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Editorial

Mesenchymal stem cells: A future for the treatment of arthritis?

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1. Background

Rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA) are chronic inflammatory diseases characterized by destruction of cartilage and bone. The destructive lesions result from both immune responses and non-antigen-specific inflammatory processes. Strong evidence indicates that autoantigen recognition by specific T cells is crucial to the pathophysiology of rheumatoid synovitis. Furthermore, in addition to direct autoimmune attack by effector T cells, arthritis may result from defective homeostatic control of immunity by regulatory T cells [1]. Although an increasing number of biologicals have been added to the therapeutic options of a rheumatologist, there is still a category of patients that fails them all and suffers from progressive disease. Recently, cellular therapy by means of autologous (haematopoietic) stem cell transplantation became a treatment option for RA and JIA patients with insufficient response to conventional treatment. This requires, however a strong immune-ablative pre-treatment with high morbidity and even mortality.

An immunosuppressive subset of the non-haematopoietic mesenchymal stem cells also called mesenchymal stromal cells (MSCs) would not require such a hazardous pre-treatment. Since a few years these MSCs are a fast growing field of interest for science: 3286 (72%) of the 4584 publications found with the search term ‘‘mesenchymal stem cells’’ are written since 2005.

2. Mesenchymal stem cells

MSCs are adult (non-embryonic) stromal cells with multipotentiality, meaning that they have the ability to differentiate into various mesodermal cell lineages which are also very important in inflammatory arthritis. Recently, it has been proved that a single-cell-derived population was capable of differentiating into different mesenchymal cell types in vivo [2]. By current definition, MSCs do at least express CD73, CD105 and CD90, but lack the haematopoietic and endothelial markers CD45, CD11b, CD31 and CD14. MSCs are capable of in vitro formation in colonies of fibroblastlike cells (CFU-F) and the frequency of these colonies is accepted as a measure of MSC-content. Since there is no uniformly accepted method for prospective isolation of MSCs yet, their ability to adhere to plastic and their differentiation in vitro into osteoblasts, chondrocytes and adipocytes is still needed for their profile. MSCs are present in relatively low numbers in bone marrow, fat, muscle and in lower numbers in many other tissues. BM-derived MSCs (BM-MSCs) are part of the haematopoietic niche, where they support haematopoiesis. The synovial membrane has shown to be a great source for the functionality of MSCs with highest multipotentiality for adipogenesis, osteogenesis, chondrogenesis and myogenesis [3]. MSCs can be expanded ex vivo up to a billion-fold without loss of their multipotent properties and are excellent vehicles for gene therapy (e.g. for specific homing or cytokine production) since they maintain the expression of transfected genes for up to 40 divisions.

MSCs are studied widely for regenerative purposes and it has been shown that a single local delivery of adult mesenchymal stem cells to injured joints stimulates regeneration of meniscal tissue and retards the progressive destruction normally seen in a caprine model of osteoarthritis [4]. Muscle damage stimulates influx of MSCs into the muscle compartment, the influx being commensurate with the damage. The recruited MSCs differentiate into myoblasts thereby repairing the damage [5]. MSCs tend to migrate to areas with local damage. Systemic administration of syngeneic MSCs or allogeneic MSCs promoted the healing of fascial and cutaneous incisional wounds. Labeling of the MSCs with iron oxides/fluorescent dye revealed that systemically administered MSCs engrafted to the wound [6]. Recently, specific homing of MSCs to the bone marrow was accomplished without genetic modification. Cellular recruitment to bone occurs within specialized marrow vessels that constitutively express vascular E-selectin. Human MSCs do not express E-selectin ligands, but express a CD44 glycoform bearing a-2,3-sialyl modifications. By enzymatic processes the native CD44 glycoform on MSCs was converted into haematopoietic cell

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E-selectin/L-selectin ligand (HCELL), which conferred potent E-selectin binding without effects on cell viability or multipotency. Real-time intravital microscopy in immunocompromised mice showed that intravenously infused HCELL + MSCs infiltrated marrow within hours of infusion, with ensuing rare foci of endosteally localized cells and human osteoid generation [7]. The engraftment in tissues of MSCs seems to be low. Only 0.1–2.9% of the human MSCs injected in the left ventricle of diabetic NOD/SCID mice were found in the pancreas after 32 days and in the kidneys this was up to 11.6% [8]. In a non-conditioned baboon however the transgene marker, enhanced Green Fluorescent Protein (eGFP), could be detected in the bone marrow 1 year after intravenous administration of gene-marked autologous BM-MSCs [9].

