Factors Defining Human Adipose Stem/Stromal Cell Immunomodulation in Vitro

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Abstract

Human adipose tissue-derived stem/stromal cells (hASCs) are adult multipotent mesenchymal stem/stromal cells with immunomodulatory capacities. Here, we present up-to-date knowledge on the impact of different experimental and donor-related factors on hASC immunoregulatory functions in vitro. The experimental determinants include the immunological status of hASCs relative to target immune cells, contact vs. contactless interaction, and oxygen tension. Factors such as the ratio of hASCs to immune cells, the cellular context, the immune cell activation status, and coculture duration are also discussed. Conditioning of hASCs with different approaches before interaction with immune cells, hASC culture in xenogenic or xenofree culture medium, hASC culture in two-dimension vs. three-dimension with biomaterials, and the hASC passage number are among the experimental parameters that greatly may impact the hASC immunosuppressive potential in vitro, thus, they are also considered. Moreover, the influence of donor-related characteristics such as age, sex, and health status on hASC immunomodulation in vitro is reviewed. By analysis of the literature studies, most of the indicated determinants have been investigated in broad non-standardized ranges, so the results are not univocal. Clear conclusions cannot be drawn for the fine-tuned scenarios of many important factors to set a standard hASC immunopotency assay. Such variability needs to be carefully considered in further standardized research. Importantly, field experts' opinions may help to make it clearer.

Keywords Human adipose stem/stromal cells · Immune cells · Coculture · Immunomodulation · Preconditioning · Experimental determinants

Introduction

Adipose tissue-derived stem/stromal cells (ASCs) are the relatively homogenous population of fibroblast-like cells that can be expanded after plating the stromal vascular fraction of adipose tissue (AT) onto standard cell culture surfaces [1]. hASCs express the surface markers of mesenchymal stem/stromal cells (MSCs), including the receptor molecules CD90 and CD105; the glycosyl phosphatidylinositol-anchored enzyme CD73 and the cell adhesion molecules CD29, CD44, CD146, and CD166. Additionally, hASCs should be negative for the hematopoietic antigens, including CD11b, CD13, CD14, CD19, and CD45, the endothelial markers CD31 and CD34, and the human leukocyte antigen (HLA)-DR [2].

ASCs hold great promise for clinical application as a personalized cell therapy because of a number of advantageous characteristics (Fig. 1).They can be easily isolated with minimal ethical issues and donor risk and they are expandable in vitro [3, 4]. hASCs exhibit more enhanced proliferation, multipotency [5], and immunosuppressive capacity [6], however, lower senescence [7], than donor-matched bone marrow (BM)-derived MSCs (BMSCs). Importantly, varied paracrine factors including inflammatory, angiogenic, anti-apoptotic, anti-oxidative, anti-fibrotic, and anti-inflammatory mediators, contribute to ASCs-mediated tissue repair [8, 9].

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Fig. 1 ASCs' regenerative characteristics. Abbreviations: ASCs: adipose tissue-derived stem/stromal cells, b-FGF: basic Fibroblast growth factor, CCL2: C–C motif chemokine ligand 2, CCL5:: C–C motif chemokine ligand 5, HGF: Hepatocyte growth factor, IL-6: Interleukin 6, IL-8: Interleukin 8, IL-1ra: Interleukin 1 receptor antagonist, IL-10: Interleukin 10, IGF: Insulin-like growth factor, IDO: Indoleamine 2, 3 dioxygenase, PGE2: Prostaglandin E2, PDGF: Platelet-derived growth factor, STC-1, stanniocalcin-1, TSG-6: Tumor necrosis factor stimulated gene-6, TGF-β: Transforming growth factor beta, VEGF: Vascular endothelial growth factor
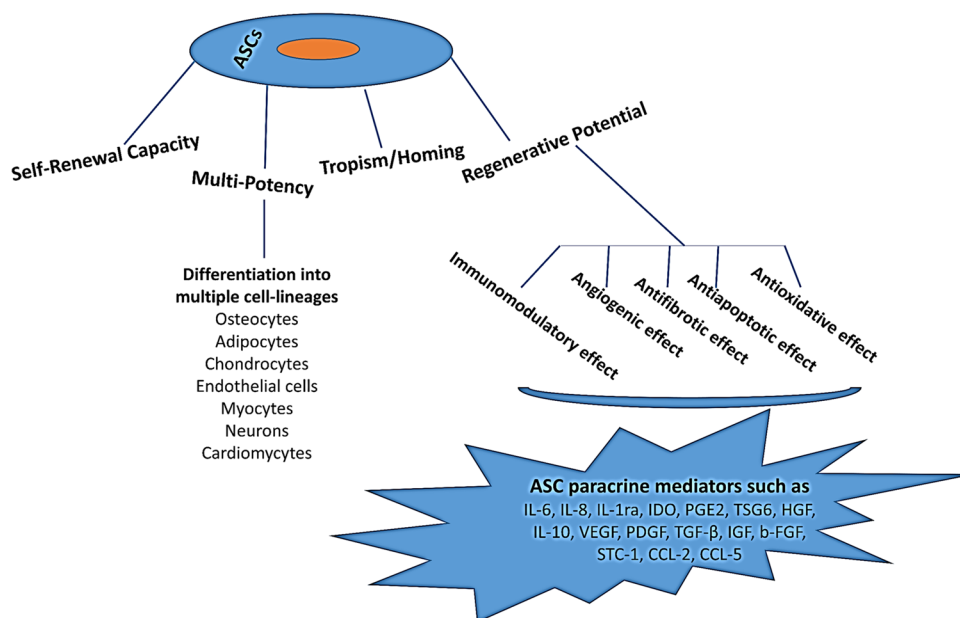
ASCs are widely reported not to induce the immune response of allogeneic lymphocytes [10–12]. Such property was attributed to the low expression of major histocompatibility (MHC) class II molecule (MHC II/HLA-DR) and co-stimulatory molecules, CD40, CD80, and CD86 [12, 13]. On the contrary, some studies illustrated that ASCs had the potential to activate the proliferation of resting allogeneic CD4 T cells under circumstances of low inflammation [14, 15], and to induce the reactivity of cytotoxic CD8 T cells educated with allogeneic ASCs [16], or the production of alloreactive-memory CD8 T cells [17], so they are not intrinsically immunoprevilliged [18].

The immunomodulatory functions of hASCs in vitro are multifaceted and include the proliferation and differentiation of a variety of immune cells [19]. In particular, the effect of hASCs on effector T helper (Th) cells and regulatory T cells (Tregs) has been widely studied [20–22] (Supplemental Table 1). ASCs inhibit the proliferation of T cells via a plethora of paracrine mechanisms, including indoleamine 2, 3-dioxygenase 1 (IDO) activity [22, 23], secretion of prostaglandin E2 (PGE2) [24], leukemia inhibitory factor (LIF) [25], tumor necrosis factor-stimulated gene 6 (TSG-6) [26], interleukin 1-receptor antagonist (IL-1RA) [27], and other several factors [19, 28], and also induce T cells to adopt a regulatory phenotype [29, 30]. Surface molecules also contribute to ASC immunosuppressive effect on T lymphocytes [31–33].

In addition, hASCs have been reported to affect the proliferation, differentiation, and immune functions of B cells [34], inhibit dendritic cell (DC) maturation [35], suppress natural killer (NK) cells cytotoxicity [36, 37], and stimulate macrophage polarization to anti-inflammatory macrophages [38, 39]. It has been recently evolved that apoptotic MSCs, after MSC infusion, are phagocytosed by macrophages that are then reprogrammed to become immunoregulatory cells [40].

