Umbilical cord-derived stromal cell therapy for rheumatoid arthritis: what does the future hold?

"*Umbilical cord-derived mesenchymal stem cells offer potential opportunities for allogeneic cell therapy for resistant rheumatoid arthritis.*"

KEYWORDS: cell therapy ■ graft-versus-host disease ■ immunoregulation ■ MSC1 proinflammatory phenotype ■ MSC2 anti-inflammatory phenotype ■ multipotential stromal cells ■ rheumatoid arthritis ■ umbilical cord-derived stromal cells

Limitations of the current therapies for rheumatoid arthritis

Rheumatoid arthritis (RA) is the most common inflammatory rheumatic disease characterized by progressive cartilage loss and bone destruction with progressive functional decline. It is thought to be an autoimmune disease with aberrant B- and T-cell function eventually culminating in an increased local production of inflammatory mediators and cytokines in particular, such as TNF-α, IL-1β, IL-6 and others. Collectively these cytokines orchestrate the molecular cascades that are activated in osteoclasts, fibroblasts and immune cells that culminate in joint destruction. An appreciation of these pathways has lead to the development of anticytokine therapies, costimulatory pathway blockade therapy and B-cell depletion therapies, which have dramatically improved patient outcomes.

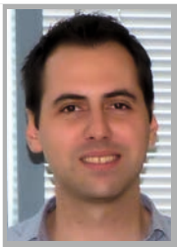
Despite these therapeutic advances, it is clear that a significant burden of RA inflammatory disease activity remains suboptimally treated. First, many patients are intolerant to the current therapies. Second, there is the issue of toxicity of existing therapies including the risks of serious infections or other noninfectious immunological perturbations that restrict therapy use. Third, many patients who benefit from the existing therapies only have a partial response and continue to experience significant joint pains owing to joint damage and functional loss. Finally, there is a small hard core of cases that have a fairly resistant inflammatory course where newer approaches are needed.

Multipotential stromal cells, also called mesenchymal stem cells (MSCs) were originally studied for their tissue regenerative capabilities. The immunomodulatory capacity of allogeneic MSCs was first documented in a pioneering paper by Le Blanc *et al.* who showed that bone marrow (BM)-derived MSCs could inhibit mixed lymphocyte cultures and mitogenic responses [1]. This has now translated to the clinical arena in severe graft-versus-host disease. This article explores the possibility that MSC-based immunomodulatory therapies may have a role in RA and, in particular, whether umbilical cord (UC)-derived MSCs may have a role in resistant RA.

Multipotential stromal cells & their immunoregulatory capacity

Although MSCs were first discovered in the BM, it was later found that many different connective tissues contain a resident population of MSCs. In 2001 Zuk *et al.* described the presence of MSCs in the adipose tissue [2] and 2 years later Romanov *et al.* documented MSCs in UC matrix [3]. To ensure standardization in the nomenclature, the International Society of Cellular Therapy has proposed four criteria to define MSCs: plastic adherence, the expression of several positive markers (such as CD73, CD90 and CD105), the lack of hematopoietic lineage marker expression (CD14, CD11b, CD79, CD34, CD45 and HLA-DR) and, finally, the ability to differentiate towards bone, cartilage and fat lineages [4].

Following the demonstration of BM-derived MSC immunomodulation as already described many *in vitro* studies have subsequently explored its potential mechanisms and found that these were in part mediated through the secretion of several soluble factors, such as HGF, TGF-β, prostaglandin E2 and tryptophan catabolising enzyme indoleamine 2,3-dioxygenase [1]. Later studies have investigated the effect of MSCs on monocytes, natural killer cells and B cells and in all cases some sort of MSC suppressive activity was documented. The lack of MHC class II expression by MSCs, as well as their prominent



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immunoregulatory function, implied that MSCs could be used for allogeneic therapeutic applications owing to their increased transplantation tolerance across MHC barriers [5]. Indeed, pioneering animal studies showed that systemically infused allogeneic BM-derived MSCs were safe in MHC-mismatched animal models [6]. This has led to the clinical use of MSC infusions to treat graft-versus-host disease of liver, skin and gut after allogeneic hematopoietic infusion [7,8].

