Umbilical cord mesenchymal stem cells: the new gold standard for mesenchymal stem cell based therapies?

El Omar R. 1*, Beroud J. 1*, Stoltz JF. 1,2, Menu P. 1, Velot E. 1, and Decot V. 1,2

1 CNRS UMR UL 7365, Bâtiment Biopôle, Faculté de médecine, 9 Avenue de la Forêt de Haye, 54500 Vandœuvre-lès-Nancy, France

2 CHU de Nancy, Unité de Thérapie Cellulaire et Tissus, allée du Morvan, 54500 Vandœuvre-lès-Nancy, France.

(*) Both authors contributed equally to this work

Corresponding author:
- Dr Decot Véronique
Email: v.decot@chu-nancy.f
Tel: 0033-383157937

- EL Omar Reine, PhD student
Email: reine.el-omar@univ-lorraine.fr
Tel: 0033-3 83 68 54 53

- Beroud Jacqueline, PhD student
Email: jacqueline.beroud@univ-lorraine.fr
Tel: 0033-383685453

- Dr Menu Patrick
Email: patrick.menu@univ-lorraine.fr
Tel: 0033-383685457

- Dr Velot Emilie
Email: emilie.velot@univ-lorraine.fr
Tel: 0033-383685451

- Pr Stoltz Jean-Francois
Email: Jf.stoltz@chu-nancy.fr
Tel: 0033-383153779
Abstract
Because of their self-renewal capacity, multilineage differentiation potential, paracrine effects and immunosuppressive properties, mesenchymal stromal cells (MSCs) are an attractive and promising tool for regenerative medicine. MSCs can be isolated from various tissues but despite their common immunophenotypic characteristics and functional properties, source-dependent differences in MSCs properties have recently emerge and lead to different clinical applications. Considered for a long time as a medical waste, umbilical cord appears today as promising source of MSCs. Several reports have shown that umbilical cord derived MSCs are more primitive, proliferative and immunosuppressive than their adult counterparts. In this review, we aim to synthesize the differences between umbilical cord-MSCs and MSCs from other sources (bone marrow, adipose tissue, periodontal ligament, dental pulp,…) concerning their proliferation capacity, proteic and transcriptomic profiles and their secretome involved in their regenerative, homing and immunomodulatory capacities. Although umbilical cord-MSCs are until now not particularly used as MSC source in clinical practice, accumulating evidences show that they may have a therapeutic advantage to treat several diseases especially autoimmune and neurodegenerative diseases.

Key words:
Mesenchymal stem cells, umbilical cord matrix, differentiation potential, paracrine effects, immunomodulation
INTRODUCTION

Mesenchymal stromal cells (MSCs) are attractive cells due to their capacity of long-term ex vivo proliferation, multilineage differentiation potential and immunomodulatory properties. These cells were first identified and isolated from the bone marrow (BM) and have emerged as powerful tools in tissue engineering and regeneration (1). Although adult BM is the most common and best characterized source of MSCs, Wharton’s jelly (WJ) of the umbilical cord provides a novel source of MSCs with higher accessibility and fewer ethical constraints than BM holding great promise as an alternative. WJ in an extra-embryonic tissue easily obtained after birth which has initially been described by Thomas Wharton in 1656 (2). While isolation of MSCs from BM requires an invasive procedure for the donor, MSCs can be non-invasively isolated from WJ (3). These WJ-MSCs are believed to be more primitive than MSCs derived from more mature tissue sources and to have intermediate properties between embryonic and adult stem cells (4). Moreover, WJ-MSCs are available in potentially large quantities, have a fast proliferation rate, a great expansion capability, do not induce teratomas and harbor strong immunomodulatory capacities (5, 6).

In this review, we will focus on the similarities and differences between WJ-MSCs and MSCs from other sources regarding their proliferation, their surface markers and their transcriptome profiles. The controversy between their paracrine effects and trans-differentiation potential will be discussed. In addition, we will particularly highlight their roles as (a) immunomodulators and the mechanisms involved in their immunosuppressive properties, as (b) anti-tumor agents, (c) migratory curative cells with (d) a special emphasis on their clinical and therapeutic applications in autoimmune and neurodegenerative diseases.

I. Main Features of WJ-MSCs
1. Isolation methods

Isolation of MSCs from WJ requires complex processing. Many isolation and expansion protocols have been demonstrated for a fast and efficient ex vivo generation of large quantities of cells. Currently, “enzymatic digestion” and “tissue explant” are the two types of methods for the isolation of WJ-MSCs (7). Those based on enzymatic digestion have mainly used collagenase alone or in combination with other enzymes (ex; trypsin, hyaluronidase) and were performed with or without the dissection of the umbilical cord into small pieces or with or without removing the blood vessels (8). Recently, Han et al. have suggested that using 0.2% collagenase II at 37°C for a digestion of 16-20 hours is an effective and simple enzyme digestion method (7). Other groups have found that enzymatic digestion can alter cell population and function and thus, have developed explant approaches without using any enzyme and taking advantage of the ability of MSCs to migrate from the tissue to adhere on the plastic (8-10). Hua et al. have, very recently, compared three explant and three enzymatic methods with regards to time of primary culture, cell number, cell morphology, immune phenotype and differentiation potential of WJ-MSCs. They have shown that 10 mm size tissue explant method was the optimal protocol for the isolation of MSCs (11).

2. Morphology and proliferation capacity

WJ-MSCs cultured in vitro shared a similar fibro-blastoid shaped morphology to BM, amniotic fluid (AF), or teeth and periodontal ligament (PDL)-MSC (12, 13).

The proliferation capacity of cells is important regarding their potential of application in cell therapy and tissue engineering. WJ-MSCs proliferation capacity seems to be different from MSCs from other sources. Indeed, for instance, Yu et al. have shown that over a period of seven days after seeding, WJ-MSCs grew much faster than PDL-MSC and had a cell doubling time of 22.23 h against 27.51 h for PDL-MSC (14). Compared to BM-MSCs, WJ-MSCs grow much faster for the early passages and have a cell doubling time (24h) almost twice shorter than BM-MSCs (40h) over 1st passage. These observations were confirmed by Abu Kasim et al. showing that WJ-MSCs and dental pulp-mesenchymal stem cells (DP-MSCs) were highly proliferative as compared to BM and adipose tissue (AT)-MSCs (15). Furthermore,
WJ-MSCs have a greater ability to form CFU-F colonies \textit{in vitro} than BM-MSCs and their formation’s frequency depends on seeding cell density (4, 16).

Other studies focusing on MSCs derived from DP-MSCs showed that cells from both sources (WJ and teeth) initially grew slowly but their proliferation rates were increased after the first subculture (17). However, WJ-MSCs growth is influenced by the number of culture passages \textit{in vitro}, as amplifying these cells until passage 10 will result in a slower cell growth compared to the same cell culture at passage 5 (18).

A very recent study has evaluated the proliferation kinetics and phenotypic characteristics of MSCs derived from WJ and AT during prolonged \textit{in vitro} expansion and found that WJ-MSCs were isolated with high efficiency and bore a substantially increased proliferation capacity, whereas AT-MSCs exhibited a reduced proliferation potential showing typical signs of senescence at an early stage (19).

3. Markers expression at protein level

A large number of studies have analyzed the surface markers of WJ-MSCs and compared their expression profiles to other sources of MSCs such as bone marrow, teeth or amniotic fluid. The following table summarizes the phenotypic profiles of these MSCs mentioned in the literature (table 1).

WJ-MSCs, like MSCs from other sources, positively express the classical mesenchymal surface markers. However, table 1 highlights the differences in the expression levels of other markers:

- Unlike BM-MSCs, WJ-MSCs weakly expressed endoglin (SH2, CD105) and CD49e at passage 8.
- WJ-MSCs and AT-MSCs express CD106 at much lower levels than BM-MSCs.
- In comparison with BM-MSCs, HLA-ABC is very weakly expressed by WJ-MSCs suggesting that these cells could be good candidates for allogeneic cell therapy.

4. Transcriptomic profile

Emerging data has compared the transcriptomic profile of WJ-MSCs to MSCs from other sources. The following table gives an overview of the main comparisons (table 2).
Some studies showing a high expression of embryonic genes such as LIFR, ESG1, SOX2, TERT, NANOG, POUF1, OCT4, LIN28, DNMT3B, GABRB3 by WJ-MSCs, suggest that WJ could be a more primitive source of MSCs (4, 6, 24-26). Furthermore, as shown in table 2, WJ-MSCs express genes encoding for proteins associated with morphogenesis: SHH, neuregulin-1 and 4, SNA2, and WNT4 (27).

