# Toll-like receptors as modulators of mesenchymal stem cells

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Mesenchymal stem cells (MSCs) have differentiation and immunomodulatory properties that make them interesting tools for the treatment of degenerative disorders, allograft rejection, or inflammatory and autoimmune diseases. Biological properties of MSCs can be modulated by the inflammatory microenvironment they face at the sites of injury or inflammation. Indeed, MSCs do not constitutively exert their immunomodulating properties but have to be primed by inflammatory mediators released from immune cells and inflamed tissue. A polarization process, mediated by Toll-like receptors (TLRs), toward either an anti-inflammatory or a pro-inflammatory phenotype has been described for MSCs. TLRs have been linked to allograft rejection and the perpetuation of chronic inflammatory diseases (e.g., Crohn's disease, rheumatoid arthritis) through the recognition of conserved pathogen-derived components or endogenous ligands (danger signals) produced upon injury. Interest in understanding the effects of TLR activation on MSCs has greatly increased in the last few years since MSCs will likely encounter TLR ligands at sites of injury, and it has been proven that the activation of TLRs in MSCs can modulate their function and therapeutic effect.

Keywords: toll-like receptor, mesenchymal stem cells, cell therapy

## ADULT MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) have emerged in recent years as therapeutic tools based on three important features: (i) differentiation potential, (ii) capacity to modulate immune responses, and (iii) low immunogenicity, which would may allow allogeneic treatments.

Mesenchymal stem cells have been isolated from multiple tissues of mesodermal origin, such as bone marrow (Friedenstein et al., 1976), adipose tissue (Zuk et al., 2002), umbilical cord blood (Romanov et al., 2003), placenta (Fukuchi et al., 2004), synovium (De Bari et al., 2001), or dental pulp (Gronthos et al., 2000), among others. Despite significant efforts, no exclusive surface markers have been identified for MSCs. To date, MSCs are defined according to the three criteria of the International Society for Cellular Therapy (Dominici et al., 2006): (a) *Adhesion to plastic*: MSCs can be isolated by adhesion to plastic and expanded *in vitro* in serum containing media with no additional requirements for growth factors or cytokines; (b) *Expression of a specific combination of surface markers*: MSCs are negative for CD45, CD34, CD14, or CD11b, CD79α, or CD19 and HLA-DR, and positive for a variety of other markers, including CD73, CD90, and CD105; (c) *Differentiation potential*: MSCs can be identified *in vitro* by their ability to differentiate into mesenchymal-type cells (trilineage differentiation into adipocytes, osteoblasts, and chondrocytes; Pittenger et al., 1999). Although sharing these main characteristics, differences between MSCs from different sources can be found. The secretome differs between cell types, and bone marrow-derived MSCs (BM-MSCs) and adipose-derived MSCs (AD-MSCs), for instance, show specific RNA and protein expression profiles (De Ugarte et al., 2003; Noël et al., 2008; Skalnikova et al., 2011).

In homeostatic conditions, allogeneic cells are rejected by the immune system upon recognition of their foreign human leukocyte antigen (HLA). Allogeneic cells can also activate T cells through an indirect pathway where their HLA antigens are presented by professional antigen-presenting cells (APC). MSCs express low levels of cell surface HLA class I molecules whereas HLA class II, CD40, CD80, and CD86 are not detectable on the cell surface which theoretically opens the possibility of allogeneic treatments without the requirement of suppression of host immunity. Stimulation with interferon (IFN)γ has been shown to increase both class I and class II molecules. However, MSCs do not express classic co-stimulatory molecules such as CD40, CD80, CD86, even after stimulation in an inflammatory milieu. These features may allow MSCs to avoid or delay immune recognition (Le Blanc et al., 2003a,b; Majumdar et al., 2003; Rasmusson et al., 2003; McIntosh et al., 2006; Chamberlain et al., 2007), although this is a question that needs to be further investigated in both experimental animal models and clinical trials (Griffin et al., 2010).