Using MSCs in animal models of arthritis

The success of ongoing graft-versus-host disease trials has ignited an increased interest in the possibility of using MSCs to treat inflammation-related immune diseases such as RA [9]. In the collagen-induced arthritis (CIA) experimental animal model, intraperitoneal BM-derived MSC administration decreased the TNF-α or IFN-γ serum levels, and the severity of the disease [10]. Furthermore, a single infusion of allogeneic BM-derived MSCs was sufficient to prevent irreversible bone and cartilage joint destruction. Of note, a pioneering study of Djouad *et al.* showed that MSC administration may not always be effective; in their CIA animal study, administration of the C3H10T1/2 MSC line did not affect T-cell proliferation nor the disease course of CIA *in vivo* [11]. In agreement, a study from our laboratory showed that long-term exposure of joint resident MSCs to chronic inflammation may negatively affect their clonogenic and differentiation capacities in RA, an issue described later in this article [12].

> "*The success of ongoing graft-versus-host disease trials has ignited an increased interest in the possibility of using mesenchymal stem cells to treat inflammation-related immune diseases such as rheumatoid arthritis.*"

Adipose tissue-derived MSCs (AT-MSCs) have been also used in various induced arthritis animal models. For example, Melief *et al.* showed that AT-MSCs and BM-derived MSCs possessed comparable immunomodulatory properties; however, AT-MSCs had a stronger cytokine secretion profile [13].

Potential advantages of UC-derived MSCs as a cell therapy for RA

Similar to AT-MSCs, UC-derived MSCs could have a superior immunomodulatory capacity compared with BM-derived MSCs. Whereas BM-derived MSCs have intermediate levels of MHC class I [14], UC-derived MSCs have low levels of MHC class I [15]. These properties underline their immunoprivileged applicability in allogeneic transplantation as fully mismatched UC-derived MSCs did not provoke a proliferative T-lymphocyte response [16]. Furthermore, the superior immunomodulatory capacity of UC-derived MSCs in the context of RA has been recently shown *in vivo* using an acute carrageenan-induced arthritis and a chronic adjuvant-induced arthritis models. UC-derived MSC administration in an acute carrageenan-induced model resulted in a reduction of animal's paw edema more efficiently than BM-derived MSC administration. In agreement, in a chronic adjuvant-induced arthritis model, intra-articular and intraperitoneal administrations of UC-derived MSCs showed faster remission of local and systemic arthritic manifestations [17].

> "*Umbilical cord-derived mesenchymal stem cells could have a superior immunomodulatory capacity compared with bone marrow-derived mesenchymal stem cells.*"

UC-derived MSCs are able to regulate the function of T-helper cells in RA patients. Their regulatory actions result in downregulation of peripheral blood mononuclear cells, an effect directly related to the occurrence of inflammatory mediators [18]. Coadministration of MSCs with other cell types or cytokines are two very interesting strategies to ameliorate arthritis progression. In a complete freunds adjuvant arthritis animal model, UC-derived MSCs coinjected intra-articularly with $CD34^+$ hematopoietic stem cells resulted in reduction in leukocytic infiltrate, hypertrophy of the synovial tissue and amelioration of pathological changes in joints [19]. In addition, coadministration of UC-derived MSCs with TNF-α inhibitor could significantly decrease cartilage destruction [20].

The mechanism of UC-derived MSCs' immunoregulatory action in arthritis was illustrated in a CIA model showing that UC-derived MSCs resulted in fibroblast-like synoviocyte proliferation inhibition and regulatory T-cell expansion. At the molecular level, MSCs downregulated TNF-α, IL-6 and MCP-1 and upregulated the anti-inflammatory/regulatory cytokine IL-10 in animals' sera [21]. Thus far, no data using MSCs for the treatment of RA patients have been reported, but in 2013 an Australian company initiated Phase II clinical trials to test the safety

and efficacy of a single intravenous infusion of allogeneic MSCs in patients with poor response to biologic inhibitors of the TNF-α pathway [101].

