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
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 to 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., 2016), 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; Scuillaro 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 (ClinicalTrials.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 (Scuillaro 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 over-expression 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 instability (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 (Ulalah 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 chemotactic 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
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-alpha (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 dorsoplanar 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).

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-

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 (PDLS). PDLS 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 trypsin 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 PDLS (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 (Ca2+) 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).

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 pro-inflammatory cytokines and promoting Th2 cytokines such as IL-4 and IL-5. More importantly, it induces Foxp3+ Tregs 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 Tregs support activation and efficiency of MSCs, which express IDO, resulting in TNF-α reduction and inducing IL-10 production in Tregs and effector cells (Engela et al., 2013). Different subtypes of Tregs 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 Tregs 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-
ing or redness in the front or hind paws at 5 weeks after first in-
fusion 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 the-
rapy that have high potential for both regenerative therapy and
immune therapy; these cells secrete trophic and immuno-
modulatory 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 alloge-
nic 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 telom-
erase 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 capaci-
ties, 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 fac-
tors secreted by them that can help recuperate homeostasis,
thus contributing to tissue healing. Accompanied by their inter-
actions on immunological signaling shown through in vitro ex-
periments, 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 co-
operation 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 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, reduced caspase-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 |

*aMSCs 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: monokine induced by interferon-gamma; MMP-9: matrix metalloproteinase-9; SDF-1: stromal derived factor-1.
**Table 2. Immunomodulatory effects of MSCs**

| Species | Source | Lesion of MSC stimulation | Affected immune cell | Secreted factor | Effect | References |
|---------|--------|---------------------------|----------------------|-----------------|--------|------------|
| Canine  | AD     | Atopic dermatitis         | PBMC, induction of T-reg | NR              | Induced T-regs | Byeon et al., 2016 |
|         |        | 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 |
| Equine  | BM, AD, | MLR                       | CD3+, CD28+ PBMC      | PGE2           | Allo-reactive CD3+, CD28+ PBMC suppression | Lee et al., 2011 |
|         | UCB UCT|                           | PBMC, T cell inhibition| PGE2, IL-10 | T lymphocytes inhibition | Carrade Holt et al., 2014 |

*Transduced with CTLA-4.

AT: adipose tissue; PBMC: peripheral blood mononuclear cells; CD: cluster of differentiation; IL: interleukin; TNF-α: tumor necrosis factor-alpha; 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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