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Roles of Mesenchymal Stem Cells in Tissue Regeneration and Immunomodulation

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Abstract

Mesenchymal stem cells are classified as multipotent stem cells, due to their capability to transdifferentiate into various lineages that develop from mesoderm. Their popular appeal as cell-based therapy was initially based on the idea of their ability to restore tissue because of their differentiation potential *in vitro*; however, the lack of evidence of their differentiation to target cells *in vivo* led researchers to focus on their secreted trophic factors and their role as potential powerhouses on regulation of factors under different immunological environments and recover homeostasis. To date there are more than 800 clinical trials on humans related to MSCs as therapy, not to mention that in animals is actively being applied as therapeutic resource, though it has not been officially approved as one. But just as how results from clinical trials are important, so is to reveal the biological mechanisms involved on how these cells exert their healing properties to further enhance the application of MSCs on potential patients. In this review, we describe characteristics of MSCs, evaluate their benefits as tissue regenerative therapy and combination therapy, as well as their immunological properties, activation of MSCs that dictate their secreted factors, interactions with other immune cells, such as T cells and possible mechanisms and pathways involved in these interactions.

Key Words: Mesenchymal stem cells, Immunomodulation, Regenerative medicine, Toll-like receptor, Prostaglandin E2, T regulators

INTRODUCTION

Mesenchymal stem cells (MSCs) were first discovered by Friedenstein in the 1970s as fibroblast-colony forming cells (Friedenstein *et al*., 1970) and since then have been subject of study, although they were not named as such until Caplan proposed it due their demonstrated capability to differentiate into other tissues such as cartilage and bone *in vitro*. The International Society for Cellular Therapy (ISCT) proposed three minimal criteria that cells must fulfill to be classified as MSCs: (1) characterized by plastic adherence; (2) >95% of the cells must express surface molecules such as cluster of differentiation (CD)105, CD73 or CD90, as well as <2% of expression of CD45, CD34, CD14, CD19, major histocompatibility complex (MHC) II; (3) show multipotent differentiation potential (Dominici *et al*., 2006).

Mesenchymal stem cells are capable to self-renew, but only for a limited time *in vitro*, and their lifespan can also vary from species to species. By the fact MSCs can be expanded *in vitro*, it makes them a good resource to apply them as therapy for different ailments. There are some companies, such as Osiris that invest all their research resources unto regenerative medicine using MSCs and by 2012 they received clearance from Health Canada to put on the market the world's first approved stem cell drug. In the case of veterinary hospitals, in the United States they have been offering stem cell therapy since 2003, using mostly bone marrow (BM) and adipose tissue (AT) MSCs; however, there is currently no approval of their use from the Food and Drug Administration (FDA) nor a full understanding of these cells (Cyranoski, 2013). Along with the use of BM and AT MSCs as stem cell therapies, the non-expanded stromal vascular fraction (SVF) is also vastly used under same name as "stem cell therapy". In this review, we describe MSCs characteristics, their role on tissue regeneration, their interaction with the immune system by discussing some effects of their stimuli-activation, interactions with immune cells, specially T lymphocytes and mechanisms involved in these interactions, which are starting to be elucidated by re-

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search conducted to date on MSCs.

CHARACTERISTICS OF MSCs

Definition

Mesenchymal stem cells, nowadays also known as mesenchymal stromal cells, are classified as multipotent stem cells, due their capability to transdifferentiate into various lineages that develop from mesoderm (Caplan, 1991). The multipotent stem cells, unlike their cousins the pluripotent stem cells, are theoretically able to differentiate to only one germ layer (Mahla, 2016) . Despite their classification, it has been documented that MSCs can differentiate *in vitro* into non-mesodermal cells, including neuron like cells (Kopen *et al*., 1999), hepatocytes, and pancreatic islet like cells (Xiong *et al*., 2014). Pancreatic islet cells transdifferentiation ability of MSCs was first reported by Chen *et al*. (2004) and it was also corroborated by other studies (Bai *et al*., 2015).

Though the actual nature or functions from MSC is still unclear, there is a new theory that claims MSCs are actually perivascular cells or pericytes, given that they can be isolated from all vascularized tissue, including menstrual blood (Alcayaga-Miranda *et al*., 2015) and they also express pericytes markers CD140a, CD140b and a-SMA (Kaewsuwan *et al*., 2012; Squillaro *et al*., 2016).