Regulatory issues & potency assays

Being an exceedingly abundant cellular material that is easy to source and culture, UC-derived MSCs represent an attractive immunomodulatory cellular therapy option for RA, but issues remain relating to potency assessment of UC-derived MSCs. Therefore, cell therapy manufacturers were mostly required to ensure product safety (i.e., microbial contamination absence) and purity (based on the 2006 International Society for Cellular Therapy definition). In relation to immunomodulation therapies, the International Society for Cellular Therapy has recently argued for the development of standard assays to assess the immunoregulatory capacity quality of MSCs that include assays for T cells, B cells and natural killer cells [22]. This is because MSCs cultivation protocols, even when derived from the same tissue, can profoundly influence their immunoregulatory capacity. Cultured MSCs derived from highly pure MSC fractions exerted more potent allosuppressive properties compared with plastic-adherent cells [23]. Furthermore, growing MSCs in platelet lysate-containing media generated batches with stronger immunoregulatory capacity compared with MSCs grown in standard serum-containing media, thus making uniform agreement on quality-control potency assays more timely and important.

Two states of MSCs *in vivo*: MSC1 & MSC2

Whereas the effect of MSCs on the immune cells is fairly well investigated, the reciprocal relationship, for example, the effect of immune cells on MSCs, is relatively less studied, and this is particularly important in relation to RA, which is characterized by activation of immune cells, both in the synovium and in circulation. A potential 'inhibitory' effect of active immune cells on the immunoregulatory capacity of injected MSCs was first shown in an above mentioned Djouad *et al.* study, which showed that the inflammatory milieu in RA animals, and particularly the abundance of IL-6, abrogated the immunosuppressive activity of injected MSCs [11]. These findings were further extended in Waterman *et al.*, who showed that MSCs could in fact be 'polarized' in response to some cytokines and Toll-receptor ligands, to adopt a so-called MSC1 or 'proinflammatory' phenotype [24]. These authors have proposed that in normal homeostasis *in vivo*, MSCs serve as sentinels of inflammation and adopt a MSC2/anti-inflammatory phenotype; however, florid and uncontrolled inflammation can 'turn' MSCs to assume the proinflammatory MSC1 'fate' suggesting that these cells would likely participate in disease worsening, rather than its resolution. In agreement, our recent study has shown that synovial inflammation is directly correlated with the loss of resident MSC proliferation and their chondrogenic capacities [12]. These new findings suggest that more work in animal models of RA, at different disease stages, is needed to establish the role of native resident MSCs in RA progression, as well as the efficacy of exogenously added MSCs if they are used as therapy.

> *"These new findings suggest that more work in animal models of rheumatoid arthritis, at different disease stages, is needed to establish the role of native resident mesenchymal stem cells in rheumatoid arthritis progression..."*

Conclusion

UC-derived MSCs offer potential opportunities for allogeneic cell therapy for resistant RA. Recent literature suggests that *in vitro* and in animal models of arthritis, UC-derived MSCs could be superior to BM-derived MSCs in their immunomodulatory capacity [17]. Nevertheless, caution is needed before these preclinical findings are translated to RA. First, the best method of UC derivation and cultivation, with optimal preservation of their immunoregulatory capacity, should be established and agreed upon. Second, more biomedical research in animal models of arthritis should be performed to investigate the fate of infused MSCs *in vivo* and their contribution to the disease process and its resolution. Particular attention should be given to a possible conversion of injected immunomodulatory MSC2s into potentially aggressive and harmful MSC1s, whilst they enter the inflammatory milieu of the RA joint. Finally, prior to the commencement of larger human clinical trials, the therapeutic dose of infused UC-derived MSCs should be better established. Therefore, whilst cautious optimism for MSC therapy in RA exists, more work is needed before these therapies can become the clinical reality.

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