The above findings recommend the potential immunomodulatory ability of ASCs in vitro [41, 42]. However, the ASC immunomodulation in culture greatly depend on multiple parameters. In the current review, the impact of the experimental settings and donor characteristics on the immunomodulatory effects of hASCs in vitro are discussed. Factors that may control hASC immunomodulatory phenotype and functions in vitro are categorized into four main groups including coculture setting parameters, ASC priming or preconditioning, ASC-related parameters, and donor-related characteristics (Fig. 2).

Coculture Setting Parameters

Autologous vs. Allogeneic

Despite the off-shelf availability of allogeneic MSCs, patient-derived (autologous) rather than allogeneic MSCs may be the safer choice in clinical perspectives, to avoid anti-donor immune responses in some cases [43, 44]. Human ASCs are able to modulate the activity of both autologous and allogeneic immune cells in vitro; however, the strength of the suppressive effect may be different [45]. ASCs isolated from patients with rheumatic diseases (RD) were able to inhibit the proliferation [46], and to attenuate the expression of the activation marker CD25 on allogeneic phytohemagglutinin (PHA)-activated CD4 and CD8 T lymphocytes [47]. Relative to that setting, the RD ASCs comparably inhibited the proliferation [46], however, a weaker inhibitory effect to downmodulate CD25 expression on autologous PHA-CD4 and CD8 T lymphocytes [47]. Such results suggest a possible weaker control of T cell activation by

Fig. 2 Factors affecting hASC immunomodulation in vitro. Abbreviations: hASCs: human adipose tissue-derived stem/stromal cells, AT: adipose tissue, 2D: two-dimensional, 3D: three-dimensional, BMI: body mass index

1. Coculture Setting Parameters

1.1. Autologus vs Allogenic
1.2. Direct vs Indirect - Cell Contact
1.3. Normoxia vs Hypoxia
1.4. Immune Cell Stimulant Type & Stimulation Timing
1.5. ASC: Immune Cell Ratio & Cellular Context
1.6. Coculture Duration

2. ASC Priming or Preconditioning

2.1. Inflammatory preconditioning
2.2. Hypoxia Preconditioning
2.3. Chemical or Pharmacological Preconditioning
2.4. Genetic Engineering

4. Donor-related Parameters

4.1. Age, BMI, Sex
4.2. Presence of Inflammatory disease
4.2.1. Effect of RD
4.2.2. Effect of Inflammatory Bowel Diseases
4.3.3. Effect of Breast Cancer
4.3.4. Effect of Miscellaneous Inflammatory diseases

3. ASC-related Parameters

3.1. Anatomical AT Harvest Location
3.2. ASC Differentiation
3.3. ASC Culture Conditions
3.3.1. Serum Supplementation
3.3.2. Three- Dimensional ASC Cultures
    A. Scaffold-free 3D ASC spheroids
    B. Scaffold-based 3D ASC spheroids
3.4. ASC Passaging and Cryopreservation Effects

autologous ASCs of RD patients in vivo [47]. In the context of diabetes mellitus, hASCs, from patients with type 2 diabetes (T2D), exhibited compromised antiproliferative potential on autologous anti-CD3/CD28-activated peripheral blood mononuclear cells (PBMCs) [48]. However, co-transplantation of autologous ASC-derived insulin-producing cells and hematopoietic stem cells showed a better response in individuals with type 1 diabetes (T1D) as compared with a similar allogeneic regimen [49]. Recently, it has been assumed that the current clinical scenario recommend the suitability of using autologous MSCs for T1D, not T2D therapy [50]. We think that numerous standardized studies to address the in vitro immunomodulatory phenotypes and functions of hASCs, from patients with different inflammatory diseases in autologous settings, are still needed, before assumption of the possibility of using autologous ASCs therapy in immune-related or inflammatory diseases.

Direct- vs. Indirect- Cell Contact

Some studies revealed the necessity of direct contact between (b/w) hASCs and immune cells to exert their immunoregulatory effect [51–54], where transwell experiments decreased the strength of inhibition of hASCs on mitogen-activated PBMCs [51, 55], not on a mixed lymphocyte reaction (MLR) [51]. Quaedackers et al. studied the impact of cell-to-cell interactions b/w ASCs and activated T cells on the immunomodulatory potential of the former cell population [56]. The authors reported that allogeneic activation of PBMCs had induced the attachment of their membranes to ASCs after 1h and 24h of coculture. The cell binding was HLA-class I or class II independent, as similar interactions had been established b/w the PBMCs and autologous

or allogenic ASCs, without the additional effect of ASC treatment with the pro-inflammatory cytokine; interferon-gamma (IFN-ɣ). Analysis of ASC-bound and suspended lymphocytes revealed that the former lymphocytes had been enriched for B cells, CD8$^+$ T cells, and CD4$^-$ CD8$^-$ T cells, whereas CD4$^+$ T cell-bound proportion had been increasing over time. The bound CD4$^+$ T cells were well-proliferative, and highly activated, in terms of CD25 elevated expression, they also expressed the regulatory transcription factor, forkhead box P3 (FOXP3), and however, expression of CD127 excluded the Treg phenotype (CD4$^+$ CD25^{high} CD127^{low} FOXP3$^+$) [57]. Cell proximity potentiated the immunosuppressive effect of the cell membrane or soluble ASC immunomodulatory proteins on IL-2-treated CD8$^+$ T cells. ASCs depressed the bound and suspended CD8$^+$ T cells' response to IL-2, as presented by a reduced increase in the level of phosphorylated signal transducer and activator of transcription (STAT)-5, with a more pronounced effect in the bound cells. The results indicated that in an active immune environment, ASCs secrete attractant and bind T lymphocytes inhibiting CD8$^+$ T cell activity and depleting the activated CD4$^+$T cells from the cell suspension compartment [56]. In another study, direct contact b/w activated CD3$^+$ T cells and hASCs led to active attachment and flattening of T cells to ASCs surfaces, specifically CD3$^+$/CD4$^+$ Th cells and NK T cells (CD3$^+$/CD56$^+$16$^+$). At the attachment sites, elevated expression of intracellular adhesion molecule 1 (ICAM-1)/CD54 was detected, indicating the formation of highly specific ligand-receptor contacts b/w interacting cells [58]. Recently, ASC spheroids modulated effectively the proliferation and cytokine production of allogenic γδ CD3$^+$ T lymphocytes in direct contact, while their conditioned medium (CM) induced much weaker inhibitory effects [59].

Other reports demonstrated that direct interaction is not essential for the hASC immunosuppressive effects, as the immunoinhibition persisted in transwell experiments where hASCs and immune cells were separated by a semipermeable membrane [22, 23, 32, 58, 60–66]. CM from IFN-ɣ treated hASCs were able to abrogate the proliferation of anti-CD3/CD28 activated CD4 T cells [67]. Such studies confirm the contribution of paracrine soluble factors and extracellular vesicles to hASC immunosuppressive effects [19].

Normoxia vs. Hypoxia

A number of investigators addressed the immunoregulatory impact of hASCs under hypoxic (1–7%) vs. normoxic or ambient oxygen (O2) levels (20%) [58, 68–73]. Low O2 level is typical for the putative site of the MSC-T cell interactions [72]. Hypoxia potentiated the direct antiproliferative effect of hASCs on PHA-activated PBMCs [70] or CD3$^+$ T cells [58, 71]. The effective hASC antiproliferative potential at 5% over 20% O2 level was attributed to the upregulated expression of *PDCD1* and *TGF-β1*, in cocultured T cells, which encode for programmed cell death protein 1 (PD-1) and TGF-β1, respectively [58], and both markers are involved in cell cycle arrest [74, 75]. Moreover, hASCs superior suppression for IFN-ɣ secretion by the directly cocultured T cells at low O2 level [58]. Conclusively, cell-to-cell contact may induce stronger hASC immunosuppression at 1% or 5% O2 over 20% O2 level [58, 70].