WJ-MSCs, compared to MSCs from other tissues, differentially express genes involved in bone development:

Transcription factors involved in osteoblast differentiation such as RUNX2 were found to be expressed at comparative levels in BM-MSCs, skin-MSCs, AT-MSCs and WJ-MSCs. However, table 2 shows that skin-MSCs are characterized by a significantly increased expression of genes (BMP4, BMP2) associated with bone and cartilage development in comparison to the other MSCs.

WJ-MSCs reveal an important expression of genes involved in liver and cardiovascular development:

The transcriptomic profile of WJ-MSCs and AF-MSCs reveals the basal expression of several mature myocardial genes: GATA-4, c-TnT, Cx43, which could be associated to the potential of differentiation into myocardial cells. Interestingly, a high expression of genes encoding for GATA-binding protein 6 (GATA6), Heart And Neural crest Derivatives expressed 1 (HAND1), ICAM1 and VCAM1 was detected in WJ-MSCs (table 2). WJ-MSCs were also shown to express genes involved in cardiovascular system development, including angiogenesis, cardiogenesis, endothelial cell development, and vasculogenesis (Table 2). In addition, other genes involved in cardiovascular development, including endoglin (ENG), GJA1, VCAM1, and GATA6 were significantly increased in BM-MSCs (28).

The transcriptomic profile reveals also that WJ-MSCs have a significantly increased expression of genes (AFP, DKK1, DPP4, DSG2) associated with liver development compared to BM-MSCs, AT-MSCs and skin-MSCs.

WJ-MSCs express genes involved in neural development:
WJ-MSCs and DP-MSCs revealed a high expression of the neuro-ectoderm lineage markers (15). De Kock et al. have studied the whole gene expression profiles of 4 human mesoderm-derived stem cell populations: AT-MSCs, BM-MSCs, skin-MSCs and WJ-MSCs. They have shown differences in gene expression between distinct stem cell types. Skin-MSCs pre-dominantly expressed genes involved in neurogenesis (NES), skin and bone (RUNX2, BMP4) (28).

Such a transcriptomic profile reveals a closer proximity between WJ-MSCs and BM-MSCs than between other combinations. Considering the genomic profile of WJ-MSCs, WJ may be considered as a reliable source of MSCs useful in cardiovascular regenerative medicine (30, 31) but also in neurodegenerative diseases. The latter will be discussed in the last part of the review.

5. Regenerative Role of MSCs: Differentiation potential Vs Secretome

A summary of the various comparisons between sources of MSCs that have been already described in the literature is shown in table 3.

WJ-MSCs differentiate into adipocytes slower than BM-MSCs (4). Bai et al. have shown that AF-MSCs and WJ-MSCs could differentiate into myocardial-like cells with an important expression of myocardial genes such as GATA-4, c-TnT, α-actin, Cx43 after myocardial induction (18).

More recently Chen et al. have worked on in vitro differentiation analysis of MSCs isolated from DP and WJ. They have shown that MSCs isolated from both sources exhibited the capacity to differentiate into osteoblasts, chondrocytes, and adipocytes. However, they have noted some differences in their differentiation potentials. DP-MSCs and WJ-MSCs had a similar potential for osteogenic differentiation, but the chondrogenic and adipogenic differentiation potentials of WJ-MSCs were more important than those of DP-MSCs (17). Meanwhile, according to Zhang et al., foetal human BM-MSCs have the highest potential of in vitro monolayer osteogenic differentiation, comes after human WJ-MSCs, human adult BM-MSCs and then AT-MSCs (23). Baksh et al. have found similar results as the previous study when comparing the in vitro differentiation potentials of WJ-MSCs and BM-MSCs (44).

Jo et al. have studied the in vivo osteogenic differentiation, in a rat model, of human MSCs isolated from different sources. No differences were detectable in osteogenesis between adult AT-MSCs,
BM-MSCs and WJ-MSCs (45). Controversial results have been described by Zhang et al. In fact, after a subcutaneous implantation of MSCs scaffolds in mice, better results were obtained with scaffolds elaborated with human foetal and adult BM-MSCs than those constructed with WJ-MSCs and AT-MSCs (23). Differences in the results between the mentioned studies are probably due to the different experimental conditions. This explains that MSCs of various tissue origins have specific characteristics of differentiation or require different conditions for osteoinduction.

Very recently, Yu et al. have shown that WJ-MSCs are not good alternatives for periodontal tissue generation compared to PDL-MSCs which have a much better osteo/dentinogenic differentiation potential (12, 14). Various studies have demonstrated the capacity of WJ-MSCs to differentiate into pancreatic islet-like cell (46-48). Kim et al. have compared this potential with other sources of MSCs and did not show significative differences between BM-MSCs and AT-MSCs (46).

Some authors have focused on the application of MSCs in vascular engineering and more particularly their capacity to differentiate into endothelial cells (EC), or to acquire pericytes markers when co-culturing with endothelial cells. Chen et al., were pioneer in studying the endothelial differentiation potential of BM-MSCs and WJ-MSCs. Both sources of MSCs were able to differentiate into EC but WJ-MSCs appear to have a greater differentiation potential since derived EC-like exhibited a higher expression of endothelial markers (5).

For a long time, it has been considered that the regenerative potential of MSCs is due to their plasticity and differentiation capacity. However, the direct link between their differentiation potential and their beneficial effects has never been proven. Indeed, recent studies suggest that benefits of MSCs transplantation may be associated to a paracrine modulatory effect rather than the replacement of affected cells, at the site of injury, by differentiated stem cells (49, 50). Emerging data suggest that stem cells could be then considered as reservoir of trophic factors released when needed to modulate and repair surrounding damaged tissues which led to a paradigm shift in regenerative medicine. Understanding the cell secretome has attracted much attention and it has been demonstrated that trophic factors could have many effects such as modulation of inflammatory reactions, immunomodulation, anti-apoptotic and pro-angiogenic capacities and many others [reviewed in (51)]. Vallone et al. have highlighted in their review the exact mechanisms that would lead MSCs to damaged tissues after
transplantation where they will exert their remedial actions (52). Katsuda et al. have also described a possible therapeutic mechanism of AT-MSCs, in Alzheimer disease, through a paracrine pathway. Vesicules secreted by these cells could carry soluble factors that may treat this pathology. Results of this study will be discussed in the final part of this review. Therapeutic effects of BM-MSCs in regenerative medicine (heart disease for example) through paracrine/autocrine mechanisms have been reviewed by Pourrajab et al. (53).

The controversy between the implication of the differentiation potential and the paracrine mechanisms of MSCs in their beneficial therapeutic actions is showed in figure 1.

Over the following sections of this review, we will highlight the effects of WJ-MSCs secretome involved in many processes such as immunomodulation, homing to damaged tissues and others.

II. WJ-MSCs as immunoprivileged cells

1. Immunological features of MSCs

MSCs have in the last decade gained considerable attention as candidates for tissue engineering, as modulators of immune responses in graft-versus-host-disease and autoimmune diseases (54), since these cells, once administered therapeutically may be able to evade the immune system of the host. They are currently being assessed as a novel anti-inflammatory therapeutic agent in numerous clinical trials (55). Two outstanding features of MSCs are relevant to their immunomodulatory effects:

**Immunosuppression**: MSCs-mediated immunosuppression describes the fact that MSCs are able to suppress several functions (proliferation, production of soluble factors and cellular cytotoxicity) exerted by diverse immune cells such as T-, B- and natural killer (NK) cells. It has been shown that immunosuppression is mediated by both cell-cell contact and paracrine signals via soluble factors.

**Immunoprivilege**: MSCs themselves are somehow protected from immunological defense mechanisms (56). Indeed, MSCs lack expression of major histocompatibility complex (MHC) class II giving MSCs the
potential to escape recognition by alloreactive CD4+ T cells but express MHC Class I molecules. This expression allows them to escape from NK cell lysis, Also, MSCs do not express co-stimulatory molecules required for effector T cell induction (57).

Even if BM-MSCs, considered as the gold standard in MSC therapy, and UC-MSCs share many similarities, emerging data suggest that WJ-MSCs could be less immunogenic than BM-MSCs, making them a good candidate for allogeneic transplantation.

