Key Metabolic Pathways in MSC-Mediated Immunomodulation: Implications for the Prophylaxis and Treatment of Graft Versus Host Disease

Andre J. Burnham1,2*, Elisabetta Manuela Foppiani1,2 and Edwin M. Horwitz1,2*

1 Aflac Cancer & Blood Disorders Center, Children’s Healthcare of Atlanta, Atlanta, GA, United States; 2 Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, United States

Mesenchymal stromal cells (MSCs) are spindle-shaped, plastic-adherent cells in vitro with potent immunosuppressive activity both in vitro and in vivo. MSCs have been employed as a cellular immunotherapy in diverse preclinical models and clinical trials, but most commonly as agents for the prophylaxis or therapy of graft versus host disease after hematopoietic cell transplantation. In addition to the oft studied secreted cytokines, several metabolic pathways intrinsic to MSCs, notably indoleamine 2,3-dioxygenase, prostaglandin E2, hypoxia-inducible factor 1 α, heme oxygenase-1, as well as energy-generating metabolism, have been shown to play roles in the immunomodulatory activity of MSCs. In this review, we discuss these key metabolic pathways in MSCs which have been reported to contribute to MSC therapeutic effects in the setting of hematopoietic cell transplantation and graft versus host disease. Understanding the contribution of MSC metabolism to immunomodulatory activity may substantially inform the development of future clinical applications of MSCs.

Keywords: kynurenine, PGE2, heme oxygenase-1, hypoxia-inducible factor 1 α, indoleamine 2,3-dioxygenase, graft versus host disease, mesenchymal stromal cell, aerobic glycolysis

INTRODUCTION

First identified in bone marrow in 1968 (1, 2), mesenchymal stromal cells (MSCs) are spindle-shaped, adherent in cell culture conditions, and widely investigated for their immunoregulatory properties and ability to contribute to tissue regeneration (3, 4). MSCs have been identified in a variety of tissue sources including bone marrow, adipose tissue, amniotic membrane and fluid, placental and fetal tissues, umbilical cord tissues, endometrium, blood, and synovial fluid (3). MSCs are present in relatively low numbers in any given tissue, and thus, prior to research or clinical use, are isolated and expanded ex vivo in cell culture media (3). Since MSCs may display several morphological and physiological characteristics in culture (3, 5), the minimum necessary criteria for MSC definition have been outlined by the International Society for Cellular Therapy (6, 7).
Indeed, MSCs are known to differentiate into chondrocytes, adipocytes, and osteocytes in vitro, suggesting that perhaps MSCs perform stem-like functions (8–12). Although tissue repair and regeneration has been reported after intravenous infusion of MSCs in a variety of disease settings (e.g., and late-onset hemorrhagic cystitis) (13–17), there are currently no in vivo data demonstrating that MSCs are true stem cells, differentiating to resident cells; however, it is possible that MSCs indirectly mediate endogenous tissue regeneration and repair mechanisms, perhaps by secreting soluble factors, via paracrine mechanisms or metabolic activity (18).

MSCs have also been shown to modulate adaptive and innate immunity in vitro and in vivo, usually after cytokine activation (e.g., IFN-γ) (4). MSCs may suppress both T and B cell proliferation as well as T cell effector activity (19–23), and MSCs are reported to inhibit proliferation by arresting T cells in the G0/G1 phases of the cell cycle or by promoting lymphocyte apoptotic pathways (24–28). Mechanistically, MSCs likely contribute to immunomodulation through cell-to-cell contact or paracrine and metabolic mechanisms (e.g., TGF-β, hepatocyte growth factor, prostaglandin E2; PGE2, and indoleamine 2,3-dioxygenase; IDO pathways), as immunosuppression has been reported in co-cultures and when cells are separated by a Transwell (26, 27, 29–32). Regarding the innate immune system, MSCs activated by monocytes or cytokines signal macrophages to promote pro- or anti-inflammatory pathways, by inducing the polarization of M2 macrophages (30, 32, 33). Moreover, inactive or apoptotic MSCs, those engulfed by phagocytic cells, or suppressed by host cytotoxic cells contribute to immunosuppression in vitro and in vivo (34–36).