Sources of MSCs

Being firstly discovered on BM, this has been the most representative source of MSCs and the most studied until now. Later on, it was made known that MSCs can be extracted from a great variety of tissues, including adipose tissue, umbilical cord (Arutyunyan *et al*., 2016), umbilical cord blood (Koch *et al*., 2007; Schuh *et al*., 2009), Wharton's jelly (Teixeira *et al*., 2015; Gaafar *et al*., 2017), amniotic fluid (Fei *et al*., 2013), skeletal muscle tissue (Kisiel *et al*., 2012), periosteum (Kisiel *et al*., 2012), gingiva, periodontal tissue (Mrozik *et al*., 2010; Otabe *et al*., 2012), liver tissue (Najimi *et al*., 2017), lung tissue (Nordgren *et al*., 2018), menstrual blood (Ulrich *et al*., 2013; Ren *et al*., 2016) and more. After BM, one of the most usual source for MSCs is adipose tissue and it has become one of the most preferred choice of adult stem cells for clinical applications due their abundance per gram of tissue and their easy accessibility compared to BM-MSC (Strioga *et al*., 2012). It has been reported a slight difference on cell yield of MSCs depending on anatomical region where adipose tissue is extracted (Bahamondes *et al*., 2017; Hakki *et al*., 2017).

Applications of MSCs

According to the U.S. National Institute of Health, MSCs are being used in more than 800 clinical trials for various diseases, in which ailments of skeletal muscle are the most targeted, while being followed by conditions that compromise the immune system (Clinical Trials.gov) like graft-versus-host-disease (GVHD), autoimmune diseases, hematological malignancies, cardiovascular conditions, neurological diseases, bones and cartilage defects, and refractory wounds in organ transplantation (Squillaro *et al*., 2016). The most recently focused ailments treated with MSCs are for acute liver failure cirrhosis and regeneration of bladder tissue, heart scar repair after attack, dental problems, bone degeneration, muscle degeneration and alopecia (Mahla, 2016).

Comparison of MSCs over other stem cells

In terms of pluripotency and self-renewal capacities, MSCs are not as powerful as ESCs. Though ESCs might seem the ideal choice for cellular therapies, the ethical issues involved in their isolation procedures, makes them a difficult alternative for their actual therapeutic use (Lo and Parham, 2009). Induced pluripotent stem cells (iPSCs), as adult cells with overexpression of pluripotency factors such as OCT4, NANOG, SOX2, c-Myc, KLF44, and more, can be highly similar to ESCs, with self-renewal properties, great differentiation potential, but might show genomic unstability (Mahla, 2016).

MSCs are multipotent, self-renewal, with easy accessibility and culturally expandable *in vitro* with exceptional genomic stability and few ethical issues, marking its importance in cell therapy, regenerative medicine and tissue repairment (Ullah *et al*., 2015). Another thing to notice about MSCs is their immuno-privileged status, by the lack of MHC II expression which is particularly important, as these molecules are usually detected by T-cells concurrent with an antigen on the surface of antigen-presenting cells (APC) and it leads to inflammatory reaction. In order for the usual recognition of antigens from any cell not recognized as "self" from the body to take place, there must be a signal interaction between CD28 expressed on the T-cell and CD80 or CD86 expressed on the APC to fully activate T cells. As MSCs lack CD80, CD86, and MHC II or have extremely low expression of the last molecule (Jacobs *et al*., 2013), they do not provoke allogeneic reactions mediated by T effector cell and therefore have great potential for use as "off the shelf" products in allogeneic therapies (Le Blanc *et al*., 2003).

ROLE OF MSCs IN POTENTIAL REGENERATIVE THERAPY

MSCs and their effect in tissue repair

Mesenchymal stem cells secrete trophic factors that reportedly promote cell survival, such as stromal derived factor-1 (SDF-1), hepatocyte growth factor (HGF), insulin-like growth factor (IGF-1), epithelial growth factor (EGF), nerve growth factor (NGF), transforming growth factor-alpha (TGF-a), and tissue angiogenesis vascular endothelial growth factor (VEGF) (Rhee *et al*., 2015) (Fig. 1). The importance of SDF-1 has been investigated in a rodent model of bronchopulmonary dysplasia in which SDF-1 knocked down MSCs showed significantly reduced beneficial effectors in alveolarization, angiogenesis and inflammation characterized by macrophage infiltration in alveolar spaces relative to non-silenced control MSCs (Reiter *et al*., 2017). In an ischemic murine skin flap model, VEGF paracrine expression was increased in 4 days after murine MSCs (mMSCs) treatment, where VEGF was highly immunodetected in MSCs and in a small cluster of cells around capillaries compared to the control group (Schlosser *et al*., 2012).