2. MSCs-mediated immunosuppression

MSCs show an absence or a low expression of MCH class II and co-stimulatory molecules, so they can be considered as immunoprivileged cells, but they also interfere with different pathways of the immune response (58). Their ability to modulate the immune system was first recognized after the fact that they could evade immunosurveillance after cell transplantation (59). Especially, human MSC populations such as BM-, AT-, or UC-derived MSCs selectively alter immune cell function by suppressing T cell proliferation, B cell proliferation and terminal differentiation (60), inhibiting NK cell proliferation and cytotoxicity, steering monocytes and dendritic cells (DCs) to an immature DC state (61).

a. MSCs and immune cell population

a.1 Adaptive Immunity

MSCs and T cells

T cells recognize antigens and are critical for cell-mediated immune response. They mature within the thymus into one of different subtypes with diverse roles. These cells are involved in the maintenance of self-tolerance, activation of other lymphocytes, lysis of infected cells, and interaction with cells of the innate immune system.

Currently, interactions of MSCs with T cells have been extensively studied. Graft versus Host Disease models presented the first evidence that MSCs can regulate immunosuppression in vivo (62). MSCs could reduce allograft rejection, which is partly mediated by T cells (63, 64). Shortly after, T cell immunosuppression mediated by MSCs was demonstrated in vitro. MSCs inhibit probably via their
induced or constitutively expressed secreted factors, T-lymphocyte activation and proliferation induced by mitogens and alloantigens (65-68) as well as T-cell activation with CD3 beads (66, 69). MSCs have been shown to equally inhibit CD4+, CD8+, CD2+ and CD3+ subsets (70). In addition, T-lymphocytes inhibited by BM-MSCs do not enter apoptosis, since they actively proliferate on re-stimulation with cellular and humoral activators (65). Many other studies have shown the ability of BM-MSCs to induce the expansion of functional regulatory T cells (Tregs) (70, 71). Recently, it has been shown that adhesion molecules ICAM-1 (inflammatory cytokine-induced intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1), which are required for a direct adhesion of MSCs to T cells, are critical for subsequent MSCs mediated immunosuppression, and are inducible by the parallel presence of IFN-γ and inflammatory cytokines (72). Another possible mechanism underlying the BM-MSCs mediated suppression of T-cells is to prevent their entry into the S phase of the cell cycle by mediating irreversible G0/G1 phase arrest through the inhibition of cyclin D2 expression (69, 73). Similarly, it has been shown that addition of DP-MSCs to phytohemagglutinin (PHA)-stimulated T cells mediated an inhibition of their response (74). Increased expression of immunodulatory soluble factors (HGF-β1, ICAM-1, IL-6, IL-10, TGF-β1, VCAM-1, VEGF) secreted by human DP-MSCs was detected in co-culture system with decreased expression levels of some pro-inflammatory cytokines and increased levels of some anti-inflammatory ones. Induction of Treg markers by human DP-MSCs was also demonstrated (75). A very recent study has examined the in vivo and in vitro immunomodulatory effects of human supernumerary tooth-derived mesenchymal stem cells (SNT-MSCs). It has been shown that, in in vitro co-cultures, these cells suppressed the viability of T-cells and also the differentiation of Th17 cells. In vivo transplantation of SNT-MSCs in systemic lupus erythematosus model MRL/lpr mice suppressed increased levels of peripheral Th17 cells and IL-17 as well as ex-vivo differentiation of Th17 cells (76).

Fetal MSCs have been reported to have similar inhibitory effects on T-lymphocytes. It has been shown that mitogen-induced T-cell proliferation in an allogeneic model transplant, as well as in a xenograft model, was effectively suppressed by WJ-MSCs with levels comparable to BM-MSCs immunosuppression (77). Also, IFN-γ and/or TNF-α produced by activated T cells stimulate production of IDO (indoleamine2,3-dioxygenase) by MSCs, which, in turn, inhibited T-cell proliferation (78). Tipnis et al. have reported that the expression of B7-H1, a negative regulator of T-cell activation constitutively
expressed by WJ-MSCs, is increased after IFN-γ treatment. In addition, IFN-γ treatment induced IDO secretion by WJ-MSCs which inhibited T-cell proliferation (79). These results were confirmed very recently by Manochante et al. showing that MSCs from amnion, placenta, and WJ can potentially substitute BM-MSCs in several therapeutic applications. Indeed, these cells inhibited alloreactive T-lymphocytes in the mixed lymphocyte reaction in a similar degree as BM-MSCs (80).

- MSCs and B cells

The research on T cell immunosuppression mediated by MSCs has attracted most of the attention in clinical applications and has been widely studied. However, B cells and humoral immune responses are more and more known as important mediators of chronic allograft rejection. Indeed, data about influence of MSCs and B cells growth, differentiation and production of immunoglobulins (Ig) is still scarce and controversial (81).

B cells play an essential role in adaptive immunity. These cells develop in the bone marrow strictly after close interaction between B cells progenitors and stromal cells that produce cytokines capable of supporting B cell survival and proliferation (82). They are directly responsible for the humoral immune response via the secretion of antibodies against pathogenic or foreign antigens. A subset of B lineage differentiates into memory B cells, which can mediate a rapid response upon secondary exposure to that same antigen.

Corcione et al. demonstrated that BM-MSCs inhibited the proliferation of B cells and decreased significantly the production of IgM, IgG and IgA (83); the same effect has been reported by Che et al. showing that UC-MSCs significantly suppressed the proliferation, differentiation, and immunoglobulin secretion of B cells in vitro (84). To understand the results of Che et al., it is essential to know that “B-lymphocite-induced maturation protein-1” (Blimp-1), “X-box binding protein-1” (Xbp-1), “B-cell lymphoma-6” (Bcl-6) and “paired box gene-5” (PAX-5) are known as the main regulators of B-cell differentiation to immunoglobulin-secreting cells. PAX-5 and Bcl-6 are required to keep B-cell phenotypes. Blimp-1 inhibits the expressions of both PAX-5 and Bcl-6 in order to let B-cells differentiate. BCR signaling involves MAPK signaling pathway and increases transcriptional activity mediated by the transcription factor activator protein-1 (AP-1) which leads to Blimp-1 expression. Che et al. have shown a suppression of
Blimp-1 expression and an induction of PAX-5 in the co-cultures of UC-MSCs and B-cells. They have also found that Akt and p38 MAPK were inhibited by WJ-MSCs (84).

However, these results have been contradicted by other groups. Rasmusson et al. have shown an increase of B cells immunoglobulin secretion when co-cultured with BM-MSCs, this effect varied depending on the type of stimulus used to trigger B cells (85). Likewise, Traggiai et al. have reported that BM-MSCs could promote B-cell expansion and differentiation after treatment with an agonist of Toll like receptor 9 (86). A recent study have demonstrated that UC-MSCs promoted proliferation and differentiation of B cells in vitro and in vivo partially through prostaglandin E2 (PGE2) axis (82).

Contradictions in the effects of MSCs on B-cells could be associated to the differences in B-cell source, the way of their purification and stimulation, the culture conditions and many other factors. However, the microenvironment plays a decisive role in determining the role that will play the MSCs.

### a.2 Innate response

- MSCs and NK cells

NK cells are major effector cells of innate immunity because they lack antigen-specific cell surface receptors (87). They mediate antibody-dependent cellular cytotoxicity as well as “spontaneous” killing of infected or transformed cells through release of perforin and granzyme from cytotoxic granules (88).

MSCs and NK cells have been shown to interact in vitro. The outcome of this interaction may depend on the state of NK-cell activation and/or the cytokines present in the culture medium. IFN-γ activated MSCs escaped NK cells mediated lysis through induction of HLA-E and NK inhibitory ligands (89, 90). Previous studies have indicated that cytokine-induced proliferation of NK cells leads to the up-regulation of HLA class I on MSCs (90). In response to this up-regulation, HLA class I molecules including human leukocyte antigen-G5 (HLA-G5), expressed by MSCs, bind to the inhibitory receptor ILT2 expressed on NK cells (91). Furthermore, other studies have shown that suppression of NK cell functions is mediated by a down-modulation of some activating NK cell receptors (NKP30, NKP44 and NKG2D) and by inhibition of NK cell lytic granule formation (92). There is growing evidence that IDO, PGE2 and TGF-β1 may control MSC-mediated inhibition of NK-cell function (93).
Boissel et al. evidenced that NK cells had a higher expansion when cultured with allogeneic and autologous WJ-MSCs as feeders in the presence of NK growth factors. WJ-MSCs feeders were rejected during the first week of co-culture. Expanded NK cells maintained an elevated cytotoxic profile and may be genetically manipulated (88). In a recent study, Zhao et al. has been interested in elucidating the effect of UC-MSCs on NK cells-mediated cytotoxicity against DCs and the mechanism involved. They found that UC-MSCs can enhance this effect possibly by inhibiting DCs maturation and up-regulating the ligands for killer activator receptor on the surface of the DCs (94). When comparing the immunosuppressive activity of MSCs derived from UC, AT and BM on lymphocytes, Ribeiro et al. have shown that all the three types of MSCs exhibited a strong inhibitory effect on CD56^{dim} NK cell subset activation (cytotoxic NK cells). UC-MSCs were the only cells that were unable to inhibit the activation of CD56^{bright} NK cell subset (a subset that has the capacity to produce abundant cytokines following activation but has a low natural cytotoxicity). Among all these MSCs, AT-MSCs had the higher inhibitory capacity. A downregulation of perforin and TNF-α ARNm by MSCs from the three sources was observed, while only AT- and BM-MSCs induced a minor reduction of granzyme B ARNm (95).

