC are true stem cells, clinical benefit from MSC may not require sustained engraftment of large numbers of cells or differentiation into specific tissues. It is possible that a therapeutic benefit can be obtained by local paracrine production of growth factors and a provision of temporary anti-proliferative and immunomodulatory properties.

Until recently, it was assumed that MSC enjoyed immune privilege in allogeneic settings [14], neither exerting nor being subject to immunological reaction, but recent data suggest, that in a non-immunosuppressed host, allogeneic MSC will be eliminated [15], especially if gene transfected [16]. In vitro results indicate that MSC possess immunosuppressive properties. Rodent, baboon, and human MSC suppress T and B cell lymphocyte proliferation in mixed lymphocyte cultures or induced by mitogens and antibodies in a dose-dependent fashion [17–24]. The suppression is MHC-independent, and in human cell cultures, the magnitude of suppression is not reduced when the MSC are separated from the lymphocytes in transwells, indicating that cell-cell contact is not required [17, 19, 25]. However, not all experiments have shown anti-proliferative effects. Traggiai et al. [26] studied the influence of bone marrow mesenchymal stem cells on highly purified B cells from healthy donors and children with systemic lupus erythematosus (SLE), and found that they promoted proliferation and differentiation into immunoglobulin-secreting cells of both transitional and naive B cells stimulated with an agonist of Toll-like receptor 9, in the absence of B cell receptor triggering. They also strongly enhanced proliferation and differentiation into plasma cells of memory B cell populations. A similar effect was observed in response to polyclonal stimulation of B cells isolated from pediatric patients with SLE.

Mechanism of Immunosuppression and Antiproliferation

The mechanism(s) underlying the immunosuppressive effect remain to be fully clarified with sometimes conflicting data probably reflecting the variable definitions and experimental conditions. Initially, the fact that MSC when exposed to medium levels of interferon gamma (IFN-γ) express class II antigen but never co-stimulation molecules, suggested that they may induce anergy or apoptosis in cell-cell contact conditions. Unlike professional antigen-presenting cells (APC), MSC have a bimodal response to IFN-γ in that low levels stimulate and high levels suppress HLA class II expression [27]. However, apart from one publication involving proliferating lymphocytes [28], induction of apoptosis has not been shown – in fact arrest of apoptosis may be a major mechanism for MSC imparting a survival signal to other cells such as neutrophils in the bone marrow niche [29]. Transwell experiments have demonstrated putative paracrine soluble factors including hepatocyte growth factor (HGF) and transforming growth factor-β1 (TGF-β1) [19], prostaglandin E_2 accounting for reduced lymphocyte proliferation [30], indoleamine 2,3-dioxygenase [31], inducible NO synthetase resulting in Stat 5 inhibition in lymphocytes [32], soluble HLA-G [33], and soluble interleukin 1 receptor [34]. Clearly a major anti-proliferative mechanism in lymphocytes is arrest of the cell cycle in G0/G1 [24].

Fate of Transplanted Mesenchymal Stromal Cells in vivo

There are scarce data. In animals, radio labeling experiments in rats show localization after intra-arterial and intravenous infusion mostly in lungs and secondarily in liver and other organs [35]. More detailed studies in 3 baboons (2 using autologous and 1 allogeneic MSC) and using the green fluorescent protein retroviral construct, showed that gastrointestinal tissues harbored high concentrations of transgene per microgram of DNA. Additional tissues including kidney, lung, liver, thymus, and skin were also found to contain relatively high amounts of DNA equivalents. Estimated levels of engraftment in these tissues ranged from 0.1 to 2.7%, similar in the autologous and allogeneic experiments.

Active homing of MSC to bone marrow occurs largely due to the stromal derived factor-1 (SDF-1) interacting with CXCR4 on the MSC surface [36], and similar mechanisms are operational in ischemic tissue [37]. A new initiative is to modify surface structures on MSC in order to increase their penetration and integration into specific target tissues. It has been shown that recruitment of cells to bone occurs within specialized marrow vessels that constitutively express vascular E-selectin, a lectin that recognizes sialofucosylated determinants on its various ligands. Sackstein et al. [38] showed that human MSC do not express E-selectin ligands, but express a CD44 glycoform bearing alpha-2,3-sialyl modifications. They converted the native CD44 glycoform on MSC into hematopoietic cell E-selectin/L-selectin ligand (HCELL) using an alpha-1,3-fucosyltransferase preparation and enzymatic conditions specifically designed for treating live cells, which conferred potent E-selectin binding without effects on cell viability or multipotency. Real-time intravital microscopy in immunocompromised (NOD/SCID) mice showed that intravenously infused HCELL(+) MSC infiltrated marrow within hours of infusion, with ensuing rare foci of endosteally localized cells and human osteoid generation. If active homing of MSC to inflamed and ischemic tissue were the case in humans, this would increase the feasibility of cellular therapy for AD, since independent of the putative soluble factor(s) produced by them, they would be delivered directly into the target tissue, reducing the need

for using either large numbers of MSC or high concentrations of biological agents systemically.