Mesenchymal stem cells have immunomodulating properties and inhibit function of immune cells (Bartholomew et al., 2002; Krampera et al., 2003; Zhang et al., 2004; Beyth et al., 2005; Glennie et al., 2005; Puissant et al., 2005; Nauta et al., 2006; Yañez et al., 2006; Cui et al., 2007; Chiesa et al., 2011; DelaRosa et al., 2012). The specific molecular and cellular mechanisms involved in the immunoregulatory activity of MSCs are still under investigation and remain poorly understood. There is evidence that the capability to modulate immune responses rely on both cell

contact-dependent mechanisms (i.e., through Jagged1–Notch1 interactions; Liotta et al., 2008) and paracrine effects through the release of soluble factors (reviewed by Doorn et al., 2012). A broad panel of soluble factors have been involved including hepatocyte growth factor (HGF), prostanglandin-E2 (PGE2), transforming growth factor (TGF)-β1, indoleamine 2,3-dioxygenase (IDO), nitric oxide (NO), interleukin (IL)-10, heme oxygenase-1 (HO-1), and HLA-G5 (Krampera et al., 2003; Beyth et al., 2005; Puissant et al., 2005; Yañez et al., 2006; Chabannes et al., 2007; Cui et al., 2007; Oh et al., 2007; Selmani et al., 2008; DelaRosa et al., 2009). Differences in the mechanisms of immunomodulation employed by MSCs from different species have been reported. Whereas IDO activity appears to be a key player in human MSC-mediated immunomodulation, mouse MSCs do not express IDO and seem to use NO as the main mediator (DelaRosa et al., 2009; Ren et al., 2009; Meisel et al., 2011). Interestingly, MSCs may also modulate immune responses through the generation of regulatory T cells (Tregs; Krampera et al., 2003; Zhang et al., 2004; Maccario et al., 2005; Nauta et al., 2006; Gonzalez-Rey et al., 2010). Whether this MSC-mediated Treg induction is due to an expansion of pre-existing Tregs, to a *de novo* induction or to a combination of both needs to be further explored.

Importantly, MSCs do not constitutively exert their immunomodulating properties but have to be "primed" by inflammatory mediators released from activated immune cells, such as IFNγ, IL1β, and TNFα (Krampera et al., 2006; Prasanna et al., 2010). Also, the functionality of MSCs can be modulated by other inflammatory mediators such as APRIL and BAFF (Zonca et al., 2012). The thinking that MSCs are only anti-proliferative and immune-inhibitory on immune cells has been recently challenged by Waterman et al. (2010) who reported a "licensing" process of MSCs toward either anti-inflammatory or pro-inflammatory phenotypes, depending on the toll-like receptor (TLR) ligand used for activation. For extensive review on the concept of MSC "licensing" see the excellent review by Krampera (2011).

The biological characteristics mentioned above make MSCs an interesting tool for cellular therapy. This is supported by a number of studies in experimental models of inflammatory diseases demonstrating an efficient protection against allograft rejection, graft-versus-host disease, experimental autoimmune encephalomyelitis, collagen-induced arthritis, sepsis, and autoimmune myocarditis (Le Blanc et al., 2004; Zappia et al., 2005; Ohnishi et al., 2007; González et al., 2009a,b; Gonzalez-Rey et al., 2009; Németh et al., 2009). As indicated previously, TLRs have been implicated in the pathology of graft transplantation and inflammatory diseases (Ishihara et al., 2006; Yamamoto-Furusho and Podolsky, 2007) and therefore may modulate MSC function *in vivo* (DelaRosa and Lombardo, 2010; Krampera, 2011).

## TOLL-LIKE RECEPTORS
Innate immunity relies on the existence of a mechanism of recognition that identifies conserved molecular structures, known as pathogen associated molecular patterns (PAMPs), broadly expressed by different groups of microorganisms. These PAMPs include lipids, lipoproteins, carbohydrates, and nucleic acids (Akira et al., 2006). The recognition of these PAMPs is mediated by a set of germ line-encoded receptors known as pattern recognition receptors (PRRs). This recognition enables eukaryotic hosts to reliably detect a microbial infection, activating a number of signaling pathways that culminate in the induction of pro-inflammatory cytokines, chemokines, and inflammatory mediators. PRRs include TLRs, Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and NOD-like receptors (NLRs). PRRs, through their modulation of innate and adaptive immune responses, are essential players in the battle for tolerance or rejection of transplanted organs (Methe et al., 2004; Penack et al., 2010). The molecular and cellular mechanisms involved remain poorly understood and represent an emerging field of research with potential therapeutic implications.