Another feature of MSCs is their ability to migrate toward injury sites through chemoattractant gradients in the stromal extracellular matrix and peripheral blood. In these injury sites, local factors such as hypoxia, cytokine milieu, and toll like receptor ligands can stimulate MSCs functions. Therefore, these stimuli all promote the formation of abundant growth factors by MSCs that converge together to augment tissue regeneration (Rhee *et al*., 2015). For example, in experimental autoimmune

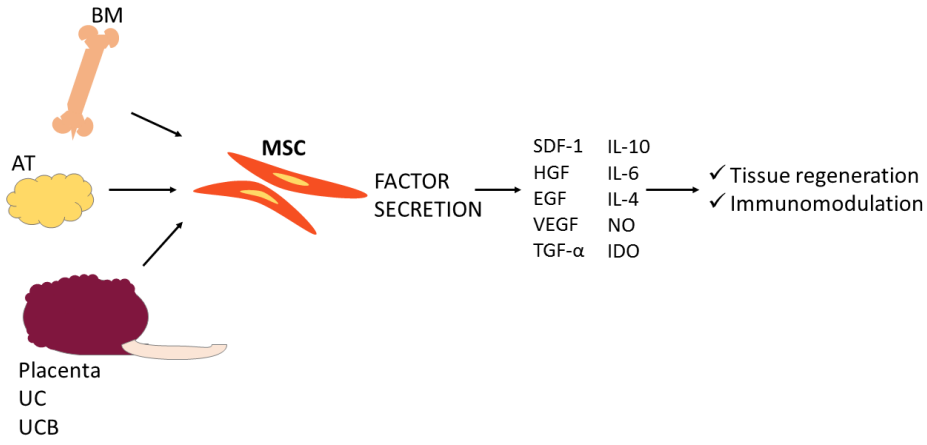

Fig. 1. MSC sources and secretome. MSC can be isolated from a variety of tissues, such as bone marrow, adipose tissue, placenta, umbilical cord, umbilical cord blood, liver, dental and more. MSC can transdifferentiate in vitro to other tissues but it is assumed that most of the benefits come from the factors they secret in the target environment.

thyroiditis, there was migration and nesting of intercellular adhesion molecule (ICAM)-overexpressing mMSCs towards the inflamed thyroid (Ma *et al*., 2017).

Since MSCs are primarily used as cell therapy for joint or limb injury in canine or equine animals (Volk and Theoret, 2013), information regarding the chondrogenic and osteogenic potential is of particular importance. In the case of chondrogenesis of hMSCs, an *in vitro* study showed that large quantities of IL-6 might also enhance transdifferentiation to chondrogenic tissue through activation of STAT3, which is part of the JAK/STAT pathway (the primary regulatory pathway for cytokine expression) (Rawlings *et al*., 2004). Unstimulated MSCs are capable to secret IL-6 and this can be beneficial or harmful depending on the target cells, organs and/or *in vivo* environment. Still, it is needed to take in account that IL-6 is commonly detected in the synovial fluid of osteoarthritic (OA) patients, and the actual quantities of IL-6 that were capable to enhance chondrogenesis, were much higher than those in OA patients; therefore, it is difficult to say that a higher IL-6 is associated with greater chondrogenic differentiation. Rather, this differentiation might rely much more on its local effects in the proximity of producing cells (Kondo *et al*., 2015). Now, STAT3 plays a role in tissue regeneration by MSCs and it has been documented that its activation or inactivation can modulate their trophic effects. If activated, STAT3 has been shown to be involved with cardiac repair and left ventricular function improvement by porcine MSCs (pMSCs). pMSCs increased expression of HGF and VEGF in the skeletal myocyte cell line C2C12. In the TO2 cardiomyopathic hamster model, intramuscular injection of pMSCs into the hamstring appeared to have a global trophic effect, since these factors were increased in the circulatory system, as well as in quadriceps, the liver, and the brain and attenuated myocardial apoptosis (Shabbir *et al*., 2010). Conversely, in the case of fibrotic injury, it is through STAT3 inactivation that MSCs beneficial effects can be seen (Matsui *et al*., 2017), like in the case of renal fibrosis (Matsui *et al*., 2017). Renal fibrosis is considered a common result of kidney diseases, and is frequently reported as a pathological diagnosis in chronic kidney disease (Lawson *et al*., 2015). When injured, an interstitial inflammatory infiltration results in production of various cytokines and growth factors, including transforming growth factor-beta (TGF-β), tumor necrosis factor-alfa (TNF-α), angiotensin-II (ANG II), and IL-18. ANG II stimulates STAT3 in tubular epithelial cells and mesangial cells (Matsui and Meldrum, 2012), which also leads to deposition of collagen, fibronectin, matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1), thus increasing fibrosis. Treatment with hMSCs induced a decrease in STAT3 activation, STAT3-dependent MMP-9 production and tubulointerstitial fibrosis (Matsui *et al*., 2017). Reduction of fibrosis by mMSCs was also reported in the cisplatin-induced acute kidney injury model, and their conditioned media reduced interstitial fibrosis, tubular cell apoptosis, and urinary kidney injury molecule-1 (Kim-1), though involved mechanisms were not reported (Abouelkheir *et al*., 2016).