MSC homing to tumors is of concern as shown by human MSC localization to a murine xenogenic breast cancer SCID mouse model via monocyte chemotactic protein-1 (MCP-1) [39] which while being a potential therapeutic delivery system for cancer therapy, may pose long-term safety issues in AD treatment. MSC concentrate in radiation damaged and ischemic tissue [40]. This may be an important advantage when treating some acute inflammatory AD with accompanying critical ischemia such as vasculitis or systemic sclerosis (SSc). In humans receiving sex mismatched allogeneic MSC, sex chromosomal analysis of MSC cells have shown to be in the gastrointestinal tract and bone marrow for many months, though extensive data are lacking.

Animal Models of Tissue Protection

It may be impossible, in fact meaningless, to separate the anti-inflammatory, immunomodulatory, and tissue protective 'trophic' effects of MSC [41]. An immunosuppressive effect of MSC in vivo was first suggested in a baboon model where infusion of ex vivo-expanded donor or third-party MSC delayed the time to rejection of histoincompatible skin grafts [22]. MSC also downregulate bleomycin-induced lung inflammation and fibrosis in murine models if given early (but not late) after the induction [42]. A similar effect was seen in a murine hepatic fibrosis model (carbon tetrachloride-induced) using a MSC line bearing the fetal liver kinase-1 (FLK1) marker [43]. More recently, fulminant hepatic failure in a rat model was abrogated by both MSC and MSC-conditioned medium, suggesting a leukocyte diversion mechanism by paracrine substances such as chemokines [44]. Tissue protective effects were also seen in a rat kidney model of ischemia/reperfusion injury in which syngeneic MSC but not fibroblasts were used [45]. Recently, the same group showed a vasculotropic effect of infused MSC in the kidney [46], which may be relevant to diseases such as SSc.

Animal Models of Autoimmunity

In the two experimental autoimmune encephalomyelitis (EAE) murine models, both clinical and histological improvement occurred. The responses were dependant on time of MSC treatment, the earlier the better, and were reversed with IL-2 treatment, indicating that anergy rather than apoptosis had occurred [47, 48]. In one model, the MSC were located in the secondary lymph organs but not the central nervous system, despite a positive clinical outcome. This is reminiscent of the induction of regulatory T cells (Treg) by antigen vaccination [49]. However, in a murine model of arthritis, collagen-induced arthritis (CIA) was not improved by the addition of MSC, and the in vitro immunosuppressive effects were reversed by the addition of TNFα. MSC were not found in the joints [50]. A second murine arthritis model showed however a positive clinical outcome [51].

Recently, a murine model of streptozocin-induced diabetes mellitus was reported to improve clinically following transplantation with a combination of bone marrow-derived cells and syngeneic and allogeneic MSC following sublethal irradiation [52]. The proposed mechanism was regeneration of recipient-derived islet cells plus immunosuppression of autoreacting T cells. Neither cell product alone was effective.

Mesenchymal Stromal Cells and Human Experience

Ex vivo-expanded allogeneic MSC have been infused in several phase I studies [53–57]. No adverse events during or after MSC infusion have been observed, and no ectopic tissue formation has been noted. After infusion, MSC remain in the circulation for no more than an hour [56]. Although durable stromal cell chimerism has been difficult to establish, low levels of engrafted MSC have been detected in several tissues [54, 57, 58].

It is possible that sufficient therapeutic benefit is obtained by local paracrine production of growth factors and the provision of temporary immunosuppression by MSC infusion. Infusion of haploidentical MSC to a patient with steroid-resistant severe acute graft versus host disease (GvHD) of the gut and liver promptly improved liver values and intestinal function [59]. Upon discontinuation of cyclosporine, the patient's acute GvHD recurred but was still responsive to a second MSC infusion. Lymphocytes from the patient, when investigated on multiple occasions after MSC infusion, continued to proliferate against lymphocytes derived from the haploidentical MSC donor in co-culture experiments. This suggests an immunosuppressive effect of MSC in vivo, rather than a development of tolerance. The European Group for Blood and Marrow Transplantation (EBMT) is currently running protocols for prevention and treatment of acute GvHD through the Developmental Committee, and interim results of the treatment trial have recently been published [60]. Thirty out of 55 steroid-resistant acute GvHD patients had a complete response with no immediate toxicity. Median dose of allogeneic MSC was 1.4×10^6/kg body weight given up to 5 times. Four patients had a recurrence or de novo malignancy, and the complete responders had a reduced transplant-related mortality at 12 months (37 vs. 72%) and higher overall 2-year survival (52 vs. 16%).