Toll-like receptors are type I membrane proteins expressed by immune and non-immune cells (i.e., monocytes, macrophages, endothelial cells) either in the plasma membrane or intracellularly (endosomes). To date, 11 human and 13 mouse TLRs have been identified that recognize distinct microbial products from bacteria, viruses, protozoa, and fungi (Moresco et al., 2011). In addition, the recognition of endogenous ligands by TLRs is thought to have an important role in the regulation of inflammation, both in infectious and non-infectious diseases. A number of endogenous ligands have been identified, including heat shock protein (HSP) 60, HSP 70 (Asea et al., 2000; Oashi et al., 2000), heparan sulfate (Johnson et al., 2002), hyaluronan (Termeer et al., 2002), fibronectin extra domain A (Okamura et al., 2001), uric acid (Liu-Bryan et al., 2005), oxidized LDL (Miller et al., 2003), intracellular components of fragmented cells (Boule et al., 2004; Barrat et al., 2005), myeloid-related proteins-8 and 14 (Vogl et al., 2007), eosinophil-derived neurotoxin (Yang et al., 2008), and human defensin-3 (Funderburg et al., 2007). As these ligands are accessible to TLRs in the setting of injury or non-infectious threat, they have been called "danger signals."

Toll-like receptor activation triggers intracellular signaling pathways that lead to the induction of inflammatory cytokines, type I IFNs, and upregulation of co-stimulatory molecules leading to the activation of the adaptive immune response. Ligand recognition results in the recruitment of intracellular adaptor proteins, including myeloid-differentiation primary-response protein 88 (MyD88), shared by all TLRs except TLR3, and Toll/IL-1R domain-containing adaptor-inducing IFNβ (Trif), employed by TLR3 and TLR4 (O'Neill and Bowie, 2007). Recruitment of MyD88 leads to the activation of the mitogen-activated protein (MAP)-kinases (MAPKs) and nuclear translocation of the transcription factor nuclear factor-κB (NF-κB; *MyD88-dependent pathway*; Hoebe et al., 2006; Meylan et al., 2006). The activation of these signaling pathways is absent in MyD88-deficient mice in response to all TLRs, except TLR4 and TLR3. This is due to the activation of an alternative pathway triggered by Trif (*MyD88-independent pathway*) that culminates in the activation of NF-κB, MAPKs, and the transcription factors interferon-responsive factors (IRFs), whose are responsible for induction of type I IFNs, in particular IFNβ (Honda et al., 2006; Stetson and Medzhitov, 2006). Besides MyD88 and Trif, two other adaptor proteins have been described: TIR-domain-containing adaptor protein (TIRAP, required for MyD88-dependent signaling by TLR2 and TLR4), and Trif-related adaptor molecule (TRAM, required for Trif-dependent signaling through TLR4, but not TLR3; Takeda and

Akira, 2005; O'Neill and Bowie, 2007). Specific adaptors used by different TLRs combined with cell type-specific signaling pathways determine differential responses: inflammatory response, cell differentiation, proliferation, or apoptosis.

## MODULATION OF MSCs THROUGH TLRs

Expression of TLR 1, 2, 3, 4, 5, and 6 has been reported in human and mice AD-MSCs and BM-MSCs, human umbilical cord blood MSCs (UCB-MSCs), human Wharton jelly's MSCs (WJ-MSCs), human dental pulp (DP), and dental follicle (DF)-MSCs (van den Berk et al., 2009; DelaRosa and Lombardo, 2010; Kim et al., 2010; Raicevic et al., 2011; Tomic et al., 2011). Expression and function of TLRs can be modulated in different ways in MSCs. Hypoxia significantly increased mRNA of TLR1, 2, 5, 9, and 10 (Hwa Cho et al., 2010). Infection of MSCs with baculoviral vectors upregulated expression of TLR3 and activated TLR3 signaling pathway (Chen et al., 2009). Interestingly, the inflammatory environment may also modulate the pattern and function of TLRs expressed by MSCs. When cultured in the presence of an "inflammatory cocktail" (made with IFNα, IFNγ, TNFα, and IL1β) expression of TLR2, 3, and 4 was increased, while TLR6 was downregulated (Raicevic et al., 2010). This modulatory effect seems to depend on the origin of MSCs as differences between BM, AD, and WJ-MSCs was found recently (Raicevic et al., 2011). Fatty acids may also modulate TLR signaling in ob/ob mouse AD-MSCs. Stearidonic and eicosapentainoic acids inhibited LPS-mediated upregulation of TLR2 through a mechanism that involves NF-κB but not ERK signaling pathway (Hsueh et al., 2011).