Immune Cell Stimulant Type & Stimulation Timing

The MSC populations act as a switcher of inflammation, at a low level of immune cell activation, they acquire a pro-inflammatory phenotype, while at a high level of inflammation, they are immunosuppresors [76]. Similarly, ASCs are plastic immunoregulators that depend on the stimuli context to acquire pro- or anti-inflammatory phenotype [77]. Thus, the hASC immunoregulatory functions in vitro are affected by the type of immune cell stimulant (mitogen vs. alloantigen/MLR) [51, 61]. ASC immunosuppression on PBMCs was the greatest against proliferation induced by PHA, then by concanavalin A, and last by OKT3 (anti-CD3 antibody) [51].

Another determinant is the level of immune cell activation which is defined by the timing of coculture relative to stimulation, i.e. simultaneous coculture and stimulation vs. coculture after stimulation induction [78]. Mancheño-Corvo et al. [78], investigated the influence of PBMC pre-stimulation on the antiproliferative effect of hASCs. T lymphocyte pre-stimulation impaired in a time-dependent manner the capacity of ASCs to inhibit proliferation. Deficient ASC potential to inhibit the proliferation of 48 h pre-stimulated PBMCs was not due to reduced *IDO* activity, but rather to the kinetics of tryptophan (Trp) degradation and the low level of Trp available in the medium at 48 h of stimulation to be degraded by IDO. Pre-activation of ASCs with IFN-ɣ or polyinocinic-polycytidykic acid (poly I:C), toll-like receptor (TLR)-3 ligand, restored their capacity to inhibit proliferation of 48 h pre-stimulated lymphocytes, with a stronger effect with IFN-ɣ [78].

ASC: Immune Cell Ratio & Cellular Context

The immunomodulatory potential of hASCs in vitro is, as well, strongly affected by cells ratio. At a hASC: immune cell ratio ranging from (1:1–1:25), hASCs exhibit potent immunosuppressive properties on diverse immune cell types. Substantial literature studies reported maximum hASC immunosuppression at high ASC: immune cell ratio (from 1:1 to 1:10) [6, 10–12, 14, 21, 24, 25, 33, 36, 51, 58, 61, 64, 67, 73, 79–88]. Others reported maximum suppression at lower ratios (1:20) [89] or (1:25) [23, 32, 35, 90]. At cell ratios 1:50, 1:100, or 1:1000, hASCs inhibited [6, 51] or failed to inhibit the proliferation [14, 23] of activated lymphocytes. The attenuated hASC immunosuppression at low ASC densities could be associated with intensive cell death [91], and autophagy-mediated apoptosis of MSC under inflammatory conditions [92]. Surprisingly, Th17 lymphocyte pathway is significantly modulated by hASC density, and it was greatly enhanced at high (1:5), compared with low (1:80) ASC: T cell ratio, contradicting the well-documented immunosuppressive effect of ASCs, specifically at high density [93].

The intensity of ASCs inhibition varied with cellular contexts, i.e. the whole PBMC population or a specific purified immune cell compartment is included in the coculture with the hASCs [22, 45, 61]. The effect of the cellular context on the in vitro ASC immunoregulation was clearly presented in cocultures of hASCs and mitogen (PHA)-stimulated PBMCs, where there was a marked decrease of IFN-ɣ and a significant increase of IL-17AF. While, in the cocultures of anti-CD3/CD28 activated CD4 + T cells with ASCs, there was some increase in IFN-ɣ and IL-17AF. As well, ASCs significantly downregulated CD25 expression on PHA-stimulated PBMCs, however, they did not affect its expression on α-CD3/CD28-activated T cells [61]. Such findings recommend that immune cells create a cytokine milieu in vitro which differs depending on the stimulation method and cellular context, and in turn differentially affects ASC immunomodulatory actions [61]. To shift Th differentiation to a functional anti-inflammatory direction, ASCs require accessory cell support, whereas their direct effect on a purified immune cell type may be a pro-inflammatory [46]. In other contradictory reports, hASCs exerted analogous immunosuppressive action on the proliferation of activated whole PBMCs population and enriched CD4$^+$ T cells [22, 67],

reducing the impact of the interplay of accessory immune cells (B cells, NK cells, and monocytes) within PBMCs on MSC immunoregulation [94].

Coculture Duration (Assessment Day)

The duration of the ASCs/immune cells interaction is another considerable issue. The type of immune cells [64] and the assessed immune cell-related parameters [33], are determinants of the coculture time. Significant inhibitory effects of hASCs have been reported on days 3 [36, 58, 70], 4 [33, 95], 5 [6, 23–25, 46, 51, 96], 7 [21], or 10 [34], of coculture. Inhibition of lymphocytes' immune responses becomes more distinct with an extension of the coculture time [45, 51].

ASC Priming/Licensing/Preconditioning

MSCs can actively sense their surroundings and modulate, accordingly, their fate and behavior. Intriguingly, it has been proposed that MSCs immunosuppressive ability is not constitutive; instead, it is induced in inflammation [97, 98] and interaction with activated immune cells [99]. Several approaches have been introduced to boost the in vitro immunosuppressive functions of hASCs isolated from healthy donors or even patients with inflammatory diseases and they are discussed below.

Inflammation Preconditioning

The cohabitation of ASCs with pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, IFN-ɣ, IL-6, IL-1β, and/or IL-17 can potentiate their effectiveness for inflammatory diseases [53, 100, 101]. Numerous investigators (Table 1) assessed the immunogenicity and/or immunosuppressive properties of hASCs after treatment with pro-inflammatory cytokine(s) [6, 13, 14, 22, 27, 61, 67, 85, 93, 102–121] or TLR agonist (s) [122–129].

Pre-conditioning of ASCs with IFN-ɣ is one of the most common approaches to enhance ASC-mediated immunosuppression [19, 67, 105]. IFN-ɣ upregulates expression of the immunoregulatory surface molecules, programmed death-ligand 1 (PD-L1)/CD274, PD-L2, and ICAM-1/CD54 by hASCs [13, 61]. Moreover, IDO activity potentially involved in the enhanced immunosuppressive effects of IFN-ɣ-treated ASCs [14, 85]. Interestingly, ASCs showed a stronger upregulated expression of *IDO* than BMSCs by 8 h IFN-ɣ exposure [6]. Enhanced activation of the JAK/STAT1 pathway mediates IFN-ɣ induced expression of PD-L1 and IDO in primed MSCs [114]. From mechanistic perspectives, priming of MSCs with IFN-ɣ increased glucose turnover leading to abundant STAT1 glycosylation and stability, thus

sustaining its downstream effects [115]. Glucose metabolic reprogramming is thus a novel modulatory mechanism for the immunosuppressive function of IFN-ɣ–challenged MSCs and this mechanism can be analyzed in the IFN-ɣ primed hASCs. Not only the IFN-ɣ licensed-ASCs, but also their CM can exert potential immunosuppression [67, 108]. Moreover, IFN-ɣ treatment using low [67] or high [109] dose enhanced the immunoregulatory phenotypes, and antiproliferative potential of ASCs from patients suffered from T2D with different ethnicities and body mass indices.