On account of this immnosuppressive activity, MSCs have been identified as promising candidates for immunosuppressive cell therapies and have been especially studied in the context of treating and preventing acute graft versus host disease (aGVHD) during hematologic cell transplantation (HCT), which occurs when donor immune cells attack recipient tissue (usually liver, gut, and skin) (37, 38). MSC-based cell products have been approved or conditionally approved for the treatment and prophylaxis of aGVHD in pediatric patients in Japan (TEMCELL), Canada, and New Zealand (Prochymal) (39). Recently, Mesoblast conducted a phase III clinical trial using a donor-derived bone marrow MSC cell therapy (RYONCIL™) to treat pediatric steroid refractory aGVHD (40). Notwithstanding these approved and pre-approved MSC-based cell products for treatment and prophylaxis of GVHD, many clinical trials have generated mixed results (40–44).

Although cell contact-dependent and secretory mechanisms have been established as the primary immunoregulatory modes of action of MSCs, recently, metabolic stress and activity have been shown to be involved in MSC immunomodulatory functions. Indeed, a great many metabolic pathways are known to be involved in MSC physiologic mechanisms. However, the IDO, PGE2, hypoxia-inducible factor 1α (HIF1α), and energy metabolic pathways have been especially implicated in the literature to play key roles in the immunosuppressive activity of MSCs (Figure 1). Herein, we review recent and notable scientific advances that indicate how the aforementioned metabolic pathways endogenous to MSCs, among others, may contribute to immunomodulation in the context of HCT and GVHD.

**FIGURE 1** | Schematic of proposed MSC-mediated metabolic immunomodulatory mechanisms reviewed herein. Dotted lines indicate incompletely understood mechanisms. Tryp, tryptophan; Kyn, kynurenines; CO, carbon monoxide; AA, arachidonic acid; mito, mitochondria; met, metabolism.
IDO: IFN-γ AND THE KYNURENINE PATHWAY

The metabolic activity of IDO seemingly plays a key role in the immunosuppressive activity of MSCs on T cells and other lymphocytes (45, 46). Naïve MSCs do not normally synthesize IDO; however, after cytokine activation (usually by IFN-γ or TNF-α), MSCs express high levels of IDO (22, 47). The enzymatic activity of IDO acts on the kynurenine pathway of tryptophan metabolism. An essential amino acid, tryptophan is recruited by the cell for protein synthesis, or may be metabolized in the serotonin or tryptamine pathways (48, 49). After induction of IDO expression, the kynurenine pathway is activated and L-tryptophan is metabolically converted into kynurenine, which may be further metabolized into biologically-active kynurenine derivatives including kynurenic acid, 3-hydroxyanthranilic acid, picolinic acid, quinolinic acid (48, 49). Notably, quinolinic acid and 3-hydroxyanthranilic acid are known to target lymphocytes and contribute to suppression of T cell proliferation (30, 51), and kynurenic acid may also modulate the immune system by agonizing aryl hydrocarbon receptor, G-protein-coupled receptor 35, and promoting anti-inflammatory cytokines (e.g., TNF-α, IL6, IL1β, and IL10) (52). Moreover, the addition of tryptophan significantly restores allogeneic T-cell proliferation (53), while adding kynurenine suppresses allogeneic T-cell proliferation (46). Although IDO-mediated catabolism of tryptophan contributes to the MSC-induced immunosuppression, additional investigation is needed in order to uncover the mechanism of action, especially in vivo.

IDO: IMMUNOMODULATION AND GVHD

It is known that MSCs elicit immunosuppressive effects when first primed with IFN-γ or a combination of IFN-γ with TNF-α, IL-1α or IL-1β (22, 47). Given that the IDO pathway is significantly upregulated with IFN-γ treatment, IDO expression and tryptophan metabolism has been implicated in suppressing T cells and controlling GVHD (54). Kim et al. recently demonstrated that compared to naïve MSCs, human MSCs primed with IFN-γ significantly upregulated IDO expression, increased immunosuppressive activity in vitro, and reduced GVHD symptoms and mortality a NOD-SCID PBMC-transplanted mouse model (55). In addition, the investigators showed that downregulating IDO in IFN-γ-primed MSCs decreased this activity, and IDO expression was driven by the JAK/STAT1 signaling pathway. In another recent study, human gingival MSCs stimulated were reported to inhibit T cell and PBMC proliferation in vitro and improve survival in a xenogenic GVHD model in the NOD/SCID mice via a combination of CD39, CD73, adenosine, and IDO signals (56).