MSCs and DCs

DCs play a key role in the initiation of primary immune responses and tolerance, depending on the activation and maturation stage of DCs. Locally produced inflammatory cytokines or microbial components promote the maturation of DCs from a processing to a presenting stage, characterized by up-regulation of MHC-class II and co-stimulatory molecules (CD80 and CD86), production of IL-12, and migration to lymphoid tissue. DCs maturation is a prerequisite to induce immunogenic T cell responses, whereas tolerance is observed when antigens are presented by immature or semi-mature DCs. Therefore, DC maturation plays a key role in initiating T cell responses.

BM-MSCs were shown to block the generation of functional antigen-presenting cells, including myeloid DCs from both monocytes and CD34+ cell precursors (96-98). Most results supported the notion that DCs at early stages of differentiation are sensitive to their inhibitory effects, while at later stages are resistant. However, WJ-MSCs inhibited DC maturation and activation even when the contact happened at the mature or immature stage. Both cell contact via surface ligands (B7H1) as well as soluble factors...
(IDO) enhanced the efficiency of suppression (79). Very recently, Saeidi et al. showed that UC-MSCs and BM-MSCs strongly inhibited differentiation and maturation of DCs with more inhibitory effect on CD1a, CD83, CD86 expression and dendritic cell endocytic activity. These cells also severely up-regulate CD14 expression. Results have indicated that UC-MSCs and BM-MSCs exerted their inhibitory effect on differentiation, maturation and function of DCs through the secreted factors and free of any cell-to-cell contacts (99).

Immunomodulatory properties of WJ-MSCs on innate and adaptive responses are resumed in the figure below (Figure 2).

b. MSCs and immumodulatory paracrine factors

Multiple reports have evidenced, first *in vitro* and then *in vivo*, the ability of MSCs to express molecules that interact with both innate and adaptive immunity, both through soluble factors (65, 100) and in a cell contact-mediated fashion probably through the interaction of membrane receptors, adhesion molecules or the cellular exchange of membrane vesicles (101). It is still a matter of debate if the regulatory effects are cell-to-cell contact-dependent, or if soluble factors are sufficient (102). The MSCs immune modulating effects will depend also on the ratio between MSCs and immune cells, and the state and stage of immune cell activation or maturation. Several factors that contribute to the MSCs-mediated effects have been identified, in particular growth factors, cytokines, chemokines and hormones, all of which exert paracrine effects on immune cells and allow homing, migration and their attachment to injured cells. Soluble factors implicated in MSCs-mediated immune-modulation include nitric oxide (NO), Indoleamine 2,3-dioxygenase (IDO), Heme Oxygenase (Hmox 1), secretion of anti-inflammatory cytokines like IL-10, Transforming Growth Factor-β (TGF-β), Hepatocyte Growth Factor (HGF), IL-6 and PGE2 (68, 70, 103, 104). A study comparing the immunomodulatory properties of MSCs derived from many sources showed that despite their similar cytokine profiles, WJ-MSCs only secrete IL-12, IL-15 and platelet derived growth factor-AA (PDGF-AA). They did not secrete VEGF like other adult MSC sources (78). The precise meaning of these differences, however, needs to be understood in WJ-MSCs/ immune cell co-cultures (105).
Specialized immune tolerance implicated at the maternal-fetal interfaces depend on expression of many molecules including galectin-1, B7 proteins, HLA-G (106), and expression of immune suppressive cytokines like Leukemia inhibitory factor (LIF) (107, 108). Because WJ-MSCs are isolated from a perinatal source, they could exhibit immune evasion mechanisms dominant at the fetal maternal interface. In fact, Najar et al. showed that higher constitutive as well as IFN-γ inducible levels of LIF are expressed by WJ-MSCs than by BM-MSCs and the suppression of lymphoproliferation can be rescued by blocking LIF in co-cultures (109). Furthermore, Prasanna et al. reported higher levels of both constitutive and IFN-γ inducible HGF in WJ-MSCs over BM-MSCs (110).

Non-classical type I HLA molecules are an interesting as yet only partly explored field in MSCs immune function. Several reports showed that BM-MSCs and WJ-MSCs express the HLA-G molecule, both as mRNA and protein level, and its soluble form HLA-G5 (29, 77, 111). Weiss et al. also showed that WJ-MSCs constitutively express high levels of the immune suppressive HLA-G6 isoform while BM-MSCs express HLA-G5 isoform constitutively and its expression is not induced by IFN-γ (77). HLA-G5 secretion has been directly implicated in induction of regulatory cells (CD4+CD25+FoxP3+ Tregs) which are characterized as key suppressors of effector responses to alloantigens (112). HLA-G5 secretion has also been linked with the suppression of NK cell production of IFN-γ in BM-MSCs co-cultures (113). As inhibition of maternal alloreactivity is due to expression of high levels of HLA-G by the fetus, the exact role of immune-suppressive HLA-G isoforms, like HLA-G6 expressed by WJ-MSCs needs to be evaluated in detail (105). Recently, the expression of HLA-E and HLA-F on the WJ-MSCs has been reported, both are implicated in tolerogenic processes occurring at the fetomaternal interface, together with HLA-G (114).

III. Homing of MSCs

Mantaining the function and the integrity of the human body, which is often subjected to injuries, is essentially due to tissue repair. Shortly after an injury, different types of immune cells (neutrophils, monocytes and lymphocytes) are conducted to the site of damage. These cells are responsible for the
secretion of various growth factors and cytokines that will attract other residing or circulating cells like MSCs. Endogenous MSCs present a pool of regenerative cells, participate to tissue repair and communicate with other cells in response to signals of cellular damage (115). Their “homing” can be defined as the arrest of MSCs within the vasculature of a tissue then crossing the endothelium (116).

Thus, homing of endogenous MSCs is being considered as a therapeutic benefit and studies are evaluating new methods for recruiting sufficient number of MSCs to exert their regenerative capacity. In cases where the reservoir of MSCs is depleted because of several diseases or the age, exogenous MSCs could be administrated to compensate the lack of endogenous MSCs [reviewed in (115, 117)]. It has been reported that in-vitro-expanded MSCs preferentially home to sites of tissue damage, where they enhance wound healing, support tissue regeneration and restore the BM microenvironment following damage by myeloablative chemotherapy or integrate into tumors (118).

Since the precise molecular mechanisms by which MSCs migrate into sites of injury are not yet fully defined, migration of leukocytes into sites of inflammation has been taken as model (119). Indeed, upon delivery into blood stream, the MSCs keep close contact with endothelial cells whose role is being extensively studied in MSCs migration. They engraft into the endothelium, and eventually pass and leave the endothelium (120). The migration of MSCs is mediated by a wide variety of molecules expressed by MSCs including growth factors, chemokines, and receptors and by chemotactic factors produced by immune cells (116). It has been demonstrated that human MSCs showed significant chemotaxis responses to several factors (including PDGF, VEGF, IGF-1, IL-8, bone morphogenetic protein BMP-4 and BMP-7) (121) and express a variety of chemokine receptors (such as CCR1, CCR4, CCR7, CXCR5 and CCR10) that might be involved in their migration into injured tissues along a chemokine gradient (122).

In addition, specific proteolytic enzymes are required so the cells can traverse the protein fibers of the extracellular matrix (ECM) and reach the target sites (118, 123). In particular, the matrix metalloproteinases (MMPs), consisting of more than 24 zinc-dependent endopeptidases, are physiologically necessary for stem cell migration, degradation and remodeling of ECM components, and are crucial for developmental events like morphogenesis, cell proliferation, apoptosis and differentiation (124-127).
Ries et al. were the first to show that human BM-MSCs use constitutively expressed MMP-2 (gelatinase A), membrane-type matrix metalloproteinase-1 (MT1-MMP), and tissue inhibitor of metalloproteinase 2 (TIMP-2) to migrate through human recombinant basement membranes. Inflammatory cytokines such as TGF-β1, IL-1β, and TNF-α are able to exert chemoattractive potential on hBM-MSCs and to up-regulate MMP-2, MT1-MMP and/or MMP-9 allowing cellular trafficking of MSCs across human ECM barriers (118). MMP-2 has been also detected in WJ-MSCs in association with MMP-9 (gelatinase B), MMP-8 and MMP-13 (respectively collagenase-1 and -2), as well as in different regions of full-term human umbilical cord and in cultured HUVEC. The wide expression of these enzymes in the umbilical cord has been attributed to their role in the degradation and remodeling of ECM and in other physiological processes (128).