Mesenchymal Stromal Cells from Human Autoimmune Disease

Autologous bone marrow-derived MSC have been shown to be potently anti-proliferative to stimulated T cells from nor-

Table 1. Clinical trials using MSC for autoimmune diseases in the US National Institutes of Health (NIH) public web site

Study title	Disease indication	Center	Interventions	Phase
Prochymal™ Adult Human Mesenchymal Stem Cells for Treatment of Moderate-to-Severe Crohn's Disease	Crohn's disease	Osiris Therapeutics, USA (manufacturer)	drug: Prochymal; adult human mesenchymal stem cells; drug: adult human MSC	phase II active, not recruiting
Extended Evaluation of Prochymal™ Adult Human Stem Cells for Treatment-Resistant Moderate-to-Severe Crohn's Disease	Crohn's disease	Osiris Therapeutics, USA	drug: adult human mesenchymal stem cells; drug: adult human mesenchymal stem cells	phase III recruiting
Prochymal™ to Treat Crohn's Disease	colitis; Crohn's disease; inflammatory bowel disease	NIH Clinical Center (CC), USA	drug: Prochymal; mesenchymal stem cells; procedure: colonoscopy; procedure: infusion	phase III recruiting
Effect of Mesenchymal Stem Cell Transplantation for Lupus Nephritis	lupus nephritis	Organ Transplant Institute, China	biological: mesenchymal stem cell	phase I/II not yet recruiting
Cotransplantation of Islet and Mesenchymal Stem Cell in Type 1 Diabetic Patients	type 1 diabetes mellitus	Fuzhou General Hospital, China	biological: cotransplanta tion of islet and mesenchymal stem cells	phase I/II recruiting
Mesenchymal Stem Cells in Multiple Sclerosis (MSCIMS)	multiple sclerosis	University of Cambridge, UK	procedure: intravenous administration of bone marrow-derived auto-logous adult MSC	phase I/II not yet recruiting
Evaulation of Prochymal ™ Adult Human Stem Cells for Treatment Resistant Moderate to Severe Crohn's disease	Crohn's disease	Osiris Therapeutics, Duke University, USA	intravenous, two-dose (4 infusions over 4 weeks) placebo controlled double blind	phase III recruiting

mal subjects and autoimmune patients (rheumatoid arthritis, SSc, Sjoegrens, SLE) [61], and in SSc patients these MSC were normal in respect to proliferation, clonogenicity, and differentiation into bone and fat [62]. However, one group has shown defective differentiation into endothelial precursors in bone marrow-derived MSC from SSc patients [63], which should be considered when choosing autologous or allogeneic MSC sources for SSc treatment. MSC are being tested widely as potential agents for increasing neovascularization in critical ischemia settings, with the attendant risk of increasing tumor growth [64]. In fact, MSC may also play a role in reducing tumor surveillance, as shown in a murine melanoma model [65].

Mesenchymal Stromal Cells for Human Autoimmune Disease

Currently, few peer-reviewed publications concerning the results of using MSC in human AD are available. A small series of 10 multiple sclerosis (MS) patients from Iran was reported using autologous intrathecal MSC. The conclusion was that it was feasible, the clinical results being mixed. A small study in Crohn's disease has been announced by Osiris Therapeutics Inc., Baltimore, MD, USA [66] – details of the outcome being not yet available.

Ongoing Studies and Future Directions

There are several running phase I/II and III clinical trials in AD including MS, Crohn's disease, and type I diabetes mellitus. Table 1 shows the AD studies registered in the US National Institutes of Health public information site (*www.clinicaltrials.gov*). A total of 43 trials are registered under MSC trials, 10 being for GvHD, 7 for cardiological indications, 7 for AD, and the rest for various tissue engineering and tissue protection indications. Important is the setting of clear therapeutic targets and harmonization of cell products, especially MSC source and type (autologous or allogeneic), cell expansion conditions, and trial protocols. In addition, long-term safety data collection across disciplines is required, and an inter-

national interdisciplinary registry of MSC-treated patients has been launched [67].

Conclusion

There are now data to suggest that the benefit/risk ratio for MSC in human is acceptable, mostly derived from acute GvHD studies. The fact that conditioning of the patient with cytotoxic and other immunosuppressive agents is not necessary prior to MSC infusion, makes such a treatment attractive in a critically ill patient who would be unable to tolerate an autologous hematopoietic stem cell transplant, an alternative strategy for severe AD not responding to conventional therapy.

The choice of patient, i.e. multisystem, inflammatory disease similar to acute GvHD or less active end stage disease, depends on whether the study aim is efficacy or feasibility/safety. Immune privilege of MSC is not guaranteed in an immune competent host, but that may not be important if the MSC home to the target organ and survive long enough to exert a positive therapeutic effect. Clearly, an international and interdisciplinary effort is required, as is being undertaken by the European League Against Rheumatism (EULAR) and EBMT [68].

Conflicts of Interest

The author has no conflict of interests.

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