Inhibition of T cell proliferation by IFN-γ-licensed MSCs is widely believed to be IDO-dependent. However, there is additional evidence that IDO metabolism and signaling may not be involved with MSC activity on effector T cell effector functions (e.g., cytokine production). Chinnadurai et al. have shown, for example, that IFN-γ-primed MSCs inhibit T cell (Th1) effector production of IFN-γ, TNF-α, and IL-2 independent of IDO. Inhibition of T cell effector function was instead mediated by B7H1 and B7DC/PD1 pathways (23). In an analysis of a MSC-based off-the-shelf cell product, intravenous infusions of Cymerus™ MSCs (Cynata Therapeutics) ameliorated disease and prolonged survival in a humanized GVHD mouse model after treating cells with IFN-γ (57). Although activating Cymerus™ MSCs with IFN-γ increased IDO expression 5-fold after a 48 h incubation, upregulation of the immune checkpoint inhibitor PD-L1, which contributes to the PD1-PDL1 signaling axis was also observed (26, 58). Therefore, while IDO-mediated metabolic activity plays a role in the immunomodulatory properties of IFN-γ-primed MSCs, other factors such as PD1 signaling and IDO-independent T cell effector activity seem to also be involved in immunosuppression induced by MSCs. Moreover, the relative contributions of each mechanism have yet to be determined.

Despite the potent immunosuppressive activity observed in IFN-γ-primed MSCs, largely due to the IDO pathway, no clinical trials to date have employed IFN-γ-primed MSCs for the treatment and prophylaxis of GVHD. However, Horwitz and colleagues have recently registered a phase I clinical trial with the NIH aimed at using IFN-γ-primed MSCs as prophylaxis for aGVHD after patients with hematologic malignancies and myelodysplasia have received HCT (NCT04328714).

PROSTAGLANDIN E2

PGE2 metabolic activity has been implicated in MSC-based immunosuppressive activity. PGE2 is an arachidonic acid derivative synthesized by cyclooxygenases COX1, COX2, and prostaglandin synthetase (59). MSC secretion of PGE2 correlates with suppression of lymphocyte proliferation (60), and PGE2 is known to promote induction of immunosuppressive interleukins (IL4, IL10, and IL6), proliferation and cytotoxicity of natural killer cells, and differentiation of Treg cells, and suppress differentiation of dendritic cells and naive T cells to Th17 cells (61–64). Additionally, IL6 may contribute to PGE2-mediated immunomodulation in MSCs by positively regulating the COX2 function and synthesis of PGE2 (65, 66). IL6-dependent PGE2 has also been shown to promote immunosuppression via changing Th1 and Th2 ratios, inhibiting maturation of dendritic cells and stimulating of Treg cells (67, 68). Thus, synthesis and secretion of PGE2 contributes to MSC immunomodulation and have immunosuppressive potential in the context of GVHD.

In vivo, MSC infusions significantly increase secretion of PGE2 both before and after the onset of GVHD (69). Auletta et al. reported that of indomethacin (IM), a COX inhibitor which decrease PGE2 synthesis, and direct pharmacologic inhibition of PGE2-EP receptor interaction reversed T cell suppression induced my BM MSCs in vitro (70). In an allogeneic BMT mouse model, the investigators show that the survival advantage of animals treated with intravenous BM MSC injections was also
reversed with a 7-day dose of IM. Similar results have been reported with MSCs isolated from other tissue sources, such as umbilical cord tissue (71). More recently, Kim et al. showed that treating BM MSCs with IM or downregulating expression of prostaglandin E synthetase (PGES) via siRNA reduced proliferation of human PBMCs, and PGES knock down MSCs were unable to reduce mortality in mice with GVHD (72). These studies present evidence that PGE2 may be a key effector of immunosuppression in GVHD and HCT clinical settings.