Another very important and largely studied feature of MSCs is the potent immunosuppressive and anti-inflammatory effects they exert in vitro and in vivo. MSCs seem to regulate lymphopoiesis and suppress the immune response as well. Bone marrow-MSCs participate in the developmental process of both T lymphocytes and B lymphocytes through growth factors, cytokines, adhesion molecules. Some crucial surface molecules are expressed on both MSCs and thymic stromal cells, indicating similarities between two different microenvironments of bone marrow compartments and thymus. Moreover, MSCs mediate immunoregulatory effects on both innate and adaptive immunity through either indirect soluble factors or direct cell–cell contact. Numerous studies convincingly demonstrate that MSCs are able to modulate the function of different immune cells in vitro, particularly involving the suppression of T-cell proliferation and the inhibition of dendritic cell maturation. This effect is dose dependent and is exerted on T-cell responses to polyclonal stimuli or to their cognate peptide. The inhibition does not appear to be antigen specific and targets both primary and secondary T-cell responses. After co-culture with MSCs, the T cells exhibited a regulatory phenotype. MSCs downregulated T cell responses through direct contact and secretion of anti-inflammatory and tolerogenic cytokines, which may involve the recruitment of regulatory T cells. The general effects are to skew the immune response towards anti-inflammatory/tolerant phenotypes, including the shift from Th1 towards Th2, downregulation of IFN-γ production from NK and reduction in the antibody productions of B cells [10]. Such immunosuppressive activity does not seem to be spontaneous but requires MSCs to be 'licensed' in an appropriate environment. IFN-γ has been shown to be a powerful inducer of such activity [11]. Immunosuppression might not be confined to MSCs only, since stromal cells (e.g. chondrocytes or fibroblasts) from different human tissues were recently shown to inhibit the proliferation of peripheral blood monocytes following polyclonal stimuli. This was in marked contrast to parenchymal cells [12].

Moreover, MSCs express low levels of human MHC class I and lack human MHC class II. In addition, MSC do not express the CD40, CD80 or CD86 co-stimulatory molecules. All these features make that MSCs are less immunogenic which enables their transplantation in an non-immunocompromised allogeneic host without rejection. However, under some experimental conditions, MSCs infused into allogeneic, MHC-mismatched mice have been rejected after some time [13]. Thus, at least in some experimental conditions, the immune privilege of MSCs is limited.

In vivo the immunosuppressive effect was first demonstrated convincingly by the prolongation of skin graft survival after systemic administration of allogeneic MSC in baboons. The most significant results so far have been obtained by the successful treatment of severe, treatment-resistant, graft-versus-host disease (GvHD) with third party MSCs which resulted in a two prospective randomized European phase III studies to further explore the therapeutic usefulness of MSCs for the treatment or prevention of acute GVHD following allogeneic stem cell transplantation [14]. Murine MSCs have been demonstrated to ameliorate experimental autoimmune encephalomyelitis (EAE), a model of human multiple sclerosis, through the induction of peripheral T cell tolerance against the pathogenic antigen [15]. The infusion of MSCs was only effective at disease onset and at the peak of the disease, but not after disease stabilization. Central nervous system pathology showed decreased inflammatory infiltrates and demyelination in mice that received transplants of MSCs. T-cell response to the specific antigen and mitogens from MSC-treated mice was inhibited and restored by IL-2 administration. Gene-marked MSCs (eGFP) were detected in the lymphoid organs of treated mice. Many phase I and II trials with the use of MSCs are going on at the moment such as for treatment of acute myocardial infarction, end-stage ischemic heart disease, or prevention of vascular restenosis through stem cell mediated injury repair. Trials for treatment of osteogenesis imperfecta, amyotrophic lateral sclerosis, periodontitis, heart failure, bone fractures, osteonecrosis and lysosomal storage diseases. Unfortunately, some of these studies are performed before the proper use of existing animal models and most of them are without randomized controls.