Recently, Balsubramanian et al. have compared chemokine and receptor gene expression between WJ- and BM-MSCs. Their results have shown that Chemokine (C-C motif) receptor 3 (CCR3) was more expressed in WJ-MSCs than in BM-MSCs, whereas the latter have presented a higher expression of CCR1, CCR7, CCRL2, Chemokine (C-X3-C motif) receptor 1 (CX3CR1) and CXCR5. MSCs from both sources had a similar expression of CCR5, CCR6 and CCRL1.

In addition, Chemokine (C-X-C motif) ligand 1 (CXCL1), CXCL2, CXCL5, CXCL6 and CXCL8 (members of the CXC chemokine family) were up-regulated in WJ-MSCs in comparison with BM-MSCs. These chemokines are known as potent promoters of angiogenesis and mediate their activity by binding CXCR2 receptor on endothelium (Figure 3). Contrariwise, CXCL12 and CXCL13, also two members of CXC chemokine family and known to contribute to immune and non-immune cell homing, were up-regulated in BM-MSCs. WJ-MSCs has shown a higher expression of IL-1A (enhance the expression of CXCL8) and TNF-α (angiogenic factor) than BM-MSCs; while IL16 (has an immunomodulatory role in asthmatic inflammation) and CCRL12 (plays a role in the control of airway inflammatory response and in lung dendritic cell trafficking) were more expressed in BM-MSCs. Moreover, results have reported a stronger expression, in WJ-MSCs, of many growth factors linked with angiogenesis like VEGF-D, PDGF-AA, TGF-β2, β-FGF and HGF (116). Their chemokine gene profile suggests that WJ-MSCs may be useful in the healing and treatment of ischemic lesions as ischemic myocardium, cerebral ischemia for example. Moreover, they could be suggested as a treatment to reduce or prevent fibrosis and scarring in tissue lesions since it has been shown that they secreted bFGF and HGF (known to have an anti-fibrotic effect).
Thus, *ex vivo*-expanded human MSCs with cytokines may be a useful method, in clinical applications, to increase their migration/homing potential after transplantation into patients, as well as the administration of cytokines to mobilize MSCs to sites of injury.

IV. Anti-tumourigenic effects of WJ-MSCs

MSCs have the capacity to migrate to tumour sites and modulate their microenvironment. Thus they have an impact on tumour behaviour (129). A great deal of evidence suggests that solid tumours generate a microenvironment similar to that associated with wound healing, as they apply physical and chemical stress to neighbouring tissues. Tumours can therefore be considered sites of tissue damage, which induces the migration of MSCs (130).

Human MSCs have been intensively studied for their potential use in cancer treatment. Their use has been limited, however, by a general concern related to their biosafety (131). Many studies have reported pro- or anti-tumourigenic effects of MSCs on the progression of primary and metastatic tumours. These contradictory results could be associated with differences in the MSC sources used, the type of tumour model, the method of administration, or other unknown factors (130, 132).

The tumour stroma consists of a complex extracellular matrix wherein inflammatory and immune cells, fat cells, fibroblasts and blood vessels reside. It plays a crucial role in tumour progression, angiogenesis and metastasis through its effects on tumour-host interactions. Tumour-associated fibroblasts (TAFs) are activated fibroblasts in the tumour stroma (131). Several reports have hypothesized that BM-MSCs selectively proliferate to tumours and contribute to the formation of tumour-associated stroma by transforming into TAFs. They also promote tumour growth and metastasis by enhancing migration and angiogenesis and inhibiting apoptosis of tumour cells (133-137).

On the other hand, the immunosuppressive effects of MSCs can impair the function of a variety of immune cells (directly or through paracrine signals). This may be an important mechanism allowing MSCs to promote tumour growth or to increase the incidence of tumour formation. For instance, by increasing
Tregs and reducing the activity of NK cells and cytotoxic T lymphocytes (CTL) (known to kill tumour cells), BM-MSCs can protect breast cancer cells (138). They also have been linked to osteocarcinomas (139), prostate tumours (140, 141), breast tumours (138, 140, 142), colon cancer (137), and others. A recent study has also demonstrated a fusion between MSCs and gastrointestinal epithelial cells, suggesting the formation of a more cancer-prone cell type (143).

Very recently, in order to examine the possible anticancer therapeutic applications of MSCs from different sources, Akimoto et al have studied the inhibitory effects of MSCs from umbilical cord blood (UCB) and adipose tissue on “glioblastoma multiforme (GBM)” (the most aggressive type of primary brain tumour in humans). They found that, both in vitro and in vivo, GBM growth was inhibited by UCB-MSCs but promoted by AT-MSCs. UCB-MSCs induced apoptosis through the tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), which is more strongly expressed by UBC-MSCs than by AT-MSCs (144). Furthermore, it has been shown that naïve WJ-MSCs are able to produce factors suppressing cancer cell growth and inducing apoptosis, and so may be a novel tool for cancer therapy in contrast to MSCs from some other sources (145). Other reports have likewise shown that WJ-MSCs can abrogate certain solid tumours (146-149). These cells decreased the growth of human breast cancer in vitro and stopped its growth when intravenously injected in an SCID mouse model (150). Later, Fan et al. showed that WJ-MSCs do not induce teratomas in immunodeficient SCID mice, nor do they induce tumours when transplanted into diseased animal models (151). In a recent study, Subramanian et al. have examined whether WJ-MSCs, like BM-MSCs, transform to the TAF phenotype in the presence of ovarian and breast cancer conditioned medium. Results have shown no expression of tumour-associated markers for hWJ-MSCs with low expression of TAF-related genes, confirming that these cells are not associated with enhanced growth of solid tumours (3). In order to determine whether WJ-MSC-mediated inhibition of cancer cell growth was not specific to breast cancer cells, the same group compared the effects of WJ-MSC extracts and cell lysate on three other types of solid tumours: breast adenocarcinoma, ovarian carcinoma and osteocarcinoma. They observed the same effects, which were probably mediated via agents in WJ-MSC extracts (152). In addition, Ma et al have shown that WJ-MSCs significantly inhibited the growth of breast cancer stem cells in vitro and in vivo, probably by inducing a cell cycle arrest and tumour cell apoptosis and inhibiting the activities of phosphoinositide 3-kinase (PI3K) and AKT (also
known as protein kinase B) (149). A more recent study has reported that WJ-MSC conditioned medium as well as its cell lysate inhibits mammary carcinoma and osteosarcoma cell growth via apoptosis and autophagy in vitro and in xenograft mice (153). In another study evaluating the tumourigenesis potential of WJ-MSCs in comparison with ESCs, animals injected with ESCs developed teratomas with increased levels of pro-inflammatory cytokines, whereas those injected with WJ-MSCs developed no tumours or inflammatory reactions at the injection sites and exhibited increased production of anti-inflammatory cytokines (154). A very recent study on the effects of WJ-MSCs on intrahepatic cholangiocarcinoma (ICC, a common form of primary liver cancer) has shown that these cells can inhibit the proliferation and induce the apoptosis of human ICC cells. Apoptosis of tumour cells is related to the inhibition of PI3K/Akt and the Wnt/β-catenin signalling pathways (155). The effects of WJ-MSCs have also been studied in hematopoietic tumours. Results obtained by Tian et al have provided a new insight on how these cells may modulate leukemic tumour growth in vitro. According to this study, p38 MAPK, a suppressor of tumour development, was required for leukemic tumour suppression by WJ-MSCs (156).

These studies, taken together, indicate that WJ-MSCs are non-tumourigenic, anti-tumourigenic and hypoinmunogenic, do not transform to the TAF phenotype that is associated with enhanced growth of solid tumours, and suppress hematopoietic tumour development. WJ-MSCs appear to be a safe and promising tool for future cancer therapy and clinical applications, but more pieces of evidence are needed to further characterize their anti-tumourigenic mechanisms and to confirm this hypothesis.