Another example of tissue healing through MSCs is in the hypertrophic scar (HTS) model. In the process of hypertrophic scarring, there is an abundant deposition of extracellular matrix (ECM) which is produced by fibroblasts and myofibroblasts, being these last ones differentiated from fibroblasts and also an important step to HTS development. This is accompanied by an increase of inflammatory mediators through infiltration of immune cells (Liu *et al*., 2014; Domergue *et al*., 2016). In the HTS model, rabbit BM-MSCs (rMSCs) were injected through the ear artery, after which they migrated toward scar tissue and led to reduction of scar elevation index (SEI) at 3, 4 and 5 weeks after injection, and also to less collagen deposition compared to control group with no rMSCs injection. rMSCs also downregulated transforming growth factor-beta receptor I (TGF-βRI) and alpha-smooth muscle actin (α-SMA) at the mRNA and protein level of fibroblasts (Liu *et al*., 2014), both of which are needed for myofibroblast differentiation (Wipff and Hinz, 2008). Moreover, p53 also seems to be one of the pathways involved in HTS, as p53-knockdown of rMSCs augmented fibroblasts and nitric oxide, which increased fibrosis (Liu *et al*., 2014).

MSCs used as conjunctive therapy for regenerative effect

Due to the rising popularity of MSCs as potential treatments, their application along with different methods or strategies is being studied to enhance the quality of administered MSCs and achieve regenerative responses. Among the investigated

methods, extracorporeal shock wave (ESWT) in eMSCs is of note. If a horse damages its limbs with no way of recuperation, it becomes economically unfavorable and difficult to care for. Therefore, it is of utmost importance to focus on treatments aimed at limb healing (Vidal *et al*., 2008; Carrade Holt *et al*., 2014). Extracorporeal shock wave treatment, which in veterinary medicine is usually applied to equines for optimization of bone, tendon and cartilage restoration, may induce a 2-fold upregulation of Erk1/2 in eMSCs, a proliferation marker, as well as enhanced adipogenic and chondrogenic differentiation potential (Raabe *et al*., 2013).

Another method of using MSCs as a conjunctive therapy is utilizing a custom, progressive, dynamic orthosis (CPDO), which has been reported in a case of gastrocnemius tendon injury in a dog. Following autologous cMSCs injection into the core lesion, its thickness was reduced along the dorsoplantar plane, and a mild return of a linear fiber pattern in small sections of the gastrocnemius tendon was observed at day 98. Although there was incomplete recuperation of the tendon fiber pattern, the functional result was considered equal to successful surgical approach outcomes (Case *et al*., 2013). Improved animal MSCs regenerative therapeutic effect, especially for chondrogenic differentiation, has also recently been achieved through their transfection with a minicircle vector containing the high-mobility-group (HMG) transcription factor, SOX9, which upregulates type 2-collagen (COL2A1) (Tidd *et al*., 2017).