3. Arthritis and MSCs

The frequency of MSCs in human synovial fluid (SF) of osteoarthritis (OA) patients is higher than in patients with early or established RA [16]. MSCs with immunomodulatory capability are present in the SF of JIA patients as well. MSCs have been shown to be capable of adhesion-independent survival and expansion as floating single cells. Furthermore, individual MSCs can survive in the viscous, antiadhesive medium of the SF of RA patients in vivo and, in spite of their pathologic environment, can maintain their multipotentiality in vitro. It was postulated that synovial fluid recruits mesenchymal progenitor cells derived from the subchondral spongious bone marrow. In collagen-induced arthritis there is an antigen-independent (innate) prearthritic phase requiring the presence of TNFα which involves the early arrival of marrow-derived mesenchymal cells [17]. At the onset of antigen-induced arthritis MSCs were attracted from the bone marrow to the joint, some of which might form fibroblastlike synoviocytes (FLS) [18]. FLS and MSCs isolated from the joint and bone marrow of RA patients provided survival factors to T and B cells in vitro. So the role of MSCs in arthritis might be contributory to

pathogenesis or the (failing) attempt to suppress the inflammatory reaction [19]. The potential of SF of RA patients for recruitment of the mesenchymal progenitor cells was impaired when compared to SF from normal or OA donors [20].

Bone marrow-MSCs (BM-MSCs) from RA patients ($n = 26$) and age-/sex-matched healthy individuals ($n = 21$) were similar in frequency, differentiation potential, survival, immunophenotypic characteristics, and protein profile. Patient MSCs, however, had impaired clonogenic and proliferative potential in association with telomere length loss. Previous treatment with methotrexate, corticosteroids, anti-cytokine and biological agents or other disease-modifying anti-inflammatory drugs did not correlate with the clonogenic and proliferative impairment of BM-MSCs [21]. In another study, however, the same clonogenicity (CFU-F frequency) was found between BM-MSCs from healthy donors and patients with RA. BM-MSCs from both healthy donors as well as from patients with an autoimmune disease reduced the proliferation of autologous and allogeneic PBMCs by up to 90% in a cell dose-dependent fashion. MSCs prevented the onset of the in vitro immune response and also downregulated an ongoing immune response. Furthermore it was independent of the clinical activity of the autoimmune disease [22].

MSCs can produce matrix proteins such as type II collagen (CII). Since CII is a major component of hyaline cartilage that acts as an autoantigen in RA, it was necessary to prove that MSCs are capable of suppressing T cells that specifically respond to collagen. One recent study in which 18 of the 37 RA patients (48.6%) had specific T-cell responses to CII in both peripheral blood and SF showed that MSCs from healthy donors significantly suppressed CII-stimulated T-cell proliferation and activation. Moreover, the MSCs inhibited production of IFN-γ and TNFα by both CD4+ and CD8+ T cells, while they increased the levels of IL-10 and restored the secretion of IL-4. TGF-β1 was confirmed to play a critical role in the inhibition [23].