### EFFECT OF TLRs ON DIFFERENTIATION OF MSCs

Adipogenic differentiation of human MSCs does not seem to be affected by TLRs (Hwa Cho et al., 2006; Liotta et al., 2008; Lombardo et al., 2009; Kim et al., 2010; Raicevic et al., 2010). Chondrogenic differentiation of human BM-MSCs has not been reported to be altered by activation through LPS, PolyIC, or R848 (Liotta et al., 2008), but was increased by TLR2 activation on human UCB-MSCs (Kim et al., 2010). The osteogenic differentiation seems to be enhanced in human BM-MSCs, AD-MSCs, and UCB-MSCs after LPS, PGN, or Poly IC activation (Hwa Cho et al., 2006; Mo et al., 2008; Lombardo et al., 2009; Kim et al., 2010), while CpG oligodeoxynucleotides (CpG ODN), have been reported to inhibit it on human AD-MSCs and BM-MSCs (Hwa Cho et al., 2006; Pevsner-Fischer et al., 2007; Liotta et al., 2008; Lombardo et al., 2009; Nørgaard et al., 2010). It has been reported recently that TNFα and TLRs activate osteogenic differentiation of AD-MSC via upregulation of transcriptional coactivator with PDZ-binding motif (TAZ; Hwa Cho et al., 2010).

On the other hand in mouse BM-MSCs, TLR2 was found to reduce differentiation into the three mesodermal lineages (Pevsner-Fischer et al., 2007). Interestingly, some reports link TLR signaling pathways with MSC multipotency. MyD88-deficient mouse BM-MSCs, when cultured in the appropriate differentiation media without additional stimulation with TLR ligands, effectively differentiated into adipocytes but failed to differentiate into osteocytes and chondrocytes (Pevsner-Fischer et al., 2007). However, TLR4-deficient mouse BM-MSCs showed higher differentiation rates compared to wild-type BM-MSCs (Wang et al.,

2010). Nevertheless, TLR2-deficient mouse BM-MSCs failed to accumulate vacuoles in differentiated adipocytes, suggesting some impairment in the terminal differentiation process (Abarbanell et al., 2010). Therefore, the role of TLR signaling pathways in MSC multipotency needs to be further clarified.

### EFFECT OF TLRs ON PROLIFERATION AND MIGRATION OF MSCs

So far, most of the studies have not found effects of TLR activation on human MSC proliferation. Only Hwa Cho et al. (2006) reported that TLR9 activation of AD-MSCs inhibited their proliferation. Interestingly, the use of TLR-deficient mouse BM-MSCs provided some insight on the role of TLRs on proliferation as TLR4-deficient BM-MSCs showed higher proliferation rates and TLR2-deficient showed reduced proliferation compared to wild-type MSCs (Abarbanell et al., 2010; Wang et al., 2010). In addition, TLR2 and TLR4 activation promoted proliferation of mouse BM-MSCs (Pevsner-Fischer et al., 2007; Wang et al., 2009).

Migration to the appropriate site of injury is believed to play a key role in the therapeutic efficacy of MSCs. Tomchuck et al. (2008) demonstrated that TLR3 activation drives the migration of human BM-MSCs in vitro. However, other reports found that TLR activation either impaired or had no effect on mouse BM-MSC migration (Pevsner-Fischer et al., 2007; Lei et al., 2011). In addition, TLR9 activation enhanced human BM-MSC invasion through a mechanism mediated, at least in part, by increased expression of MMP-13 (Nurmenniemi et al., 2010).