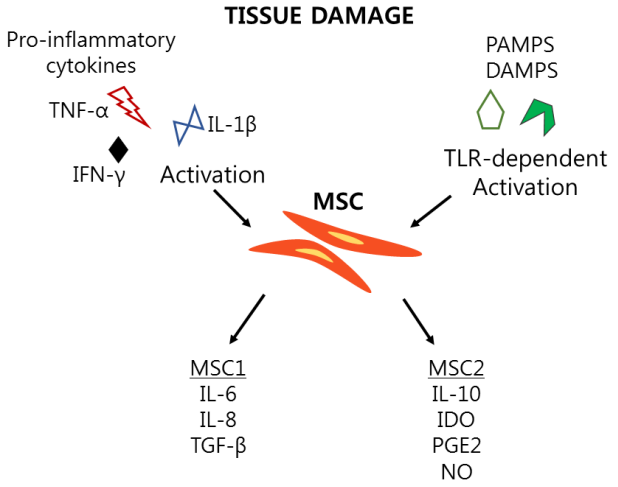

Fig. 2. MSC Activation. After there is tissue damage, different pro-inflammatory cytokines produced by lymphocytes can "activate" MSC to start producing anti-inflammatory factors like indoleamine 2,3-dioxygenase, prostaglandin E2, nitric oxide, interleukin 10. Pathogen-associated molecular patterns (PAMPS) and Damage-associated molecular patterns (DAMPS) can also activate MSC by ligating with toll-like-receptors on the surface of MSC. Depending on species, different TLR ligation can commit MSC to express either a pro-inflammatory phenotype (MSC1) which secretes pro-inflammatory cytokines like interleukin 6, interleukin-8, transforming growth factor beta, or an anti-inflammatory phenotype (MSC2).

MSCs AND THEIR EFFECT IN THE IMMUNE SYSTEM

Although the exact mechanisms underlying MSCs immunomodulation are not entirely understood, it is assumed that the main factors are cell-to-cell contact and/or release of soluble factors (Sharma *et al*., 2014). Mesenchymal stem cells can be found in their default niche in a resting state, where they present bystander anti-apoptotic and immune homeostatic features mostly inclined towards suppression. These features can be enhanced via MSCs activation by certain environmental stimuli (Krampera *et al*., 2013).

Activation of MSCs

The activation of MSCs is accomplished through individual or combined cytokines such as IL-1β, TNF-α, and IFN-γ (Singer and Caplan, 2011). For example, IFN-γ can be found in supernatants of cMSCs with stimulated leukocytes from mixed lymphocyte reaction (MLR), and subsequently, cMSCs become able to inhibit lymphocyte proliferation (Kang *et al*., 2008). However, MSCs have little or no effect on unstimulated peripheral blood mononuclear cells (PBMC) (Le Blanc *et al*., 2003). Once activated, MSCs secrete various immunomodulators including nitric oxide (NO) (Sato *et al*., 2007), IDO, PGE2, IL-6, and IL-10. All of these influence immune cells, including dendritic cells (DC), natural killer (NK) cells, macrophages, B cells and both $CD4^+$ and $CD8^+$ T cells (Rhee *et al*., 2015). After MSCs activation with MLR, in presence of serum, Clark reported inhibition of lymphocyte proliferation by eMSCs through PGE2 secretion in co-culture supernatant, but in the absence of serum, this secretion was shown to be markedly decreased, though anti-inflammatory IL-10 production was increased. Nevertheless, in both serum and serum free media (SFM), PGE2 seemed to be one of the main inhibitors of T-lymphocyte.

MSCs activation by TLR: MSCs can produce different cytokines depending on different stimuli. Among these stimuli, is to notice the pathogen-associated molecular patterns (PAMPS) such as lipopolysaccharides (LPS) and damage-associated molecular patterns (DAMPS), which act through toll like receptors (TLRs). Although MSCs are immunosuppressive biased, it has been proposed that polarization of MSCs through activation by different kinds of TLRs can result in different MSCs phenotypes (Fig. 2). For example, hMSCs can be either proinflammatory MSC1, which might secrete increased levels of IL-6, IL-8 or TGF-β, involving TLR4, or anti-inflammatory MSC2, which secrete IL-10, indoleamine 2,3-dioxygenase (IDO), and prostaglandin (PGE2), involving TLR3 (Burr *et al*., 2013; Krampera *et al*., 2013; Lim *et al*., 2016). However, differences by species may exist. For example, Lei *et al*. (2011) reported that TLR2 ligation of mMSCs completely restored and even stimulated lymphocyte proliferation on MLR. TLR2 ligation of mMSCs also reduced expression of CXCL10, which is involved in MSCs migration, and even reduced mMSCs-mediated expansion of $CD4^+CD25^+FoxP3^+$ T regs. Conversely, non-treated mMSCs and TLR4 ligated mMSCs inhibited lymphocyte proliferation, maintained ability to promote T regs generation and increased CXCL10 expression from day 1 (Lei *et al*., 2011). Therefore, it is possible that TLR2 is involved in proinflammatory MSC1 phenotyping on mMSCs and not TLR4.