The first study describing collagen-induced arthritis treated with mesenchymal stem cells showed that intravenously administered MSCs from the murine C3H10T1/2 MSC line (C3 MSC) did not confer any clinical, radiological or histological benefit in the incidence or severity of arthritis [24]. MSCs tended to worsen the clinical symptoms when injected at high dosages (4 million) or later injection. Both the clinical and the immunologic findings suggested that MSCs were associated with augmentation of the Th1 response. Using luciferase-expressing MSCs, they were unable to detect labeled cells in the articular environment of the knee, suggesting that worsening of the symptoms was unlikely due to the homing of MSCs in the joints. Experiments in vitro showed that the addition of TNFα was sufficient to reverse the immunosuppressive effect of MSCs on T cell proliferation, and this observation was associated with an increase in interleukin-6 secretion. The yet unpublished results from the same group using freshly cultured MSCs instead of the use of the C3 mesenchymal stem cell line are much more promising. Timing seemed to be very important; a preventive systemic injection did suppress arthritis, but when the first injection was administered at stages of established arthritis no positive effect was seen (personal communication with C. Jorgensen). Another study demonstrated that preventive iv injections of MSCs three times weekly for 24 days significantly decreased the onset and severity of collagen-induced arthritis in mice for IL-10 producing MSCs only and not for normal MSCs [25]. A recent study showed that a single intraperitoneal injection of high dose (5 million) allogeneic MSCs significantly reduced the incidence and severity of collagen-induced arthritis and prevented the occurrence of bone and cartilage erosions in the joints of immunized mice [26]. Treatment with MSCs in established arthritis also prevented exacerbation of the disease; the incidence and disease score were comparable with those of animals that received MSC therapy at the time of first immunization. T cells from immunized mice that were treated with MSCs showed significant reduction in in vitro proliferation, mitogen-induced proliferation, and CII-recalled proliferation. The serum concentration of TNFα was significantly decreased in immunized mice treated with allogeneic MSCs. In contrast, secretion of TNFα by in vitro stimulated CII-primed T lymphocytes was significantly increased when they were cocultured with allogeneic MSCs. One week after intraperitoneal injection of MSCs, they were found as ghost cells in the spleen. At day 11 both the peritoneum and spleen were negative. MSC treatment in immunized mice induced proliferation of antigen-specific clones of Tregs with a CD4+, CD25+, CD27+, FoxP3+ phenotype, suggesting that the immunosuppressive activity of MSCs could be prolonged by the action of Treg clones that can be activated by an antigen-specific stimulus. In another animal model of RA (proteoglycan induced arthritis) we showed that a single injection of MSCs, especially high dose (5 million) intraperitoneal MSCs, decreased progression of arthritis score in mice with established arthritis. The MSCs that were injected in the right knee, remained viable in the joint for at least five weeks and might have exerted their immunosuppressive action in a paracrine manner. Migration to sites of inflammation of MSCs injected intravenously or intraperitoneally was not seen with Bioluminescence imaging. Currently, we are investigating the effect of both systemical and local repeated injections of MSCs on clinical score and histology of proteoglycan induced arthritis.

4. Future directions

We conclude that patients with inflammatory arthritis have functional MSCs in their bone marrow as well as in their synovial fluid. The ongoing inflammation despite their presence could be due to a low number of MSCs, the inhibitory effect of the cytokine environment or a combination of both.

There is a definite need for standardized protocols for isolation, culture and expansion of MSCs, since many groups have their own which makes the results much harder to interpret. It is nowadays possible to expand the MSCs in animal-protein-free media (e.g. platelet-lysate or autologous serum) which is preferable because fetal calf serum is immunogenic. Acute toxicity in humans appears to be minimal, but there are no data on the long-term toxicity. Since MSCs could become neoplastic after extensive culturing and

(karyotypic) abnormal cells might exist in the donor BM at rare frequency there seems to be a need for karyotyping as a release criterion for clinical use of MSC [27]. Uncontrolled differentiation of MSCs (e.g. into invasive fibroblastlike synoviocytes or osteocytes) might result in extra damage to the joint. It is conceivable that allogeneic donor MSCs are safer since they are likely to become rejected a few weeks after their immunomodulatory action. The ideal source (bone marrow, fat or synovium) of MSCs for treatment in RA remains to be elucidated.

In conclusion, MSCs are promising candidates for therapy in inflammatory arthritis, but since it is not a life threatening disease and there have been conflicting data, more animal studies are needed before the use of MSCs in clinical trials for patients with RA or JIA.

References

[1] Fournier C. Where do T cells stand in rheumatoid arthritis? Joint Bone Spine 2005;72:527–32.

[2] Anjos-Afonso F, Bonnet D. Nonhematopoietic/endothelial SSEA-1+ cells define the most primitive progenitors in the adult murine bone marrow mesenchymal compartment. Blood 2007;109:1298–306.

[3] Sakaguchi Y, Sekiya I, Yagishita K, et al. Comparison of human stem cells derived from various mesenchymal tissues – superiority of synovium as a cell source. Arthritis Rheum 2005;52:2521–9.

[4] Murphy JM, Fink DJ, Hunziker EB, et al. Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum 2003;48:3464–74.