**HEME-OXYGENASE-1**

Heme oxygenase intracellularly metabolizes heme to biliverdin, CO, and free divalent iron, and HO-1 is reported to have anti-inflammatory and immunosuppressive properties (73–75). Chabannes et al. (76). were the first to demonstrate that HO-1 may play a role in MSC-mediated immunosuppression, and report a reversal of PBMC suppression after adding the HO-1-specific inhibitor tin protoporphyrin (SnPP) (76). Reduced T cell suppression in *vitro* and improved survival in *vivo* was also observed after inhibiting rat MSC HO-1 in combination with nitric oxide synthase (NOS). Human MSCs displayed similar results in *vitro*, but were not tested in *vivo*. Interestingly, the nitric oxide synthesizing pathway, which, like the IDO pathway, may also be stimulated in MSCs by IFN-γ, is implicated in rodent rather than human systems (77–79). Infusion of murine MSCs transduced with murine HO-1 have also been shown to increase the number of Treg cells in spleen and lymph nodes, and significantly reduce severity of clinical aGVHD in mice (80).

In contrast, Galipeau and colleagues have reported that human BM MSCs express low levels of HO-1 both before and after priming with IFN-γ, TNF-α, and/or TGF-β, and MSCs treated with SnPP had no effect on T cell suppression, possibly due to the notion that the IDO pathway may require heme as a cofactor (81). Therefore, the role of HO-1 in MSC-mediated immunomodulation, notably in the context of suppressing the immune system, is inconsistent in the literature. It is possible that this inconsistency may be an *in vitro* artifact, or a result of mixing experimental approaches. For example, studies described herein that reported an effect of HO-1 on MSC-mediated immune suppression, largely studied animal MSCs, while Galipeau et al. investigated humans MSCs. In any case, additional investigation is required in order to understand the role of HO-1 in MSC-mediated immunomodulation, especially *in vivo*.

**HYPOXIA-INDUCIBLE FACTOR 1 α**

*In vivo*, MSCs are thought to be located in perivascular niches under relatively hypoxic conditions, where oxygen tension is low. Hypoxia plays a crucial role in maintaining homeostasis throughout the body from early stages of embryonic development, and the metabolic regulatory mechanisms of hypoxia are largely driven by oxygen-sensitive transcription factors, including hypoxia-inducible factor 1 (HIF-1) (82). HIF-1 is a heterodimer consisting of an oxygen-regulated α-subunit and a constitutively expressed β-subunit. Under hypoxic conditions, hydroxylation of HIF-1 α by prolyl hydroxylase is suppressed, leading to the accumulation and nuclear translocation of HIF-1 α (83, 84). Activation of HIF-1 α has been shown to regulate transcription of genes necessary for carbohydrate, fatty acid, and other metabolic pathways involved with energy production (85). Metabolic activity and regulation of HIF-1 α in MSCs has been implicated in MSC differentiation potential (86–89), migration and chemotactic localization (90), the inflammatory response (91), tissue repair, and angiogenesis (92–94).

Hypoxia and HIF-1 α metabolism in MSCs may also play a role in immunomodulation. In response to hypoxia, it has been shown that MSCs produce an increased level of anti-inflammatory cytokines (e.g., IL-10), decreased pro-inflammatory cytokines (e.g., TNF α), and demonstrate enhanced suppression of PBMCs (95–97). Recently, Kim and colleagues have shown that human MSCs expanded under hypoxia, promoted T cell suppression, and when IV-administered to a humanized mouse GVHD model, improved survival and reduced symptoms of GVHD were also observed (97). These data support the notion that hypoxia priming or increased expression HIF-1 α in MSCs could be a viable strategy to promote donor and host immunomodulation and reduce GVHD during HCT. However, hypoxia- and HIF-1 α-mediated immunoregulation by MSCs is a relatively new avenue of research, and this transcription factor regulates many metabolically active genes. Thus, additional studies aimed at uncovering a mechanism of action are needed.

**ENERGY METABOLISM**

Energy-generating metabolic pathways (i.e., lipid and carbohydrate metabolism) have also been implicated in MSC-mediated immunomodulation. Contreras-Lopez et al. recently demonstrated that metabolism of peroxisome proliferator-activated receptor (PPAR) β/δ, which plays and key role in lipid and glucose metabolism and homeostasis, may be important for MSC immunomodulation of T cells (98). The investigators reported that PPARβ/δ knock out MSCs had enhanced suppression of Th1 and Th17 proliferation via enhancement of glycolysis, and inhibition of mitochondrial generation of ATP promoted aerobic glycolysis in WT MSCs and consequently improved immunosuppressive activity. Similarly, Lui et al. recently demonstrated that priming MSCs with IFN-γ induces a metabolic switch towards aerobic glycolysis and strengthens T cell suppression (99). Moreover, oxidative glucose and lipid metabolism have been shown to contribute only 3% of ATP production in MSCs, while glycolysis generates 97% of cellular ATP (100). The studies together suggest that regulation of energy metabolism in MSCs, notably by reprogramming a switch from mitochondrial activity towards glycolysis, promotes immunosuppression.
**CONCLUSION**