MSCs and their interaction with peripheral blood mononuclear cells

As suggested by Caplan, MSCs can similarly function as an "injury drugstore" and they present diverse interactions involving immune cells. Described first by Bartholomew, MSCs

are able to suppress alloantigen induced proliferation in MLR (Bartholomew *et al*., 2002), and as previously mentioned, can be attributed in part to MSCs produced soluble factors, such as prostaglandins (English and Mahon, 2011), NO, IDO, as well as to the inflammatory environment with IL-1β, TNF-α, and IFN-γ. For example, the presence of IFN-γ can reduce MLR by MSCs through induction of IDO (Ryan *et al*., 2007). IDO acts through kynurenines, which catalyze tryptophan depletion from the environment, leading to cell cycle arrest. IDO subsequently induces apoptosis by caspase 8 activation and mitochondrial cytochrome-c release, and they might also induce FoxP3$^+$ T regulatory cells (Fallarino *et al*., 2002). Cell cycle arrest on PBMC is also assumed to be induced by periodontal ligament stem cells (PDLSC). PDLSC are derived from mature periodontal ligament of canine dental tissues, which are of ectomesenchyme origin and possess both neural crest and mesenchymal markers (Zhu and Liang, 2015). Periodontal ligament stem cells have shown potential to inhibit allogeneic and even xenogeneic PBMC proliferation, which is assumed to occur because of cell cycle arrest. This was measured with trypan blue, which showed that only 20 to 30% were apoptotic cells in every group of PHA-stimulated PBMC that were co-cultured with or without cMSCs and PDLSC (Kim *et al*., 2010).

cMSCs incubated with mitogen-stimulated leukocytes can inhibit their proliferation from a ratio of 1:1 to 1:10 (cMSCs:leukocytes). However, not only do the cells have this effect, the supernatant from cMSCs is also able to block leukocyte proliferation. This indicates that soluble immunomodulatory factors are also present in conditioned media. Indeed, PGE2 and IDO, which are well known immune tolerance key factors, are found in higher levels in conditioned media obtained from cMSCs co-culture with leukocytes than in supernatant from cMSCs alone (Kang *et al*., 2008; Lee *et al*., 2011), once again confirming that MSCs immunomodulation is enhanced after activation by certain stimuli.

Interaction with T cells: One of the cell interactions of utmost importance on immunosuppression by MSCs soluble factors is with the T lymphocytes. In the case of NO secretion by MSC, is reported to cause T cell suppression through cell cycle arrest (Glennie *et al*., 2005), or also apoptosis (Plumas *et al*., 2005). In eMSCs, this mechanism can vary according to the MSCs source. Carrade reported that equine tissue derived MSCs (AT and cord tissue) inhibited T cell proliferation through apoptosis. On the other hand, in equine BM and cord blood MSCs, this occurred through induction of cell cycle arrest in G0/G1 (Carrade Holt *et al*., 2014). Sato *et al*. (2007) reported that one of the suppression mechanisms of NO secreted from mMSCs is through the decrease of STAT5 phosphorylation in T-cells. In here, after mMSCs are stimulated in the presence of activated T lymphocytes, instead of targeting T-cell receptor complex, it induces a decrease in the phosphorylation of STAT5, working in a downstream signaling activating protein kinase C and calcium (Ca^{2+}) influx. This is similar to the mechanism macrophages perform in order to decrease T-lymphocyte in inflammatory environment (Sato *et al*., 2007).

These inhibitory effects toward T cells might also be helpful in the treatment of canine hypothyroidism. Hypothyroidism can be caused by idiopathic atrophy or immune mediated destruction of the thyroid gland because of mononuclear cell infiltration, consisting mostly of T-lymphocytes, which first causes thyroiditis by T cells (Lee *et al*., 2004). In an experimental autoimmune thyroiditis mouse model, mMSCs were found to influence immunocytes by downregulating IFN-γ and IL-17, as well as upregulating the anti-inflammatory cytokines IL-10 and IL-4 on T cells. These findings suggest that a change from Th1 towards their regulatory phenotype, Th2, occurred (Ma *et al*., 2017).