V. Therapeutic applications

1. Treatment of autoimmune diseases

The immune properties of WJ-MSCs suggest that they may be a therapeutic option to treat autoimmune diseases such as type 1 Diabetes or Crohn’s disease.

a. Type 1 diabetes
Diabetes is a metabolic disease listed among the leading causes of death in some countries. It is characterized by absolute or relative insulin deficiency. Type 1 diabetes is characterised by an absolute insulin decrease due to T cell-mediated destruction of insulin-producing pancreatic β-cells (157). This autoimmune destruction of pancreatic islet β-cells reduces the patient’s ability to regulate blood glucose, leading to a high frequency of vascular complications that compromise quality and expectancy of life (158).

Transplantation of pancreatic islet cells (PICs) as a potential cure for type I diabetes has been hampered by immune rejection and recurrent attacks against islets by the underlying autoimmunity. Studies have shown the capacity of WJ-MSCs to differentiate into mature islet-like cell clusters. These islet-like cell clusters have been shown to contain human C-peptide and to release insulin in vitro and in vivo in response to physiological glucose levels. Real-time PCR analysis has shown the enhancement of insulin and other pancreatic β-cell related genes, such as pdx1 (pancreatic and duodenal homeobox 1), hlx9 (homeobox HB9 ou MNX1), nkx2.2 (NK2 homeobox 2), nkx6.1 (NK6 homeobox 1), and glut-2 (glucose transporter 2) in these cells (159). Various publications have confirmed the pancreatic islet-like cell differentiation potential of WJ-MSCs (47, 48, 160). Kim et al, comparing the capacities of MSCs from various sources (WJ, BM, AT and periosteum) to differentiate into PICs, have confirmed that all cell lines were well differentiated with an increased insulin mRNA expression, but only PICs derived from periosteum progenitor cells showed insulin secretion to a high glucose concentration (160).

More recently, Hu et al have studied the therapeutic potential of WJ-MSCs in patients with type 1 diabetes, evaluating the effects of these cells over a longer treatment time. They followed two groups of patients, the first of which received a basic treatment combined with WJ-MSC implantation, while the second received a basic treatment combined with normal saline therapy. Patients were followed for two years after the operations. During the follow-up period, patients treated with WJ-MSCs showed better Hba1 and C-peptide expression levels than patients in the second group. Although the precise mechanisms involved are unknown, WJ-MSC therapy appears to have a promising effect on type 1 diabetes patients and to be a good strategy for treatment of this disease (158).
b. **Type 2 diabetes**

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes and is characterized by insulin resistance and pancreatic β-cell dysfunction. Hu *et al.* studied the effect of intravenous infusion of human WJ-MSCs as therapy, administering them alone and in combination with sitagliptin (a dipeptidyl-peptidase IV inhibitor known to increase insulin release and decrease glucagon levels by having an impact on α and β cells in the pancreatic islets) in a T2DM rat model. Compared to the control groups (a diabetic control group and a sitagliptin-only group), rats treated with WJ-MSCs only and those treated with a combination of WJ-MSCs and sitagliptin exhibited increased numbers of β-cells. Glucagon level was decreased in the sitagliptin-only group and the WJ-MSCs + sitagliptin group compared to the WJ-MSCs-only group and the diabetic control group. These results suggest the therapeutic potential of WJ-MSCs in β-cell regeneration (161).

c. **Crohn’s disease**

Crohn’s disease (CD) is an inflammatory chronic disease caused by a dysregulation of immune tolerance and characterized by an idiopathic inflammation of the gastrointestinal tract. Frequent complications in CD are abscess and stricture formation, intestinal obstruction, and fistulas (abnormal connective passages from the epithelial lining of the intestines to another organ or to the skin caused by inflammation). Anti-TNF-α therapy is the first choice in the treatment of patients with perianal fistulas. Even with this treatment, however, perianal fistulas often lead to physical and emotional distress, and only 46% of cases heal completely (162).

Therapeutic effects of AT-MSCs and BM-MSCs on CD have already been proven (163, 164). For instance, Garcia-Olma *et al.* performed a clinical study on patients suffering from Crohn’s enterocutaneous fistulas in which they compared the therapeutic effects of autologous expanded AT-MSCs and unexpanded cells corresponding to the stromal vascular fraction (SVF) when cells of each type were implanted in the fistulas. Three out of four cases treated with expanded AT-MSCs were healed, compared to only one out of four cases treated with SVF. The authors have suggested that the use of expanded AT-MSCs would be more advantageous, and that the immunosuppressive properties of these cells were responsible for their healing effects in the treatment of CD. Other studies in progress will enable us to
better understand the link between the expansion of AT-MSCs and their beneficial effects (163). In another study, Cicconioppo et al examined the effect of ex-vivo expanded BM-MSCs in CD. All ten cases in which BM-MSCs were injected into the fistula exhibited signs of healing. In addition, a pro-apoptotic effect of BM-MSCs on mucosal T cells has been observed (164).

Recently, studies have focused on two granulomatous disorders, intestinal tuberculosis (ITB) and Crohn’s disease. Both diseases present similar clinical signs and are difficult to distinguish. The current challenge is the early identification of the correct disease in order to treat it efficiently and quickly to avoid complications or death. Working from the fact that recruited MSCs within granulomas in ITB can evade the host immune response, Banerjee et al have been pursuing the possibility of analyzing MSC markers in the two types of granulomas (i.e., those derived from patients with CD and those derived from patients with ITB). Their results have shown that the mesenchymal marker CD73 is expressed only in MSCs within tuberculous granulomas, identifying CD73 as a possible marker of ITB. This would explain the essential pathogenic mechanisms in ITB as being based on the recruitment of MSCs with high CD73 expression. These observations suggest that MSCs with increased CD73 expression could be a future candidate for therapeutic intervention in CD. Given their phenotypic profile, WJ-MSCs could have real potential for therapeutic applications in CD (165).

2. **Treatment of neurodegenerative diseases**

Neurodegenerative diseases are chronic and progressive disorders of the central nervous system (CNS), characterized by a steady loss of neurons in the region of the brain and spinal cord that affects the mental and motor abilities of affected people. According to the World Health Organization, in 2040 the devastating diseases known as Alzheimer’s and Parkinson’s will represent the second leading cause of death worldwide. Multiple sclerosis is another neurodegenerative disease.

a. **Parkinson’s disease**

Parkinson’s disease (PD) is a neurodegenerative disorder more common in the elderly. Its symptoms (tremor, rigidity, bradykinesia and postural instability) are caused primarily by the degeneration of dopamine (DA) neurons in the substantia nigra (166). Current therapies mostly relieve symptoms but do
not restore the function of the lesioned side of the brain or the efficacy lost due to disease progression. Embryonic stem cells have been investigated as a renewable source of dopamine-producing cells. However, technical and ethical obstacles have limited the application of this therapy (167).

Several groups have been interested in determining the effect of MSCs on the CNS. Ribeiro et al have shown that AT-MSCs and WJ-MSCs are able to release trophic/neuroregulatory factors that could improve the metabolic viability of hippocampal neurons in vitro. These two types of MSCs do not have similar functionality, however, because their secretomes act differently on cell viability and on the densities of hippocampal neurons. Indeed, AT-MSCs require exogenous factors such as bFGF to be added in the primary cultures of hippocampal neurons in order to influence the metabolic viability and neuronal cell densities, whereas WJ-MSCs are able to promote neuronal survival without the addition of exogenous factors (168).

Weiss et al. have characterized WJ-MSCs and compared them to MSCs derived from other sources. In their study, they tested the therapeutic effects of WJ-MSCs in parkinsonian rats. Their initial results demonstrated that WJ-MSCs express growth factors and angiogenic factors, suggesting that they may be useful for the treatment of neurodegenerative diseases. Indeed, the characterization of WJ-MSCs reveals that they produce glial cell line-derived neurotrophic factor (GDNF) located in the cytoplasm. WJ-MSCs also express nestin, a marker of primitive neural stem cells (4). After neural induction of WJ-MSCs, the expression of nestin was lower in differentiated cells than in undifferentiated cells, whereas the expression of tyrosine hydroxylase (a mature neural marker of catecholaminergic neurons) was greater in differentiated cells. Moreover, it has been shown that WJ-MSCs express some genes encoding for proteins with a neurotrophic effect: CNTF (ciliary neurotrophic factor), VEGF, FGF20, and TRKC (neurotrophic tyrosine kinase). In addition, when they are transplanted into parkinsonian rats, WJ-MSCs can partially reverse the parkinsonian behavioural phenotype (27). Yan et al have managed to differentiate WJ-MSCs into neural-like cells in vitro and have subsequently tested the therapeutic potential of differentiated cells by implanting them into the striatum and substantia nigra of methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) lesioned hemi-parkinsonian rhesus monkeys. PD monkeys transplanted with the induced cells showed an improvement on behavioural measures. Furthermore, pathological and immunohistochemical data have indicated the presence of neuronal-like cells in the right
brain hemisphere of PD monkeys, suggesting that they may be dopaminergic neurons (167). Nearly identical results were obtained when WJ-MSCs were replaced by AT-MSCs (169). The beneficial effect of BM-MSCs on a parkinsonian rat model was shown by Ye et al. (170).

b. **Multiple sclerosis**

Multiple sclerosis (MS) is a progressive neurodegenerative disorder of the central nervous system characterized by chronic inflammation, demyelination and neuronal damage. Currently, there is no medical cure for MS, mainly owing to an incomplete understanding of its pathophysiology (171).