Individual treatment with TNF-α activated nuclear factor kappa B (NF-κB) pathway in hASCs to promote the cell survival. Transcriptome analysis revealed that TNF-α treated ASCs differentially expressed genes involved in the differentiation of ASCs into mononuclear leukocytes (*NFKB1, IRF8, RELA, RELB, IRF7*) and in the antitumor immunity (*TLR2* and *PTGS2*) [111]. Regarding treatment with IL-1β, priming of hASCs from patients with osteoarthritis (OA) with this cytokine (1 ng/ml for 24 h) induced the differential expression of genes enriched in inflammation modulation and extracellular matrix (ECM) remodeling. Moreover, compared to non-primed cells, IL-1β primed hASCs superiorly modulated the CD80+/CD206+ cell ratio in co-culture with polarized M1 macrophages, promoting an increase of the anti-inflammatory CD206+ M2a macrophages [112].

The synergistic effect of priming ASCs with a mixture of different inflammatory cytokines have been tested [13, 14, 27, 54, 93, 103, 106, 110, 116]. Full genome expression analysis was carried out for hASCs cultured for 7 days under control conditions and two different inflammatory conditions; either with alloactivated PBMCs (MLR) in a transwell setting or with a cocktail containing IFN-ɣ, TNF-α, and IL-6 [14]. Partial overlapping in the significant gene expression changes, induced by both inflammatory conditions, was demonstrated indicating different ASCs responses to alloactivated PBMCs than to pro-inflammatory cytokines. Human ASCs cocultured with MLR showed a significant upregulation of the PGE2-producing enzyme; cyclooxygenase 2 (*COX-2*) (tenfold). However, priming with the pro-inflammatory cytokines cocktail significantly induced the expression of *IDO* (394fold) in ASCs [14]. In another report, priming with a mix of IFN-ɣ, TNF-α, and IL-1β induced significantly the surface expression of CD274 and the secretion of PGE2 by hASCs [103]. The increased CD274 level under inflammatory conditions may be one of the mechanisms by which ASCs counteract the immunogenic effect of the upregulation of CD40 in inflammation [12, 103, 117]; these changes in expression may ultimately lead to inhibition of activated lymphocytes [103]. In addition to *IDO* upregulation, priming of hASCs with the cocktail (IFN-ɣ, TNF-α, IL-1β, and IFN-α) was found to upregulate the transcriptional levels of the immune regulatory semaphorins SEMA4D and SEMA7A [116]. The combination of IFN-ɣ/

Table 1 Studies that addressed the influence of inflammatory and/or hypoxia priming on the immunoregulatory phenotypes and functions of hASCs

Treatment conditions	Treatment period	ASCs' response	The immunomodulatory functions of the primed ASCs	Reference
IFN-γ (50 ng/ml) + TNF-α (20 ng/ml) + IL-6 (10 ng/ml)	168 h	↑IDO,CXCL-9, CXCL-10, CXCL-11	–	[14]
TNF-α (10 ng/ml)	48 h	↑IL-6, IL-8, MCP-1 (CCL-2), CXCL-6, CXCL-2	Conditioned medium of TNF-α treated ASCs has also been reported to promote monocyte migration in vitro	[102]
IFN-γ (200 U/ml)	96 h	↑ PD-L1 (CD274), PD-L2, and ICAM-1 (CD54)	-Enhanced antiproliferative effect	[61]
IFN-γ (10^3 U/ml) + TNF-α (50 ng/ml) + IL-1β (25ng/ml) + IFN-α (3×10^3 U/ml)	Overnight	↑ PD-L1 (CD274)	'/–	[103]
IFN-γ (500 U/ml)	4, 8, 24 h	↑ IDO	–	[6]
IFN-γ (10 ng/mL) + TNF-α (15 ng/mL)	40 h	-↑ the immunosuppressive molecules [IDO, PTSG2 (Cox-2), NOS2 (iNOS), and TNFAIP6 (TSG-6) - ↑ IDO activity	-Enhanced T and B-cell-suppressive capacities	[12]
IFN-γ (100 ng/ml)	24 h	↑ IDO expression and activity	-Enhanced antiproliferative effect on PBMCs, CD4 and CD8 T cells and enhanced reduction of IFN-γ, IL-6 and IL-1β production by stimulated PBMCs -Promoted immunosuppressive effect in a humanized skin graft model	[105]
IL-1β (20 ng/ml) + IL-6 (40 ng/ml) + IL-23 (20 ng/ml)	96 h	↑ TGF-β ↑ CD45	This priming strategy promoted CD45 expression by ASCs up to 80% and the CD45+ ASCs significantly abrogated the proliferation in MLR	[54]
IFN-γ (50 ng/ml) + TNF-α (50 ng/ml) + IL-1β (25ng/ml) + IFN-α (10 ng/ml)	Overnight	↑ IDO, and the immunoregulatory semaphorins SEMA4D and SEMA7A	–	[116]
IFN-γ + TNF-α (10, 20, or 40 ng/ml)	48 h	↑ IDO (dose-dependent increase) ↑ PGE2, IL-10 and IL-8 (20ng/ml) ↑ IL-6, CCL2 (40ng/ml)	-Exosomes from pre-treated ASCs with enhanced potential to revert M1 macrophages differentiation and to promote M2 macrophages polarization	[106]
IFN-γ (200 IU/mL)	24 h	↑ IDO	-Enhanced antiproliferative effect in vitro and immunoregulatory potential in the GVHD model	[85]
IFN-γ (10^3 U/ml) + TNF-α (50 ng/ml) + IL-1β (25ng/ml) + IFN-α (3×10^3 U/ml)	Overnight	↑ secretion of distinct Th17 related cytokines including IL-6, IL-1β, IL-17F, IL-21, and IL-23	-inflammatory priming and cell ratio significantly modulated the ASCs-mediated regulation of Th17 differentiation	[93]
Cytomix (TNF-α + IFN-γ + IL-1β, all at 5 ng/mL) + 21% O2 or 2% O2	48 h	↑ IL-1RA, IL-8, IL-6 and MCP-1 ↑PTGS2, TNFAIP6, and STC-1	At normoxia (21% O2), cytomix-treated ASCs induced more potent immunosuppressive functions than the relevant group at hypoxia	[27]

Table 1 (continued)