MSCs contribute to adaptive and innate immunomodulation through cell-to-cell contact, secretory and paracrine signaling mechanisms, as well as intracellular metabolic pathways. IFN-γ, IDO and kynurenine, PGE2, HIF1α, HO-1, as well as energy-generating metabolic pathways have been implicated in MSC-mediated immunosuppression. Some studies have reported conflicting results, particularly regarding specific mechanisms of action and downstream targets. Moreover, the role of HO-1 in immunomodulation by MSCs remains an open question. IDO-kynurenine metabolism presents one of the most compelling mechanisms by which MSCs suppress the immune system. However, given that IDO is only expressed after MSCs are primed with IFN-γ or other cytokine combinations, which may regulate expression and activation of other factors (e.g., PDL-1), IDO may be only one of many contributors of MSC-based immunoregulation. Interestingly, aerobic glycolytic pathways, rather than oxidation of energy-generating substrates via mitochondria, have recently been hypothesized to play a key role in MSC immunomodulation, adding to the studies that promote the importance of IDO and kynurenine metabolism via IFN-γlicensing. Understanding how MSC metabolism modulates immune cell activity may have significant applications in the development of MSC-based therapeutics, especially in the context of HCT and aGVHD.

**AUTHOR CONTRIBUTIONS**

AJB and EMH conceived and designed the review. AJB wrote the original manuscript draft. EMH, EMF, and AJB edited and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

**FUNDING**

This work was supported by the National Institutes of Health (grant R56HL147867) and Aflac Cancer & Blood Disorders Center.