Recently, Payne et al. have assessed the therapeutic efficiency of BM-MSCs, AT-MSCs and WJ-MSCs against MS, using recombinant myelin oligodendrocyte glycoprotein (rMOG)-induced experimental autoimmune encephalomyelitis (EAE), a model of MS in which both T- and B-cells contribute to the disease pathogenesis. They have demonstrated that BM-MSCs exerted more potent immunomodulatory effects *in vitro* compared to AT-MSCs and WJ-MSCs. Unexpectedly, however, BM-MSCs did not impact the disease course, although the transplantation of AT-MSCs ameliorated clinical signs in two animal models of EAE. Furthermore, only AT-MSCs and WJ-MSCs expressed integrin-α4 (CD49d); BM-MSCs, which may not be able to adhere to VCAM-1, a critical step in the extravasation of cells into the central nervous system during EAE, did not express integrin-α4 (172). In their recent work, Liu et al. showed that WJ-MSCs could potentially play a therapeutic role in MS and could be an alternative to BM-MSCs, which have been extensively studied with regard to the treatment of MS (173-175). Thus it is believed that these cells could restore behavioural functions and attenuate the histopathological deficits of experimental autoimmune encephalomyelitis mice over the long term (50 days) (176). These results confirm those of Liang et al, who transplanted WJ-MSCs to a patient with refractory progressive MS and subsequently observed stabilization of the disease (177).

c. **Alzheimer’s disease**

Alzheimer’s disease (AD) is a progressive and fatal neurodegenerative disorder characterized by a loss of memory and a deterioration of cognitive ability. Cumulative evidence supports the hypothesis
that accumulation of amyloid-β peptide (Aβ) in the brain and oxidative stress play critical roles in AD pathogenesis.

Very recently, Liang et al have attempted to differentiate WJ-MSCs into cholinergic-like neurons. Cholinergic neurons are neurons of the autonomic nervous system and are one of the causes of cognitive disorders such as AD. To induce differentiation, Liang et al used a neural stem cell conditioned medium supplemented with Bone Morphogenetic protein (BMP4) and fibroblast growth factors 8 (FGF8). First, they observed morphological changes of WJ-MSCs after culture in conditioned medium. These cells, which under normal conditions have a bipolar spindle-like morphology, changed into a bulbous shape with thin extensions touching each other to a certain extent after 16 days of differentiation. These observations already suggest a structural organization into axons. Moreover, they confirmed their hypothesis by showing an expression of cholinergic neuron markers including CHAT (choline acetyltransferase) and NF by immunofluorescence and RT-PCR 20 days after the beginning of WJ-MSC induction. These in-vitro results demonstrate that WJ-MSCs can be induced into cholinergic-like neurons, which suggests that WJ-MSCs may be a very good candidate for the treatment of AD (178). Patients suffering from AD show a decrease in the expression and activity levels of neprilysin (NEP: neural endopeptidase), which is one of several proteases involved in the proteolysis of Aβ. Thus, NEP has been intensively studied as a potential therapeutic target for AD. Since MSCs have the ability to synthesize vesicles (generated from the membrane), which also have a real therapeutic potential, Katsuda et al have studied vesicles derived from AT-MSCs and identified their effects in co-cultures with cells over-producing Aβ. They observed a decrease in the amount of Aβ in the presence of AT-MSC vesicles. This phenomenon is explained by the initial expression on AT-MSC membranes of the NEP that is later present as an active form in the vesicles. Furthermore, they observed that AT-MSCs expressed NEP at a higher level than BM-MSCs did, suggesting that AT-MSCs could be a better candidate than BM-MSCs for the treatment of AD (179).

In another recent in-vivo study, Yang et al sought to determine the therapeutic impact and mechanisms of action of neuron-like cells differentiated from WJ-MSCs in AD. They induced the differentiation of human WJ-MSCs into neuron-like cells using tricyclodecan-9-yl-xanthogenate (D609), then transplanted them into a transgenic AD mouse model. The resulting beneficial effects were linked to
an “alternatively activated” microglia (M2-like microglia). Treated mice showed increased M2-like microglial activation, associated with an increase in the expressions of IL-4 (an anti-inflammatory cytokine) and NEP and a decrease in the expressions of IL-1β and TNF-α (pro-inflammatory cytokines). Hence, according to this study, transplantation of neuron-like cells differentiated from WJ-MSCs might be a promising cell therapy for AD (180). Later, the same group studied the therapeutic impact of systemic administration of WJ-MSCs in a transgenic AD mouse model, and found that WJ-MSC infusion improved spatial learning and alleviated memory decline by reducing oxidative stress. All these results, taken together, suggest that WJ-MSCs in their differentiated (neuron-like cells) and undifferentiated forms may have beneficial effects in the prevention and treatment of AD.

VI. Conclusion

The number of diseases which in their final stages require organ transplant or cellular therapy is increasing. Many of them derive from or are accompanied by an unbalance in the organ inflammatory or immune state. In this regard, the use of a cellular therapy vehicle that can provide both organ recellularization and restoration of a physiological microenvironment without being rejected by the patient would benefit patients significantly.

To date, BM-MSCs have been considered the gold standard among therapeutic MSC-based therapies, yet BM-MSCs eventually degrade, exhibiting loss of proliferation and senescence. In the search for an alternative therapy lacking this flaw, different sources of MSCs have been explored. MSCs from adipose tissue, dental pulp and amniotic fluid have variable proliferation potentials and multilineage differentiation capacities, suggesting that the source of MSCs should be chosen carefully depending on the clinical applications targeted. Over the last few years, MSCs derived from Wharton's jelly have gained much attention in regenerative medicine. The overview of the literature presented in this review has
described their high differentiation potential as well as their important trophic, immunomodulatory and anti-tumourigenic effects, which must be confirmed in different animal models. Taken together, all these works clearly show that the immunological features of various types of MSCs may affect their applications in regenerative medicine in ways that may be essential. As these two properties rely in turn on paracrine effects, it is essential to further study the composition of MSCs secretome. This task will probably represent a major part of the relevant publications over the next 10 years.

The great question concerning MSC therapy that still remains to be answered concerns its biosafety. Clinical trials are still needed to evaluate this particular aspect, especially in new sources of MSCs like WJ-MSCs, even if they seem to be promising tools for the treatment of incurable degenerative diseases.

Disclosure statements
No competing financial interests exist

REFERENCES


11. Hua, J., Gong, J., Meng, H., Xu, B., Yao, L., Qian, M., He, Z., Zou, S., Zhou, B., and Song, Z. Comparison of different methods for the isolation of mesenchymal stem cells from umbilical cord matrix: proliferation and multilineage differentiation as compared to mesenchymal stem cells from umbilical cord blood and bone marrow. Cell Biol Int, 2013.


tissue, or bone marrow encapsulated in fibrin gels containing TGF-beta3. Biomaterials 32, 8139, 2011.


89. Rasmusson, I., Ringden, O., Sundberg, B., and Le Blanc, K. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. Transplantation 76, 1208, 2003.

90. Spaggiari, G.M., Capobianco, A., Becchetti, S., Mingari, M.C., and Moretta, L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood 107, 1484, 2006.


144. Akimoto, K., Kimura, K., Nagano, M., Takano, S., To'a Salazar, G., Yamashita, T., and Ohneda, O. Umbilical cord blood-derived mesenchymal stem cells inhibit, but adipose tissue-derived mesenchymal stem cells promote, glioblastoma multiforme proliferation. Stem Cells Dev 22, 1370, 2013.


156. Tian, K., Yang, S., Ren, Q., Han, Z., Lu, S., Ma, F., and Zhang, L. p38 MAPK contributes to the growth inhibition of leukemic tumor cells mediated by human umbilical cord mesenchymal stem cells. Cell Physiol Biochem 26, 799, 2010.


Figure Legends
Figure 1: Summary of the potential therapeutic roles of MSCs.