Treatment conditions	Treatment period	ASCs' response	The immunomodulatory functions of the primed ASCs	Reference
IFN-ɣ (50ng/ml)	48 h	↑IDO and ICAM-1	-Enhanced antiproliferative effect of IFN-ɣ-ASCs and their CM -CM from INF-γ licensed ASCs had increased capacity to control the T-cell response	[108]
IFN-ɣ (50ng/ml) + TNF-α (50 ng/ml) + IL-1β (25ng/ml) + IFN-α (10ng/ml)	18 h	↑HLA-ABC, CD40 CD54, CD106, CD274 and CD112	–	[13]
IFN-ɣ (10ng/ml)	72 h	↑IDO expression and activity	-Priming enhanced the antiproliferative effects of hASCs on PBMCs and purified T cells	[22]
IFN-ɣ (100ng/ml)	48 h	↑IDO, CD274 and IL-RA ↓IL-1β	–	[109]
TNF-α (10 ng/ml) + IL-1β (10 ng/ml)	6 h followed by 48 h incubation in serum-free medium	↑IL-6, IL-8, MCP-1 (CCL2), CXCL6, CXCL2 and ECM proteins		[121]
IFN-ɣ (100ng/ml) + TNF-α (10 ng/ml)	24 h	↑IDO, HLA-E, CD54 (ICAM-1), CD106 (VCAM-1), CD274 (PD-L1) and CD73 (NT5E)	–	[110]
TNF-α (50 ng/mL)	24 h vs 96 h	↑Genes related to immunomodulation (*TLR2* and *PTGS2*) and cell survival (*TRAF1, NF-kB,* and *IRF7*)	–	[111]
IL-1β (1ng/ml)	24 h	↑Genes involved in inflammation modulation such as *CXCL6, CXCL8, LIF,* and *STC1* and extracellular matrix remodeling such as *MMP3, MMP10,* and *MMP1*	- IL-1β primed hASCs superiorly modulated the CD80$^+$/CD206$^+$ ratio in co-culture with polarized M1 proinflammatory macrophages, promoting an increase of the CD206$^+$ M2a anti-inflammatory marker	[112]
IFN-ɣ (10ng/ml)	24 h	↑IDO, CD274 and ICAM-1	The secretome of primed ASCs from health and T2D donors significantly suppressed the CD4 T cell proliferation	[67]
IFN-ɣ (3 ng/ml) or Poly 1:C (10μg/ml)	48 h	↑IDO activity	-Enhanced antiproliferative effect on 48 pre-stimulated T lymphocytes	[78]
TNF-α (10 ng/ml) or LPS (100 ng/ml)	24 h	↑IL-6, IL-8, and MCP-1	LPS-primed ASCs displayed enhanced therapeutic efficacy in skin flap survival in a diabetic rat model than did unprimed ASCs	[124]
LPS (10ng/ml) or Poly I: C (1μg/ml)	1 h	↑IL-8	–	[126]
LPS (10ng/ml)	Up to 24 h	↑TNF-α and GM-CSF in the secretome of the primed female line ↑IL-6 in the secretome of the primed male line	The secretome of the female LPS-ASCs which contained a high level of GM-CSF promoted the CD14 monocyte differentiation in THP-1 cells	[127]

Table 1 (continued)

Treatment conditions	Treatment period	ASCs' response	The immunomodulatory functions of the primed ASCs	Reference
LPS (1μg/ml) or Poly I: C (10ng/μl)	ND	-TLR4 agonist ↑TLR4, IDO, TDO2, COX2, TGF-β1, and HGF	–	[128]
LPS (1μg/ml) or Poly I: C (10ng/μl)	1 h	-TLR3 agonist ↑TLR3, IDO1, TDO2, COX2, TGF-β1, and HGF		[129]
5% O2 (Hypoxia)	72 h Followed by 16 h incubation in serum- free medium	↓ Type 2 cytokines (IL-13, MCP-1, and CD40 ligand)	Secretome with enhanced inflammation mitigation in fibrosis	[15]
IFN-γ (50 ng/mL)+ TNF-α (20 ng/mL)+ 1% O2	6, 24, 72 h	↑ IDO, CXCL10, PD-L1	-Enhanced inhibition of CD4+ and CD8+ T-cell proliferation	[71]
IFN-γ (100 ng/mL)+1% O2	48 h	IFN-γ induced ↑ (IDO, PD-L1, HLA-E, HLA-G Hypoxia enhanced glucose consumption and production of T-cell inhibitory lactate levels	–	[130]

ASC adipose tissue-derived mesenchymal stem/stromal cells, *CD* Cluster of Differentiation, *CM* Conditioned medium, *COX/ PTSG* Cyclooxygenase/ Prostaglandin-endoperoxide synthase, *CXCL* chemokine (C-X-C motif) ligand, *ECM* Extracellular matrix, *GVHD* Graft versus host disease, *GM-CSF* Granulocyte macrophage-colony stimulating factor, *H* Hours, *HGF* Hepatocyte growth factor, *HLA* Human Lekocyte antigen, *hASCs* human ASCs, *IL* Interleukin, *IDO* Indoleamine 2,3 dioxygenase, *iNOS/NOS* Inducible nitric oxide synthase/ Nitric oxide synthase, *IFN-γ* Interferon-gamma, *IFN-α* Interferon-alpha, *ICAM* Intracellular cell adhesion molecule, *IRF* Interferon regulatory factor, *ILF* Leukemia inhibitory factor, *LPS* Lipopolysaccharide, *MCP* Monocyte chemotactic protein, *MMP* Matrix metalloproteinase, *NT5E* 5'-Nucleotidase, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *O2* oxygen, *PD-L* Programmed death ligand, *PGE2* Prostaglandin E2, *PBMCs* peripheral blood mononuclear cells, *poly I:C* Polyinosinic-Polycytidylic Acid, *STC* Stanniocalcin, *T2D* Type 2 diabetes, *TNF-α* Tumor necrosis factor alpha, *TLR* Toll-like receptor, *TSG-6/TNFAIP6* TNF-stimulated gene 6/TNF-α induced protein 6, *TGF-β* Transforming growth factor beta, *Th17* CD4 T helper 17 cells, *TRAF* TNF receptor-associated factor, *TDO2* Tryptophan-2, 3-dioxygenase 2, *VCAM* Vascular cell adhesion molecule

TNF-α induced significantly the release of PGE2, IL-10, and IL-8 by ASCs (at ≥ 20 ng/ml) and that of IL-6 and CCL-2, only at 40 ng/ml [106]. Cytomix treatment (IFN-ɣ, TNF-α, IL-1β, all at 5 ng/ml) at normoxia robustly induced the most potent immunosuppressive functions of ASCs, likely via induced release of IL-1RA, among other mediators [27].

The effect of human Th17 cell polarizing pro-inflammatory factors such as IL-1β, IL-6, and IL-23 on the immunophenotype and immunomodulatory properties of ASCs vs. BMSCs was studied [54]. It was found that priming of both MSC types with those factors promoted the expression of CD45 by about 80%. CD45⁺ ASCs and BMSCs maintained similarly the antiproliferative functions of the respective CD45⁻ MSCs on MLR, in contact- or contactless- dependent manner. Pro-inflammatory cytokines treatment did not modulate the secretion of IFN-ɣ, TNF-α by both MSC types, however, decreased that of IL-4 and increased that of TGF-β. Such increase in TGF-β recommends that ASC pro-inflammatory conditioning strengthens their immunoregulatory properties [54].

Chemokines play important roles in the recruitment of leukocytes leading to various immune responses [118, 119]. The neutrophil, monocyte and eosinophil chemoattractants, at mRNA levels, including chemokine C-X-X motif ligand *(CXCL)-1* and *-6* were increased in ASCs cultured with MLR, whereas, the genes of T- lymphocytes attractants including *CXCL-9, CXCL-10, CXCL-11* were upregulated in ASCs treated with IFN-ɣ, TNF-α, and IL-6 [14]. Individual IFN-ɣ priming enhanced the secretion of chemokines such as monocyte chemotactic protein 1 (MCP-1) and human interferon-inducible protein 10 (IP-10)/ CXCL-10 [61], IL-8 and CCL-5 [83] or CXCL-9, CXCL-10 and CCL-8 [85] by treated ASCs, promoting immune cells recruitment to their close proximity to exert immunomodulatory functions [61, 120]. CM of TNF-α treated hASCs has also been reported to promote monocyte migration in vitro via the enhanced secretion of IL-6, IL-8, CXCL-6, CXCL-2, and MCP-1/CCL-2 (chemokine C–C motif ligand 2) [102]. The synergistic treatment with TNF-α and IL-1β enhanced the expression, by MSCs from different sources including AT, of a number of ECM proteins and chemokines including, among others, CXCL-2, CXCL-6, IL-8, CCL-2 [121].