**ACKNOWLEDGMENTS**

Figure graphics were generated at Biorender.com.
34. de Witte SFH, Luk F, Sierra Parraga JM, Gargesha M, Merino A, Korevaar SS, Bernardo ME, Fibbe WE. Mesenchymal stromal cells: Sensors and switchers
33. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, et al. Bone
32. Y. 
31. Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal
30. Plumas J, Chaperot L, Richard MJ, Molens JP, Bensa JC, Favrot MC.
29. Han KH, Ro H, Hong JH, Lee EM, Cho B, Yeom HJ, et al. Immunosuppressive
28. Ryan JM, Barry F, Murphy JM, Mahon BP. Interferon-γ does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. Clin Exp Immunol (2007) 149:353–63. doi: 10.1111/j.1365-2249.2007.03422.x
27. Shi Y, Hu G, Su J, Li W, Chen Q, Shou P, et al. Mesenchymal stem cells: A new strategy for immunosuppression and tissue repair. Cell Res (2010) 20:510–8. doi: 10.1038/cr.2010.44
26. Wang L, Yue Q, Han L, Guan J, Wang L, et al. Human bone marrow mesenchymal stem cells suppress T cell responses by upregulating PD-1 expression. J Immunother (2018) 41:174–82. doi: 10.1007/s12075-018-0654-7
25. Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of T cell responses by human mesenchymal stromal cells: Implications for immunosuppression. Transplantation (2003) 75:1009–12. doi: 10.1097/01.TP.0000066684.04686.e7
24. Le Blanc K, Tammik L, Sandberg B, Haynesworth SE, Ringdén O. Mesenchymal Stem Cells Inhibit and Stimulate Mixed Lymphocyte Cultures and Mitogenic Responses Independently of the Major Histocompatibility Complex. Scand J Immunol (2003) 57:11–20. doi: 10.1046/j.1365-3083.2003.01176.x
23. Chimadurair, R, Copland IB, Patel SR, Galipeau J. IDO-Independent Suppression of T Cell Effector Function by IFN-γ-Licensed Human Mesenchymal Stromal Cells. J Immunol (2014) 1236:1991–501. doi: 10.4049/jimmunol.1301828
22. Galipeau J. Concerns arising from MSC retrieval from cryostorage and effect on immune suppressive function and pharmaceutical usage in clinical trials. ISBT Sci Ser (2013) 8:100–1. doi: 10.1111/voxs.12022
21. Mboneke JC, Nicholas DA, Torrez TW, Kim NS, Firek AF, Langridge WHR. The role of indoleamine 2,3-dioxygenase in immune suppression and autoimmunity. Vaccines (2015) 3:703–29. doi: 10.3390/vaccines3030703
20. Ryan JM, Barry F, Murphy JM, Mahon BP. Interferon-γ does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. Clin Exp Immunol (2007) 149:353–63. doi: 10.1111/j.1365-2249.2007.03422.x
19. Shi Y, Hu G, Su J, Li W, Chen Q, Shou P, et al. Mesenchymal stem cells: A new strategy for immunosuppression and tissue repair. Cell Res (2010) 20:510–8. doi: 10.1038/cr.2010.44
18. Hofland E, Overlie G, Winberg S. Tryptophan metabolic pathways and brain serotoninergic activity: A comparative review. Front Endocrinol (Lausanne) (2019) 10:158. doi: 10.3389/fendo.2019.00158
17. Jones PA, Guillen GI, Bower BI. The kynurenine pathway in stem cell biology. Int J Tryptophan Res (2013) 6:57–66. doi: 10.4137/ITR.S12662
16. Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. J Exp Med (2006) 199:459–68. doi: 10.1084/jem.2000212
15. Fallarino F, Grohmann U, Hwang KW, Arbona C, Yacca C, Bianchi R, et al. Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol (2004) 5:1206–12. doi: 10.1038/nri1003
14. Wirthgen E, Hoeflisch A, Rebh A, Günther J. Kynurenic Acid: The Janus-faced role of an immunomodulatory tryptophan metabolite and its link to pathological conditions. Front Immunol (2018) 9:1957. doi: 10.3389/fimmu.2017.01957
13. Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow mesenchymal stem cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood (2004) 103:4619–21. doi: 10.1182/blood-2003-11-3909
12. Godoy JAP, Paiva RMA, Souza AM, Kondo AT, Okamoto OK. Clinical Translation of Mesenchymal Stromal Cell Therapy for Graft Versus Host Disease. Front Cell Dev Biol (2019) 7:235. doi: 10.3389/fcell.2019.00255
11. Kim DS, Jang IK, Lee MW, Ko YJ, Lee DH, Lee JW, et al. Enhanced Immunosuppressive Properties of Human Mesenchymal Stem Cells Primed by Interferon-γ. EBioMedicine (2018) 28:261–73. doi: 10.1016/j.ebiom.2018.01.002
10. Huang F, Chen M, Chen W, Gu J, Yuan J, Xue Y, et al. Human gingiva-derived mesenchymal stem cells inhibit xeno-graft-versus-host disease via CD39-CD73-adenosine and IDO signals. Front Immunol (2017) 8:668. doi: 10.3389/fimmu.2017.00608
9. Ozay EL, Vijayaraghavan J, Gonzalez-Perez G, Shanthalingam S, Sherman HL, Garrigan DT, et al. Cymerus™ iPS-MSCs significantly prolong survival in a pre-clinical, humanized mouse model of Graft-vs-host disease. Stem Cell Res (2019) 35. doi: 10.1016/j.scr.2019.101401
8. Yan Z, Zhuansun Y, Liu G, Chen R, Li J, Ran P. Mesenchymal stem cells suppress T cells by inducing apoptosis and through PD-1/B7-H1 interactions. Immun Lett (2015) 162:248–55. doi: 10.1016/j.imlet.2014.09.013
7. Kalinski P. Regulation of Immune Responses by Prostaglandin E2. J Immunol (2012) 188:21–8. doi: 10.4049/immunol.1101029
6. Carter D, Tyrell A, Bubnic S, Marcelino M, Kedzierski K, Monroy R, et al. Characterization of MSC Potential To Treat GVHD Using Molecular Markers Linked to MSC-Mediated Immunosuppression In Vitro. Blood (2005) doi: 10.1063/3.1321, 10.3382/blood.10061322, 10.1389/blood.10061322
5. Williams JA, Pontzer CH, Shacter E. Regulation of macrophage interleukin-6 (IL-6) and IL-10 expression by prostaglandin E2: The role of p38 mitogen-
activated protein kinase. *J Interferon Cytokine Res* (2000) 20:291–8. doi: 10.1089/107999000312423