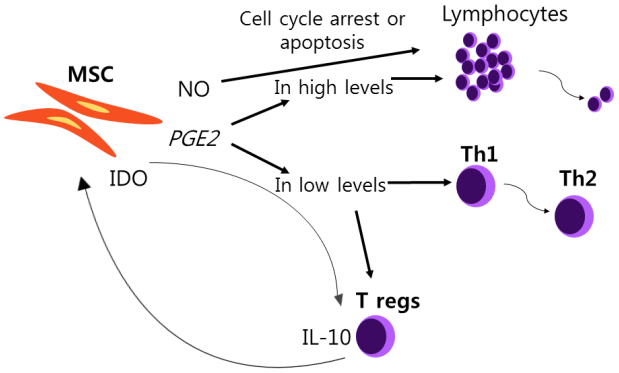

Fig. 3. Role of PEG2 and NO produced by MSC for immunosuppression. As MSCs are activated, they secrete various factors, among them, the anti-inflammatory prostaglandin E2. Depending on the environment, secretion levels of PEG2 will vary. If found in high levels, it can inhibit lymphocyte proliferation by reducing interleukin-2. In low levels, it can change Th1 to Th2 by blocking pro-inflammatory cytokines and it can also help induce T regs. At the same time, T regs can induce IDO secretion of MSC, and this works in synergy by helping T regs to produce the anti-inflammatory IL-10. Nitric Oxide can also produce an inhibition of T cell proliferation by inducing cell cycle arrest or apoptosis.

It has been suggested that the immunosuppressive function of MSCs depends mainly on their ability to induce an effect on generation or function of regulatory T lymphocytes (Engela *et al*., 2012). As previously mentioned, PGE2 from MSCs heavily impacts functions of cells associated with cellular-mediated immunity, such as T cells (Fig. 3). If PGE2 is found in high levels, T cell proliferation is inhibited through decreased IL-2 and downregulation of IL-2 receptor. This leads to impaired DNA-binding activity of transcription factors via Janus kinase-3 signaling suppression (Burr *et al*., 2013). Conversely, if found in lower concentrations, PGE2 shows a more regulatory function by helping shift Th1 towards Th2/Th17 by blocking proinflammatory cytokines and promoting Th2 cytokines such as IL-4 and IL-5. More importantly, it induces Foxp3$^+$ T regs through prostaglandin E receptor activation, this last one supposedly due to activation of nuclear factor-kB pathway (Kalinski, 2012; Burr *et al*., 2013).

This fact is important because it indicates the opportunity to use both MSCs and T regulatory cell in a combinatorial therapy. This is because they do not interfere with each other, but instead work in a synergistic interaction as T regs support activation and efficiency of MSCs, which express IDO, resulting in TNF-α reduction and inducing IL-10 production in T regs and effector cells (Engela *et al*., 2013). Different subtypes of T regs generated by MSCs have been identified, including CD4$^+$CD25$^+$Foxp3$^+$ regulatory T and IL-10 producing type 1 regulatory T (Tr1). The combination of mMSCs with either type of T regs results in reduced splenic T cell proliferation compared with single cell lines. Indeed, in a rheumatoid arthritis mouse model, MSCs infusion with Tr1 cells prevented swell-

Table 1. Regenerative therapeutic effect of MSCs

Species	Source	Lesion or disease	Secreted factor	Effect	References
Canine	AD	Semitendinous muscle lesion	NR	Improved lameness	Brown *et al*., 2012; Gibson *et al*., 2017
	AD	Osteoarthritis	NR	Improved lameness	Guercio *et al*., 2012
	BM*	Gastrocnemius strain	NR	Reduced thickness of lesion in dorsoplantar plane and at day 98, a mild return of a linear fiber pattern in small sections of the gastrocnemius tendon	Case *et al*., 2013
	BM	Chronic Chagas cardiomyopathy	NR	Increased peak velocity of aortic flow, reduced pre-ejection period, isovolumic relaxation time, and Tei index of myocardial performance	Sousa *et al*., 2011
Murine	BM	Ischemia	VEGF, MCP-1, MIP-1α, MIG	Promoted angiogenesis, reducedcaspase-3 activity	Boomsma and Geenen, 2012
	BM	Wound healing	MMP-9, VEGF	Angiogenesis, wound healing	Kim *et al*., 2011
	BM	Bronchopulmonary dysplasia	SDF-1	Improved alveolarization, angiogenesis and decreased alveolar space macrophage infiltration	Reiter *et al*., 2017
Equine	AD	Tendinitis	NR	Promoted tendon fiber organization, diminished inflammatory infiltration, increased type I collagen	de Mattos Carvalho *et al*., 2011