An additional boosting strategy is to precondition ASCs with TLR agonists. TLRs are members of a large family of receptors (e.g. TLR1-10), among which TLR3 and TLR4 are highly expressed by human MSCs [126]. In the context of treatment of hASCs with lipopolysaccharide (LPS)/ TLR-4 agonist, it has been reported that hASCs retained short-term memory when exposed to TNF-α or LPS. Transient treatment with TNF-α or LPS dramatically increased the release of IL-6, IL-8, and MCP-1, and all cytokine levels remained elevated, even after re-plating and culture of hASCs in the absence of stimulating factor. A second round

of stimulation induced quick secretion of the cytokines. Importantly, LPS-primed ASCs displayed enhanced therapeutic efficacy in skin flap survival in a diabetic rat model than did unprimed ASCs. Three miRNAs (mir-146a, mir-155, and mir-150) and 5 hydroxymethyl cytosine, epigenetic regulatory molecules, mediated the observed short-term memory of hASCs to LPS or TNF-α [124].

Some studies have suggested that LPS promotes ASCs to acquire a pro-inflammatory phenotype [123, 125], however, the immunomodulatory functions of LPS-ASCs in coculture with activated immune cells in such studies were not tested. Models of acute or chronic inflammation of hASCs were established by treatment of cells with 1 μg/mL LPS for 6 h or 4 weeks, respectively [125]. LPS activated the TLR4/TLR2/NF-κB/ STAT3 signaling pathway to produce the inflammatory cytokines IL-6, IL-1β, and TNF-α via most likely downregulating miR-223. LPS also significantly down-regulated the expression of miR-2909, to upregulate its target, the transcription factor; Kruppel-like factor 4 (KLF4) which in turn significantly, in the presence of activated NF-κB, upregulated the indicated pro-inflammatory cytokines expression. The authors demonstrated that miR-223 and miR-2909 play important roles in the immune-regulatory activity of hASCs, forming a complex regulatory network with pro-inflammatory factors and signaling pathways in ASCs stimulated by LPS [125]. The discrepancy of LPS-ASC immunoregulatory role from immunosuppressive to immunosupportive may be attributed to the differential LPS concentration and treatment duration [131].

Many reports demonstrate that priming of MSCs with the TLR3 agonist, Poly I: C promotes their anti-inflammatory phenotypes and functions [132, 133]. In the context of hASCs and Poly I: C treatment, studies have been conducted and inconsistent effects have been reported [78, 85, 108, 122, 126, 128]. Some authors revealed the expression of several immunosuppressive and inflammatory cytokines by Poly (I:C)-hASCs [126, 128], while, others reported that TLR3 signaling in ASCs did not significantly influence their immunoregulatory phenotype [85, 108, 122]. More studies are thus needed to investigate the immunosuppressive functions of TLR3-primed ASCs in vitro to drive the effective Poly I: C concentration. TLR3 priming of ASCs to promote their Treg-mediated generation as earlier demonstrated with BMSCs [134], can be considered as an interesting research. The synergistic effect of incubation of hASCs with a combination of a pro-inflammatory cytokine and one of the TLR agonists should also be studied.

Hypoxia Preconditioning

Hypoxia preconditioning generates ASCs with improved therapeutic immunosuppression [68, 71, 101, 130]. One of

the suggested mechanisms that mediate the enhanced MSC biology and function in hypoxia is the induced expression of hypoxia inducible factor 1 alpha [100, 135] and the production of various growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), or insulin-like growth factor (IGF) [9]. Additionally, hypoxic ASCs were able to upregulate strongly the expression of the immunomodulatory molecules IDO and PD-L1 upon stimulation with IFN-ɣ and TNF-α [71]. Hypoxia also maintained the chemoattractive properties of ASCs, as evidenced by the enhanced expression of the lymphocytes attractant CXCL-10 [71]. In another report, dual priming of hASCs with IFN-ɣ and hypoxia potentiated their T cell inhibition and the underlying mechanisms were unraveled to be the augmented expression of IDO, PD-L1 and HLA-G via IFN-ɣ and the hypoxia-mediated shift of the hASC metabolism to glycolysis, causing rapid glucose turnover and production of T-cell inhibitory lactate levels [130]. An advanced in vitro culture system for maintained constant O2 levels is crucial to ensure quality-controlled hypoxia-treated ASCs, which would contribute to reproducible results [101].

Chemical or Pharmacological Preconditioning

Different chemical treatments potentiate the immunosuppressive phenotype and/or action of hASCs on immune cells [136–141]. High concentrations of nanocurcuminoids (12–100 μM) enhanced the expansion and the frequency of CD4Tregsin PBMCs in coculture with hASCs. Additionally, nanocurcuminoids at low doses (below 12 μM) in ASCs/PBMCs cultures were able to decrease the expression of the inflammatory cytokines, IL-17, IFN-ɣ, and IL-6. As well, low doses of nanocurcuminoids augmented the antioxidative capacity of hASCs, as manifested by increased superoxide dismutase activity [136]. Interestingly, priming of ASCs with curcumin before cryopreservation potentiated the viability and functional potency of thawed ASCs [142]. In another report, treatment of hASCs with the active form of vitamin B6, pyridoxal-5'-phosphate (PLP) (at a concentration of 50 ng/ml) enhanced their immunosuppressive effect on CD3+ CD8+ T lymphocytes via activation of IDO mediated- Trp metabolism and promotion the accumulation of kynurenine (a Trp catabolic metabolite). Specific blocking of TLR4 reduced CD3+ CD8+ T lymphocytes inhibition by 50 ng/ml PLP-treated hASCs indicating the involvement of the TLR4/NF-κB axis in the PLP-stimulation hASC immunomodulation [137].

Treatment of MSCs, from BM and AT, with specific epigenetic regulatory modulators such as 5-aza-2-deoxycytidine (5-aza-dC) could modulate their immunoregulatory capability via upregulating the mRNA expression of the immunomodulator, HLA-G [138]. Another study reported that preconditioning of hASCs with the iron chelator deferoxamine (DFX), a hypoxia mimetic agent, induced a significant increase in the ASC secretion of anti-inflammatory factors as IL-4 and IL-5 [139]. Importantly, incubation with astragaloside IV, a traditional Chinese medicine at 30 or 60 μg/ml restored significantly the expression of *PD-L1* and *TGF-β* and attenuated the expression of *IFN-ɣ* by hASCs from patients with psoriasis vulgaris [140]. Short-term incubation times to include 24 h [136, 140], 48 h [137, 139] or 72 h [138], have been reported. However, no study addressed the challenge time as a determinant for effect of the applied pharmacological or chemical treatment on ASC immunosuppressive phenotype or action. Recently, long term (14 days) treatment of hASCs with Naltrexone hydrochloride (NTX), an antagonist of mu-, delta-, and kappa-opioid receptors, upregulated the IL-6 secretion by hASCs and promoted the induction of IDO and *PD-L1* in IFN-ɣ treated hASCs. Such effects proportionated directly with the NTX concentration [141]. Treatments of hASCs with cannabinoid compounds have been reported to protect the cells from tunicamycin-induced endoplasmic reticulum stress and inflammation, which resemble those associated with metabolic and inflammatory diseases [143, 144]. However, the stimulating effect of those compounds on hASC immunosuppressive functions in vitro has not been tested. In conclusion, the above-mentioned studies recommend priming of hASCs with chemical or pharmacological molecules could augment their immunosuppression and/or oxidative stress resistance in the settings of inflammatory diseases.