62. Baratelli F, Lin Y, Zou L, Yang S-C, Heuze-Vourc'h N, Zeng G, et al. Prostaglandin E2 Induces FOX3 Gene Expression and T Regulatory Cell Function in Human CD4 + T Cells. *J Immunol* (2005) 175:1483–90. doi: 10.4049/jimmunol.175.3.1483

63. Follin B, Juhl M, Cohen S, Perderer AE, Kastrup J, Ekbloff A. Increased Paracrine Immunomodulatory Potential of Mesenchymal Stromal Cells in Three-Dimensional Culture. *Tissue Eng Part B Rev* (2016) 22:322–9. doi: 10.1089/ten.teb.2015.0532

64. Najer M, Raicvic G, Farayd-Kazan H, Bron D, Toungouz M, Lagneaux L. Mesenchymal stem cells and immunomodulation: A gathering of regulatory immune cells. *Cytotherapy* (2016) 18:160–71. doi: 10.1016/j.jcyt.2015.10.011

65. Boufi C, Bony C, Courties G, Jorgensen C, Noel D. IL-6-dependent PG2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis. *PloS One* (2010) 5. doi: 10.1371/journal.pone.0014247

66. Rummel C, Sachot C, Poole S, Luheshi GN. Circulating interleukin-6 induces fever through a STAT3-linked activation of COX-2 in the brain. *Am J Physiol Regul Integr Comp Physiol* (2006) 291. doi: 10.1152/ajpregu.00301.2006

67. Najer M, Rouas R, Raicvic G, Boulker HI, Lewalle P, Meuleman N, et al. Mesenchymal stem cells promote or suppress the proliferation of T lymphocytes from cord blood and peripheral blood: The importance of low cell ratio and role of interleukin-6. *Cytotherapy* (2009) 11:570–83. doi: 10.1016/j.1465324009007397

68. Naut A, Krusselbrink AB, Lurvik E, Willemsz R, Fibbe WE. Mesenchymal Stem Cells Inhibit Generation and Function of Both CD34 - Derived and Monocyte-Derived Dendritic Cells. *J Immunol* (2006) 177:2080–7. doi: 10.4049/jimmunol.177.4.2080

69. Jang YK, Kim Y, Lee YH, Oh W, Yang YS, Choi SJ. Optimization of the therapeutic efficacy of human umbilical cord blood-mesenchymal stromal cells in an NSG mouse xenograft model of graft-versus-host disease. *Cytotherapy* (2014) 16:298–308. doi: 10.1016/j.jcyt.2013.10.012

70. Auletta JF, Eid SK, Wuttisarnwattana P, Silva J, Metheny L, Keller MD, et al. Human mesenchymal stromal cells attenuate graft-versus-host disease and maintain graft-versus-leukemia activity following experimental allogeneic bone marrow transplantation. *Stem Cells* (2015) 33:601–14. doi: 10.1002/stem.1867

71. Cutler AJ, Limbari V, Girdlestone J, Navarrete CV. Umbilical Cord-Derived Mesenchymal Stromal Cells Modulate Monocyte Function to Suppress T Cell Proliferation. *J Immunol* (2010) 185:6617–23. doi: 10.4049/jimmunol.2010.02239

72. Kim DS, Lee WH, Lee MW, Park HJ, Jang IK, Lee JW, et al. Involvement of TLR3-Dependent PGES Expression in Immunosuppression by Human Bone Marrow Mesenchymal Stem Cells. *Stem Cell Res Rep* (2018) 14:286–93. doi: 10.1007/s12288-017-9793-6

73. Otterbein LE, Choi AMK. Heme oxygenase: Colors of defense against cellular inflammation. *Am J Physiol Lung Cell Mol Physiol* (2000) 279. doi: 10.1152/ajlun.2000.279.6.L1029

74. Chauveau C, Bony C, Courties G, Jorgensen C, Noel D. IL-6-dependent PG2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis. *PloS One* (2010) 5. doi: 10.1371/journal.pone.0014247

75. Dengler VL, Galbraith MD, Espinosa JM. Transcriptional regulation by hypoxia inducible factors. *Crit Rev Biochem Mol Biol* (2014) 49:1–15. doi: 10.3109/10409238.2013.838205

76. Gaspar JM, Velloso LA. Hypoxia inducible factor as a central regulator of metabolism i implications for the development of obesity. *Front Neurosci* (2018) 12:813. doi: 10.3389/fnins.2018.00813