*cMSCs in combination therapy with custom, progressive dynamic orthosis.
AT: adipose tissue; BM: bone marrow; NR: not reported; VEGF: vascular endothelial growth factor; MCP-1: monocyte chemoattractant protein 1; MIP-1α: macrophage inflammatory protein-1 alfa; MIG: monokyne induced by interferon-gamma; MMP-9: matrix metalloproteinase-9; SDF-1: stromal derived factor-1.

ing or redness in the front or hind paws at 5 weeks after first infusion and led to a lower degree of mononuclear cell infiltrate and pannus formation with superficial cartilage damage when compared to controls (Lim *et al*., 2016).

CONCLUSION

As we have seen, MSCs are a promising alternative therapy that have high potential for both regenerative therapy and immune therapy; these cells secrete trophic and immunomodulatory factors (Table 1, 2) that interact according to the environment and environmental cues.

As mentioned before, MSCs are multipotent, self-renewal, with easy accessibility and culturally expandable *in vitro* with exceptional genomic stability and few ethical issues, marking its importance in cell therapy, regenerative medicine and tissue repairment (Ullah *et al*., 2015). Another thing to notice about MSCs is their immuno-privileged status, by the lack of MHC II expression which means that they do not provoke allogeneic reactions mediated by T effector cell and therefore have great potential for use as "off the shelf" products in allogeneic therapies (Le Blanc *et al*., 2003). On the other hand, some of disadvantages are that MSCs are considered to have self-renewal properties, but as the subculture number increases, they start losing their potency, due to their decrease on telomerase activity which causes telomere shortening, resulting in cellular senescence (Bonab *et al*., 2006). The therapeutic value of MSC can be influenced by donor age, as MSCs from old donors show a decreased proliferation potential (Ganguly *et al*., 2017). In terms of pluripotency and self-renewal capacities, MSCs are not as powerful as ESCs.

Although there is no *in vivo* evidence that these cells exert their regenerative effects through differentiation to target cells, there is an interaction between cytokines and/or growth factors secreted by them that can help recuperate homeostasis, thus contributing to tissue healing. Accompanied by their interactions on immunological signaling shown through *in vitro* experiments, their tendency to confer immunosuppressive cues is the reason they are being mainly appointed for treatments of hypersensitivities or autoimmune diseases. There is still a large list of unknown facts that are involved with MSCs cooperation with other cells. Accordingly, there is great demand for elucidation of their mechanisms and additional research is needed to develop accurate strategies to enable their efficient use in cellular therapy.

CONFLICT OF INTEREST

The authors do not have any conflicts of interest to declare.

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Table 2. Immunomodulatory effects of MSCs

Species	Source	Lesion or MSC stimulation	Affected immune cell	Secreted factor	Effect	References
Canine	AD	Atopic dermatitis	PBMC, induction of T-regs	NR	Induced T-regs	Byeon *et al*., 2016
	AD	Severe acute pancreatitis	Decrease CD3+ T cells, Increase of FoxP3 T regs.	IL-4, IL-10	Reduced pancreatic edema, inflammatory cell infiltration, acinar cell necrosis, decreased TNF-α, IL-1β, IL-6, -12, -17, -23, IFN-γ	Kim *et al*., 2016
	AD*	Steroid-refractory pemphigus foliaceus	NR	NR	Improved pruritus, leukocytosis, anemia, liver enzymes, body condition, no recurrence of skin lesions,	Han *et al*., 2015
	AD	MLR	CD3+, CD28+ PBMC	PGE2	Allo-reactive CD3+, CD28+ PBMC suppression	Lee *et al*., 2011
Equine	*BM, AD, UCB, UCT*	MLR	PBMC, T cell inhibition	PGE2, IL-10	T lymphocytes inhibition	Carrade Holt *et al*., 2014

*Transducted with CTLA4.
AT: adipose tissue; PBMC: peripheral blood mononuclear cells; CD: cluster of differentiation; IL: interleukin; TNF-α: tumor necrosis factor-alfa; IFN-γ: interferon gamma; MLR: mixed lymphocyte reaction; PGE2: prostaglandin E2; BM: bone marrow; UC: umbilical cord blood; UCT: umbilical cord tissue; NR: not reported.

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