Genetic Engineering

Direct transduction of immunomodulators and anti-inflammatory factors in ASCs by gene editing may represent a promising approach to enhancing the immunosuppressive functions of ASCs. Among the immunoregulatory factors have been delivered in human ASCs are IL-4 [145], fusion proteins comprising the extracellular domain of cytotoxic T-lymphocyte-associated protein 4 and the CH2-CH3 domains of immunoglobulin (CTLA4Ig) [146], glial-derived neurotrophic factor (GDNF) [38], TGF-β1 [147], or HLA-G1 [148]. In vitro cultured GDNF-ASCs induced a shift in macrophage phenotype from the inflammatory (M1) phenotype to the reparative (M2) phenotype [38]. TGF-β1 transduced-ASCs displayed strong IFN-γ-mediated immunosuppressive. ASCs overexpressing TGF-β1 significantly upregulated the expression of IL-10 in CD4+ T cells and downregulated the expression of IL-17A, IL-21, and IL-22 [147].

ASC-related Parameters

Anatomical AT Harvest Site

For developing effective ASC immunotherapy, it is important to consider the effect of fat depots. Whether derived from visceral (v.) or subcutaneous (sc.) AT, ASCs have differential biological characteristics, metabolic properties, multipotency [149, 150], and response to inflammation [151]. ASCs exhibit depot-specific gene expression profiles [152]. Additionally, within the sc. AT, the harvesting area is a strong determinant of the quality of hASCs, influencing cell viability and yield [153]. Limited studies investigated the immunomodulatory phenotype [154, 155] and/or functions of ASCs [156, 157], derived from different origins under the same experimental setup. Serena et al. reported that CM from healthy sc. ASCs, with normal weight, effectively suppressed activated T cell proliferation, however, that of healthy v.ASC failed to do. CM from sc.ASCs or v.ASCs from patients with T2D and obesity did not attenuate T cell proliferation. Interestingly, v.ASC reflected the inflammatory status associated with the metabolic disturbances (T2D and obesity) than sc.ASCs did, by expressing higher levels of IL-1β, IL-6, MCP-1, TNF-α, inflammasome components and caspase-1 [156]. Further, CM of superficial and deep sc.ASCs promoted equally the polarization of M2 anti-inflammatory macrophages in THP-1 monocytes, possibly via PGE2 and TSG-6 dependent mechanisms [157]. Omentum ASCs have been reported to have a secretome with enhanced anti-inflammatory capacity and higher cytokines levels, except for IL-8, relative to that of sc.ASCs, despite being with lower yield [155]. In another report, a comparative analysis for the expression of immune-related surface and soluble markers by sc.ASCs isolated from two different anatomical locations, (abdomen vs. breast), from different donors, was performed [154]. The results revealed a significant elevation in the expression of the two potent immunosuppressive genes, *IL-10* and *IDO* as well as the expression of the multifaceted immunomodulatory adipokine, visfatin, in breast vs. abdominal ASCs. Such data shed light on the possible therapeutic applications of breast ASCs in inflammatory diseases [154]. Conclusively, the fat depot location, whether subcutaneous or visceral or subcutaneous from different anatomical sources, may impact the immunomodulatory properties of hASCs.

ASC Differentiation

Some studies addressed the immunogenic and/or immunosuppressive properties of hASC-differentiation derivatives [79, 158–162]. Osteogenically differentiated hASCs and BMSCs retained similarly low expression of HLA-DR and costimulatory molecules (CD40, CD40L, CD80, and CD86) [79]. They did not induce proliferation of HLA mismatched PBMCs in MLR, even after induction of HLA-DR expression via licensing with IFN-ɣ and TNF-α [159]. Addition of biomaterials that stimulate bone tissue formation did not alter MSC immune-related properties [159]. In the context of the chondrogenic lineage, ASC- and BMSC- derived chondrocytes retained hypoimmunogenicity, as manifested by non-induction of PBMC alloproliferative response, even after licensing of MSCs with the inflammatory cytokines IFN-ɣ and TNF-α [160]. Both MSC-derived chondrocytes displayed dose-dependent immunosuppressive functions on T cells and NK cells. Interestingly, ASC-derived chondrocytes, but not BM-derived respective, inhibited strongly allostimulated PBMCs at low cell dosages, suggesting that ASCs would be better than BMSCs for cartilage repair. Noteworthy that the maintained immunoregulatory potential of ASC-derived preosteoblasts [159] or chondrocytes [160] was mediated partly via the expression of HLA-G5 and its level was boosted in immune-active environments. In another study, chondrogenic differentiation or IFN-ɣ treatment potentiated the immunosuppressive effects of hASCs on mitogen-treated PBMC activation and proliferation via secreting expression of higher levels of IL-10 and the surface immunomediator, jagged-2 [161].

Moreover, adipogenic differentiation of hASCs kept their ability to inhibit neutrophil and lymphocyte recruitment to TNF-α-treated endothelial cells in an IL-6/suppressor of cytokine signaling 3 (SOCS3)-dependent manner [162]. On the contrary, adipocytes derived from BMSCs lost their immunoprotective effects on neutrophils, but not lymphocytes. The authors proposed that soluble bioactive molecules, generated by BMSC-derived adipocytes in coculture with the target immune cells, induce a reduction in TGF-β1 response, modulating IL-6 signaling, such that it is no longer immunoprotective. Abnormal adipogenesis of MSCs in inflammation thus adversely affect MSC behavior; loss their immunosuppressive effects and contribute to the pathogenic recruitment of leukocytes [162]. It has been reported that adipocytes, like their progenitors, from obese donors promoted the IL-6 mediated-Th17 differentiation in vitro (in coculture with PHA-PBMCs) and in a murine model [163]. Similarly, adipocytes differentiated from sc.ASCs or v.ASCs from T2D patients exhibited elevated IL-1β phenotype as their precursors [156]. Further studies to explore the immunogenicity and immunoregulatory properties of ASC-derived cell products in health and disease may be a crucial step, beside the cell product functionality, to develop an effective applicable ASC derivatives-based therapy.

ASC Culture Conditions

Diverse culture conditions could impact variably hASC immunomodulation, including among others the serum supplement of the culture medium [17, 53, 164, 165], suspended spheroid vs. mono-adherent layer culture [86, 166], and passage number (early vs. late) [13, 52, 167–170].

Serum Supplementation

To date, most studies use fetal bovine serum (FBS)—based culture media to address the immunomodulatory functions of hASCs in vitro. However, safety concerns have been raised regarding FBS addition for the manufacturing of clinical grade MSC products [171], most of them related to immunological risk or possibility of disease transmission due to prions, bacteria, or viruses [172]. Regulatory-complaint xeno-free media such as chemically defined media [173], or media supplemented with human serum (HS) [67], or human platelet lysate (hPL) [173, 174] are promising alternatives to preserve or even enhance the ASC immunomodulatory functions in vitro.

Patrikoski et al. [53] assessed the immunogenic and immunosuppressive properties of hASCs expanded in serum and xenobiotic-free medium (SF/XF) or medium supplemented with FBS or HS. hASCs expanded in any of the three conditions did not lose their hypoimmunogenicity and they were able to suppress the proliferation of PBMCs in two-way MLR. However, the significantly strongest suppression was observed by those expanded in FBS and such effect was attributed to the higher expression of ICAM-1 and IL-6 [53]. In another study and relative to those expanded in FBS, platelet poor Plasma (PPP)—cultured ASCs exhibited compromised potential to generate Tregs and that was correlated with limited PPP-ASC potential to produce soluble immunomodulatory factors [165]. In contrast, PPP supplementation promoted the expression of vascular cell adhesion molecule-1 (VCAM-1)/CD106 and ICAM-1 on ASC surface hinting possibly toward maintained direct immunosuppressive mechanisms. These data confirm the strong effect of culture media composition on ASC immunomodulatory behavior as well as serving as an alert regarding the complexity of replacing FBS in MSC culture [53, 165].