### EFFECT OF TLRs ON INTERACTION OF MSCs WITH IMMUNE CELLS

Mesenchymal stem cells have been shown to possess the capacity to inhibit proliferation of immune cells upon mitogenic or allogeneic activation. In recent years, inconsistent results have been reported regarding the role of TLR ligands on MSCs capacity to modulate immune responses. We and others found no significant effect of TLR activation on human AD-MSC or mouse BM-MSC-mediated immunosuppression (Pevsner-Fischer et al., 2007; Lombardo et al., 2009). However, other groups have reported that TLR activation may modulate the immunosuppressive properties of human BM-MSCs, although in very different ways. Liotta et al. (2008) found that TLR3 and TLR4 activation reduce the inhibitory activity of human BM-MSCs on T cell proliferation without influencing IDO activity or PGE2 levels, but downregulated expression of Jagged1, suggesting that the Notch signaling pathway mediates cell contact-mediated immunosuppression by MSCs. In contrast, Opitz et al. (2009) reported that TLR3 and TLR4 engagement enhances the immunosuppressive properties of human BM-MSCs through the indirect induction of IDO1. Induction of IDO1 involved an autocrine IFNβ signaling loop, which was dependent on protein kinase R (PKR) and independent of IFNγ. The role of IDO seems to be species dependent as Lanz et al. (2010) reported recently that IDO activity is not required for mouse BM-MSC immunosuppressive capacity both in vitro and in vivo, using IDO-deficient MSCs. Interestingly, TLR2 activation has been reported to impair the capacity of mouse BM-MSCs to induce the generation of regulatory T cells (Lei et al., 2011). Adding more uncertainty, Raicevic et al. (2010) reported that preactivation of human BM-MSCs with TLR3 or TLR4 ligands reduced production of HGF and PGE2 which impaired their capacity to

inhibit lymphocyte proliferation. However, these authors found in a later report, that triggering of TLR3 or TLR4 on human MSCs from BM, AD, and Wharton jelly´s did not affect their immuno-suppressive capacity (Raicevic et al., 2011). Dental pulp (DP) and Dental follicle (DF)-MSCs can also modulate lymphocyte proliferation *in vitro*, which is potentiated by TLR3 activation in both cell types, whereas TLR4 activation increased the suppressive role of DF-MSCs and reduced it in DP-MSCs (Tomic et al., 2011). Immunomodulating properties of human umbilical cord blood (UCB-MSCs) were not affected by prestimulation with TLR4 or TLR5 ligands (van den Berk et al., 2009).

Toll-like receptors may polarize MSCs toward pro-inflammatory and antigen-presenting-like phenotypes leading to release of pro-inflammatory cytokines and chemokines capable of enhancing recruitment of inflammatory immune cells (Romieu-Mourez et al., 2009). In line with this, a "licensing" process of MSCs toward either pro-inflammatory (MSC1) or anti-inflammatory (MSC2) phenotypes, which depends on the ligand concentration, timing, and kinetics of activation, has been proposed (Waterman et al., 2010). TLR4 priming results in upregulation of mostly pro-inflammatory cytokines such as IL6 or IL8 (MSC1 phenotype), while TLR3 priming results in production of anti-inflammatory molecules such as IL4, IDO, or PGE2 (MSC2 phenotype). TLR3-activated MSCs maintained the capacity to inhibit lymphocyte proliferation *in vitro*, while TLR4-primed MSCs activated T lymphocytes. As suggested by the authors, the polarizing effects of TLR priming may also explain the contradictory results obtained so far on the effects of TLRs on immunomodulation by MSCs.

There are other immune functions mediated by MSCs which have been found to be modulated by TLRs. BM-MSCs and parotid-derived MSCs have been shown to support neutrophil survival and chemotaxis in a ratio dependent manner through the release of soluble factors (Raffaghello et al., 2008; Brandau et al., 2010). Recently, Cassatella and colleagues found that TLR3 and TLR4 ligands enhanced the capacity of MSCs to delay neutrophil apoptosis through the induction of IL6, IFNγ, and GM-CSF. Moreover, TLR activation of BM-MSCs strongly increased respiratory burst of neutrophils. This supportive role on neutrophil function was confirmed using MSCs from thymus, spleen, or adipose tissue (Cassatella et al., 2011).

TLR2 and TLR4 mediate the capacity of human BM-MSCs to support short-term expansion of umbilical cord CD34+ cells, promoting myeloid-differentiation through the induction of hematopoietic growth factors (Wang et al., 2012). Moreover, it has been recently reported that resident mouse BM-MSCs, by producing MCP-1 in response to LPS, induce monocyte emigration from bone marrow into circulation to confront potential infections (Shi et al., 2011). These findings suggest an important role for TLRs in the modulation of the immune system by resident MSCs since BM-MSCs could function as sensors of circulating TLR ligands and determine, by expressing MCP-1, the frequency of circulating inflammatory Ly6Chigh, CCR2+ monocytes.