77. Keith B, Simon MC. Hypoxia-Inducible Factors, Stem Cells, and Cancer. *Cell Stem Cell* (2010) 6:465–72. doi: 10.1016/j.stem.2009.07.019

78. Zhang C, Yang F, Cornelia R, Wang W, Swisher S, Kim H. Hypoxia-inducible factor-1 is a positive regulator of Sox9 activity in femoral head oстеonecrosis. *Bone* (2011) 48:507–13. doi: 10.1016/j.bone.2010.10.006

79. Robins JC, Akeno N, Mukherjee A, Dalal RR, Aronow BJ, Koopman P, et al. Hypoxia induces chondrocyte-specific gene expression in mesenchymal cells in association with transcriptional activation of Sox9. *Bone* (2005) 37:313–22. doi: 10.1016/j.bone.2005.04.040

80. Wąggeg M, Gaber T, Lohanatha FL, Hahne M, Strehl C, Fangradt M, et al. Hypoxia Promotes Osteogenesis but Suppresses Adipogenesis of Human Mesenchymal Stromal Cells in a Hypoxia-Inducible Factor-1 Dependent Manner. *PloS One* (2012) 7. doi: 10.1371/journal.pone.0046483

81. Hung SC, Pochampally RR, Hsu SC, Sanchez CC, Chen SC, Spees J, et al. Short-term exposure of multipotent stromal cells to low oxygen increases their expression of CX3CR1 and CXCR4 and their engraftment in vivo. *PloS One* (2007) 2. doi: 10.1371/journal.pone.0000416

82. Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor. *J Biol Chem* (1995) 270:1230–7. doi: 10.1074/jbc.270.3.1230

83. Razban V, Lotfi AS, Soleimani M, Ahmadi H, Massumi M, Khajeh S, et al. Hypoxia-1 alpha overexpression induces angiogenesis in mesenchymal stem cells. *Stem Cells Transl Med* (2012) 1:174–83. doi: 10.1002/stem.2012.9995

84. Robins JC, Akeno N, Mukherjee A, Dalal RR, Aronow BJ, Koopman P, et al. Hypoxia induces chondrocyte-specific gene expression in mesenchymal cells in association with transcriptional activation of Sox9. *Bone* (2005) 37:313–22. doi: 10.1016/j.bone.2005.04.040

85. Jiang CM, Liu J, Zhao CY, Xiao L, An S, Gou YC, et al. Effects of hypoxia on the expression of CX3CR1 and CXCR4 and their engraftment in vivo. *PloS One* (2007) 2. doi: 10.1371/journal.pone.0000416

86. Westman J, Lindell M, Deisseroth J, et al. The role of HIF-1 alpha in bone marrow-derived mesenchymal stem cells on traumatic brain injury. *Eng Life Sci* (2018) 18:401–7. doi: 10.1002/elsc.201800015

87. Noronha NC, DURDON J, CAIDA C, COMINAI JA, ROCHA JM, COVAS DT, et al. Promising approaches to improve the efficacy of mesenchymal stem cell-based therapies. *Stem Cell Res Ther* (2019) 10. doi: 10.1186/s13287-019-1224-y

88. Lan WW, Choo KB, Chen CM, Hung TH, Bin CY, CH H, et al. Hypoxia preconditioned mesenchymal stem cells attenuate bleomycin-induced pulmonary fibrosis. *Stem Cell Res Ther* (2015) 6. doi: 10.1186/s13287-015-0081-6

89. Bovens C, Menke J, Zhang L, Zhao X, Liao Y, Sun H, Lu J, et al. Hypoxia-inducible factor-1a regulates angiogenesis and stem cell function in hypoxia-1alpha-overexpressing stromal cells. *Stem Cell Rep* (2018) 2:694–707. doi: 10.1016/j.stemcr.2018.04.019

90. Liu Y, Yuan X, Muñoz N, Logan TM, Ma T. Commitment to Aerobic Glycolysis Sustains Immunomodulation of Human Mesenchymal Stem Cells. *Stem Cells Transl Med* (2019) 8:93–106. doi: 10.1002/stem.180007
100. Fillmore N, Huqi A, Jaswal JS, Mori J, Paulin R, Haromy A, et al. Effect of fatty acids on human bone marrow mesenchymal stem cell energy metabolism and survival. PloS One (2015) 10. doi: 10.1371/journal.pone.0120257

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.