[5] LaBarge MA, Blau HM. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. Cell 2002;111:589–601.

[6] McFarlin K, Gao X, Liu YB, et al. Bone marrow-derived mesenchymal stromal cells accelerate wound healing in the rat. Wound Repair Regen 2006;14:471–8.

[7] Sackstein R, Merzaban JS, Cain DW, et al. Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. Nat Med 2008;14:181–7.

[8] Lee RH, Seo MJ, Reger RL, et al. Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. Proc Natl Acad Sci U S A 2006;103:17438–43.

[9] Devine SM, Bartholomew AM, Mahmud N, et al. Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. Exp Hematol 2001;29:244–55.

[10] Chen X, Armstrong MA, Li G. Mesenchymal stem cells in immunoregulation. Immunol Cell Biol 2006;84:413–21.

[11] Krampera M, Cosmi L, Angeli R, et al. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells 2006;24:386–98.

[12] Jones S, Horwood N, Cope A, et al. The antiproliferative effect of mesenchymal stem cells is a fundamental property shared by all stromal cells. J Immunol 2007;179:2824–31.

[13] Eliopoulos N, Stagg J, Lejeune L, et al. Allogeneic marrow stromal cells are immune rejected by MHC class I- and class II-mismatched recipient mice. Blood 2005;106:4057–65.

[14] Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004;363:1439–41.

[15] Zappia E, Casazza S, Pedemonte E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 2005;106:1755–61.

[16] Jones EA, English A, Henshaw K, et al. Enumeration and phenotypic characterization of synovial fluid multipotential mesenchymal progenitor cells in inflammatory and degenerative arthritis. Arthritis Rheum 2004;50:817–27.

[17] Marinova-Mutafchieva L, Williams RO, Funa K, et al. Inflammation is preceded by tumor necrosis factor-dependent infiltration of mesenchymal cells in experimental arthritis. Arthritis Rheum 2002;46:507–13.

[18] Li XL, Makarov SS. An essential role of NF-kappa B in the "tumor-like" phenotype of arthritic synoviocytes. Proc Natl Acad Sci U S A 2006;103:17432–7.

[19] Dallos T, Krivosikova M, Luszczyna L, et al. Mesenchymal stem cells from bone marrow of rheumatoid arthritis patients provide survival signals to T- and B-cells in vitro. Joint Bone Spine 2007;74:217.

[20] Endres M, Neumann K, Haupl T, et al. Synovial fluid recruits human mesenchymal progenitors from subchondral spongious bone marrow. J Orthop Res 2007;25:1299–307.

[21] Kastrinaki MC, Spanoudakis M, Sidiropoulos P, et al. Bone marrow mesenchymal stem cell properties in patients with rheumatoid arthritis. Ann Rheum Dis 2006;65:A37–8.

[22] Bocelli-Tyndall C, Bracci L, Spagnoli G, et al. Bone marrow mesenchymal stromal cells (BM-MSCs) from healthy donors and auto-immune disease patients reduce the proliferation of autologous- and allogeneic-stimulated lymphocytes in vitro. Rheumatology (Oxford) 2007;46:403–8.

[23] Zheng ZH, Li XY, Ding J, et al. Allogeneic mesenchymal stem cell and mesenchymal stem cell-differentiated chondrocyte suppress the responses of type II collagen-reactive T cells in rheumatoid arthritis. Rheumatology (Oxford) 2008;47:22–30.

[24] Djouad F, Fritz V, Apparailly F, et al. Reversal of the immunosuppressive properties of mesenchymal stem cells by tumor necrosis factor alpha in collagen-induced arthritis. Arthritis Rheum 2005;52:1595–603.

[25] Choi JJ, Yoo SA, Hong KH, et al. Mesenchymal stem cells expressing IL-10 attenuate the severity of arthritis in murine collagen-induced arthritis. APLAR J Rheumatol 2006;9(Suppl. 1):A223.

[26] Augello A, Tasso R, Negrini SM, et al. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. Arthritis Rheum 2007;56:1175–86.

[27] Wang Y, Huso DL, Harrington J, et al. Outgrowth of a transformed cell population derived from normal human BM mesenchymal stem cell culture. Cytotherapy 2005;7:509–19.

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