### EFFECT OF TLRs ON THERAPEUTIC EFFECTS OF MSCs *IN VIVO*
Several studies have reported beneficial effects of MSC treatment in animal models of sepsis or LPS-induced lung injury (in which MSCs were administered within 1 h following LPS challenge; Mei et al., 2007, 2010; Xu et al., 2007; Gonzalez-Rey et al., 2009; Németh et al., 2009, 2010). Based on the therapeutic benefit observed in these experimental models, it can be interpreted that high concentrations of LPS did not polarize MSCs toward a pro-inflammatory phenotype, in apparent contradiction to the reported polarizing process observed *in vitro* (Waterman et al., 2010). However, Waterman et al. (2010) reported that MSC1 and MSC2 cells were used in mouse models of lung injury and MSC1 aggravated the inflammatory injury, whereas MSC2 improved it, when compared to unstimulated BM-MSCs.

Conflicting results have been reported regarding the modulation of MSC-mediated cardiac protection by TLRs. LPS preconditioning of mouse BM-MSCs can, when compared to unconditioned MSCs, improve their survival and engraftment and increases the release of vascular endothelial growth factor (VEGF) in a model of rat acute myocardial infarction leading to enhanced therapeutic effects (Yao et al., 2009). These effects can be mediated through a TLR4-mediated protection of MSCs from apoptosis induced by oxidative stress (Wang et al., 2009). In contrast, TLR4-deficient mouse BM-MSCs had increased cardiac protection which was mediated by activated STAT3 signaling, leading to expression of higher levels of angiogenic factors such as VEGF and HGF (Wang et al., 2010). TLR2 activity also seems to be involved in cardioprotective effects by mouse BM-MSCs after ischemia/reperfusion injury. TLR2-deficient mouse BM-MSC showed impaired capacity to recover heart function, which correlates with reduced production of VEGF in hearts treated with TLR2-deficient MSCs compared to wild-type controls (Abarbanell et al., 2010). Therefore, further investigation in experimental animal models is required to clarify the role of TLRs in the licensing process as well as in the therapeutic potential of MSCs *in vivo*.

### CONCLUDING REMARKS
Despite discrepancies and inconsistencies reported by authors, some general conclusions can be made: (a) TLR expression: MSCs from different sources express TLRs at the mRNA level, although expression at a protein level seems to be low (i.e., compared to monocytes), and often makes difficult detection by flow cytometry, (b) MSC differentiation: in human MSCs, adipogenic differentiation does not seem to be affected by TLRs but osteogenic differentiation seems to be enhanced by TLR2, TLR3, or TLR4, while inhibited by TLR9. In mouse MSCs, TLR signaling might be linked to multipotency of MSCs as MyD88-deficient BM-MSCs failed to efficiently differentiate into chodrogenic and osteogenic lineage, (c) MSC proliferation: in human MSCs, only TLR9 activation has been reported to affect AD-MSC proliferation, (d) immunomodulatory capacity of MSCs: contradictory results have been reported that can be explained, at least in part, by the experimental conditions and the source of MSCs. The fact that differences in the experimental settings may lead MSCs to behave differently, suggests that MSCs can adjust their response in a dynamic way to the specific environmental conditions they face. In this regard, Waterman et al. (2010) challenged the concept of MSCs being always immunosuppressive and suggested that a polarizing process toward a pro-inflammatory or anti-inflammatory phenotype may occur depending on the TLR activated. However, the anti-inflammatory and therapeutic effects reported in mouse

models of sepsis and lung injury, where MSCs were exposed to high levels of LPS, seems to be in apparent contradiction to the polarizing process described *in vitro*. Therefore, the *in vivo* modulation of MSC biology by TLR ligands deserves to be further investigated and clarified.

The inflammatory conditions MSCs face when administered *in vivo* is now believed to play a fundamental role in their successful therapeutic use. Research on modulation of MSCs by TLRs can strongly contribute to better understand the immunomodulating properties of MSCs under different inflammatory environments and to characterize the features an inflammatory milieu should have for MSCs to best modulate immune reactions (i.e., composition, ratio or activity of immune cells, cytokines or other inflammatory mediators such as TLR ligands).

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