Beneficial effects of MSCs have been attributed to their differentiation potentials. However, attention has been shifted to their paracrine effects (via vesicles and soluble factors) rather than their plasticity. The role that MSCs will play is determined by the microenvironment where they reside.
Figure 2. Immunomodulatory effects of the WJ-MSCs on innate and adaptive immunity which can be summarized as follows: inhibition of the maturation and activation of DCs as well as the proliferation of T cells, activation of the expansion and cytotoxicity of NK cells. Effects of WJ-MSCs on B cells are still contradictory; they can stimulate or block the proliferation and differentiation of B cells and the secretion of Ig (Ig: immunoglobulin).
Figure 2: Immunomodulatory effects of the WJ-MSCs on innate and adaptive immunity.

The effects can be summarized as follows: inhibition of the maturation and activation of DCs as well as the proliferation of T cells, activation of the expansion and cytotoxicity of NK cells. Effects of WJ-MSCs on B cells are still contradictory; they can stimulate or block the proliferation and differentiation of B cells and the secretion of Ig (Ig: immunoglobulin).
Figure 3. Proposed mechanisms involved in the homing of WJ-MSCs to sites of tissue injury and their angiogenesis capacity.

MSCs return to the site of injury along gradients created by inflammatory chemokines and several factors. They express a variety of chemokine receptors that can mediate their migration into injured sites. Expression of a set of MMPs by WJ-MSCs contributes to ECM degradation, allowing them to cross the ECM and reach the site of injury. They also express a variety of chemokines, some known as angiogenesis mediators and act by binding to their receptor CXCR2 on the endothelium.
Figure 3: Proposed mechanisms involved in the homing of WJ-MSCs to sites of tissue injury and their angiogenesis capacity.

MSCs home to site of injury along gradients created by inflammatory chemokines and several factors. They express a variety of chemokine receptors that might be involved in their migration into injured sites. The expression of a set of MMPs by WJ-MSCs contributes to the extracellular matrix degradation which allows them to cross the extracellular matrix and reach the site of injury. They also express a variety of chemokines that are known as angiogenesis mediators and exert their function by binding to their receptor CXCR2 on the endothelium.

Table 1: Phenotypic profile of WJ-MSCs compared to MSCs other sources.

<table>
<thead>
<tr>
<th>WJ-MSCs MARKERS</th>
<th>COMPARED TO [ ]</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive: CD29, CD105, HLA-ABC, Oct-4, Gata-4, Cx43, α-actin, cTnt</td>
<td>[AF-MSCs]</td>
<td>(18)</td>
</tr>
<tr>
<td>Negative: CD34, HLA-DR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive: CD44, CD13, CD56, CD61, CD73, CD105, CD90, CD166, CD29, HLA-ABC, CD59</td>
<td>[dental pulp of milk and adult wisdom teeth derived MSCs]</td>
<td>(17)</td>
</tr>
<tr>
<td>Negative: HLA-DR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive: CD73, CD105, CD90,</td>
<td>[PDL-MSCst]</td>
<td>(14)</td>
</tr>
<tr>
<td>Negative: CD34, HLA-DR, CD45, CD19, CD11b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive: CD68</td>
<td>[promyelocytic cell line (HL-60) : known to express CD68]</td>
<td>(20)</td>
</tr>
<tr>
<td>Negative: CD34, CD45, CD163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive: CD13, CD29, CD44, CD105, CD106,</td>
<td>[Bone marrow MSCs]</td>
<td>(13, 16, 21)</td>
</tr>
</tbody>
</table>
CD73, CD166, HLA-ABC, CD90

**Negative:** CD14, CD34, CD38, CD45
CD31, HLA-DR

**Similar markers expression except:**
- CD106: WJ<<BM
- HLA-ABC: WJ<<BM

**Positive:** CD105, CD146, CD73, CD90

**Negative:** CD14, CD34, CD31, CD45, CD3

[Human MSCs from: tibial plateau (TP), trabecular bone, iliac crest (IC), BM and WJ umbilical cord]

Similar level expression for all markers except CD46 (twice more expressed for IC than for WJ and TP)

**Positive:** CD44, CD73, CD105, CD90, CD106, CD29, vimentin, laminin, Oct-4, Nanog

**Negative:** CD34, CD14, CD45, CD31, vWF

[Adult and foetal Bone marrow (aBM-MSCs and fBM-MSCs) and adipose tissue derived-MSC (AT-MSCs)]

Similar markers expression except Oct-4 and Nanog expressed only by BM-MSCs and WJ-MSCs

### Table 2: Transcriptomic profile of WJ-MSCs compared to MSCs from other sources.

The table below presents the difference of genes expression, for various markers, between the different MSCs sources. The difference of expression between the sources is presented by +++, ++, +, -, and ND corresponds to a non-determined comparison.

#### Genes related to bone development and neurogenesis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene identification/function</th>
<th>WJ-MSCs</th>
<th>BM-MSCs</th>
<th>PDL-MSCs</th>
<th>AF-MSCs</th>
<th>AT-MSCs</th>
<th>Skin-derived MSCs</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP4</td>
<td>induce endochondral osteogenesis</td>
<td>++</td>
<td>+</td>
<td>ND</td>
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<td>ND</td>
<td>-</td>
<td>+++</td>
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**Gene related to liver and cardiovascular systems**

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<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>++         (18, 28, 29)</td>
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<tr>
<td>c-TnT</td>
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<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND         (18)</td>
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<td>VEGF</td>
<td>cardiovascular development</td>
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<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND         (27)</td>
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<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND         (18, 20)</td>
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<tr>
<td>VCAM1</td>
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<td>+++</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
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<td>AFP</td>
<td>Liver development</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>++</td>
<td>+          (28)</td>
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<td>-</td>
<td>ND</td>
<td>ND</td>
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<td>-          (28)</td>
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<td>AT-MSCs</td>
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**Adipogenic and chondrogenic genes**

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Genes implicated in morphogenesis, adhesion, cell structure and other mesodermal markers

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<td>ND</td>
<td>ND</td>
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<td>+/-</td>
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<td>CXCR4</td>
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<td>CD44</td>
<td>PECAM1</td>
<td>CD9</td>
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PPIA : *Cyclophilin* A, *Homo sapiens* peptidylprolyl isomerase A; ITGB1 (*Integrin, b1* :*fibronectin receptor, b polypeptide*); WNT4 (*Wingless*-type MMTV inte SHH (*Sonic hedgehog homolog (Drosophila)) gration site family, member 4); SNAI2 (*Snail homolog 2 (Drosophila)*); VGA (*Vascular gene*); SNAI3 (*Snail homolog 3 (Drosophila)*); TGFBR1 (*Transforming growth factor, β receptor I*); TERT (*Telomerase reverse transcriptase*); ESG1 :*ESTs, weakly similar to embryonal stem cell specific gene 1*; Cx43 (*connexin 43*); ACTA (*α-actin*); ACTB (*β-actin*); CDH2 (*cadherin 2*); OPN (*osteopontin*); LIFR (*leukemia inhibitory factor receptor*); SOX2 (*Sex-determining region Y (SRY)-box*).
Table 3: Differentiation potential of WJ-MSCs compared to MSCs from other sources. This table shows differences in differentiation potential of MSCs from many sources towards a specific cell type which is indicated by ++++, ++, +, -., and ND corresponds to a non-determined comparison. For example, PDL-MSCs have the greatest potential to differentiate into chondrocytes while DP-MSCs have the lowest differentiation potential towards this type of cells.

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<th>Chondrocyte</th>
<th>Osteocyte</th>
<th>Adipocyte</th>
<th>Endothelial cell</th>
<th>Neuron</th>
<th>Myocardial cell</th>
<th>Pancreatic islet-like cell</th>
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<td>++ (22, 32)</td>
<td>++ (22, 24)</td>
<td>+++ (17)</td>
<td>++ (27)</td>
<td>++ (25)</td>
<td>++ (12)</td>
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<td>Adipose tissue</td>
<td>++ (22, 33)</td>
<td>++ (23, 36)</td>
<td>+++ (23, 34, 36)</td>
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<td>+++ (36)</td>
<td>++ (25)</td>
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<td>Dental pulp</td>
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<td>++ (12, 14, 17)</td>
<td>+ (17)</td>
<td>ND</td>
<td>++ (37, 36)</td>
<td>-- (10)</td>
<td>-- (39)</td>
</tr>
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<td>++ (14, 17, 36)</td>
<td>+ (4, 14, 36)</td>
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<td>++ (27, 36)</td>
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<td>+++ (14)</td>
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<td>++ (42)</td>
<td>++ (32, 43)</td>
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