Other authors reported enhanced or at least maintained hASC immunosuppressive functions in the absence of FBS [12, 67, 173, 175]. Effective inhibition by hASCs on CD4 T cell proliferation, activation, and functions in a 5% HS-supplemented culture medium, have been recently reported [67]. As other alternatives, ASCs isolated from human infrapatellar fat pad (hIFP-ASCs) were processed in the presence of hPL, chemically reinforced medium (Ch-R), or FBS [173]. hIFP-ASCs cultured in the regulatory-compliant conditions (hPL or Ch-R) displayed enhanced anti-inflammatory surface and paracrine phenotype which was intensified by priming of hIFP-ASCs with IFN-ɣ and TNF-α. In the two indicated regulatory-compliant conditions, hIFP-ASCs upregulated CD10/Neprilysin expression which degraded substance P in vitro and in vivo, relieving experimental synovitis [173]. Interestingly, hASCs expanded in clinical grade hPL were more potent in inhibiting T-cell growth, than BM counterparts via a superior IFN-ɣ-mediated IDO activity [175]. Additionally, resting and inflammatory primed ASCs-PL expressed higher transcriptional levels of TSG-6 [12], which is a suppressor to neutrophil recruitment in acute inflammation [176]. In another report, fabricated hASC sheets in the presence of human PL exhibited enhanced deposition of ECM and inhibition of stimulated macrophages migration [177]. hPL- ASCs are thus suggested as interesting anti-inflammatory cell therapy for further preclinical and clinical evaluations.

Three-Dimensional ASC Cultures

The traditionally cultured MSCs in a two-dimensional (2D) adherent monolayer exhibit limited physiological relevance and altered genetic and epigenetic signatures [178]. Thus, cultivation of MSCs in three-dimensional (3D) systems, such as low attachment surfaces, hydrogels, or scaffolds, to resemble the in vivo spatial organization with increased cell–cell and cell–matrix interactions, have gained attention [171, 179]. The methods to generate 3D MSC spheroids, including ASC ones, and the limitation and challenges of different MSC spheroid generation platforms have been recently reviewed [171, 180].Enhanced therapeutic and anti-inflammatory mechanisms have been reported in different preclinical models treated with human ASCs cultured by a 3D strategy [86, 100, 178, 181–190].

Scaffold-free 3D ASC Spheroids Limited studies addressed the immunomodulatory phenotype and/or functions of scaffold free- 3D ASC spheroids in vitro [59, 86, 166]. hASC aggregates, obtained by the hanging drop technique, exhibited a stronger ability than 2D counterparts, to modulate macrophage polarization from M1 (pro-inflammatory) to M2 (anti-inflammatory) phenotype in vitro, presumably via elevated PGE2 production [86]. Recently, ASC spheroids significantly abrogated the proliferation and IFN-ɣ secretion and promoted the production of the anti-inflammatory cytokines IL-9 in coculture with stimulated γδ CD3+ T lymphocytes [59]. γδ CD3+ T lymphocytes represent a bridge b/w innate and adaptive T cell responses and produce high levels of IL-17 in obesity contributing to AT inflammation and insulin resistance [77]. The detected anti-inflammatory effects were greater than those observed by adherent ASCs due to higher levels of the immunomodulators IL-5, IL-10, IL-4 and IL-13 in the ASC spheroid secretome [59]. In another report,

comparative gene expression analysis in 2D vs. 3D (in ultra-low attachment surfaces) ASC cultures revealed superior expression, of genes involved in stemness (*SOX2, POU5F1,* and *NANOG*), anti-aging (*SIRT1*), and anti-inflammation (*TGF-β1*), however, lower expression of those involved in oxidative stress (*ALDH3*), in 3D ASC spheres [166].

Scaffold-based 3D ASC Spheroids Some authors addressed the immunomodulatory properties of hASCs encapsulated in hydrogels [185, 188, 191]. hASCs encapsulated in inject-able alginate hydrogel modified with arginine-glycine-aspartate- motifs did not induce DC maturation and they exhibited potentiated inhibitory lymphocyte proliferation. Importantly, hASCs in alginate responded like hASC monolayer to IFN-ɣ licensing [185]. Such results indicate that the combination of ASCs and alginate is non-immunogenic, however, immunosuppressive. That might be attributed to the elevated secretion of the chemokine IL-8 by 3D ASCs in inflammation [185]. As well, incorporating the hASCs spheroids in alginate microbeads in thermosensitive hydrogels [188] enhanced significantly the expression of immunosup-pressive cytokines such as IL-10 and/or TGF-β1 for controlling inflammation in wound healing. As well, hASC-spheroids in non-crosslink hyaluronic acid gel (4%) displayed a promoted expression of some angiogenesis growth factors, pluripotency markers, the anti-inflammatory factors (*IL1RN, IL11,* etc.), in addition to compared to the adherent ASC cultures [191]. Such studies recommend that aggregation of ASCs in hydrogels stimulates paracrine signaling and the level of anti-inflammatory factors [19], and potentiates the cell functionality [192]. The positive effects can be amplified by incorporating of the ASC spheroids in tunable immuno-tolerant polymeric hydrogels to tailor the target therapeutic effects [193]. Interestingly, sustained MSC licensing could be achieved by the chemical modification of the hydrogel capsule to present an inflammatory cytokine [194] or by incorporating the cytokine in the hydrogel matrix [193]. Such strategies might ensure enhanced MSC persistence and immunomodulation in vivo.

Anoikis is the apoptosis of adherent cells due to the lack of a scaffold, so the cultivation of MSCs on a fabricated scaffold can prevent it [195]. However, matrix stiffness (mechanical properties), construct dimensions, fiber align-ments, and/or fluid forces may affect ASC characteristics [196, 197] including immunomodulatory ones [198, 199]. Wan et al. studied the effect of fiber orientation (random or aligned), as one of the physical features of the scaffold, on the ASC immunomodulatory paracrine mechanisms. hASCs seeded on aligned fibers secreted significantly higher levels of immunomodulators, including among others, COX-2 and TSG-6, than those cultured on random fibers and that was correlated with a superior promotion of M2 macrophages.

Aligned fibers stimulated ASC immunomodulatory function by activating mechanotransduction pathways; focal adhesion kinase (FAK)-extracellular regulated kinase 1/2 (ERK1/2) and YAP/TAZ [198]. FAK has been reported to mediate the cellular responses to the biomaterial physical cues [200, 201], and has been directly linked to transcriptional regula-tion of COX-2 [202]. The inhibition of YAP/TAZ nuclear translocation reduced the gene expression of crucial immu-nomediators including *COX-2, TSG-6, IL-1RA, and MCP-1* in hASCs cultured on aligned fibers [198]. Signaling mech-anisms regulating the MSC response, specifically those derived from bone marrow, to the physical cues of scaffolds are recently reviewed [199]. In the context of hASCs, further transcriptomic, proteomic and functional studies to address the immunomodulatory capacity of scaffold-free- or based-hASC spheroids are demanded.

ASC Passaging and Cryopreservation Effects

Expansion of hASCs till passage 6 (P6) P6 did not reduce the immunomodulatory properties of hASCs, whereas, cryopreservation significantly did [52]. Analysis of hASC immunophenotype including the immuneregulatorymarkers;CD200, CD274, CD271, CD73, and CD29 [203, 204] over 8 passages revealed that hASCs maintained the expression of these markers at variable lev-els over the whole culture period without significant differ-ences except for CD271 which decreased by culture [170]. On the other hand, a larger literature cohort recommends that only ASCs of low passage rounds would be ideal for immunomodulatory therapeutic purposes [13, 167–169, 205]. ASCs at late passages failed to inhibit IFN-ɣ produc-tion by PBMCs and to abrogate neutrophil activity and the levels of pro-inflammatory markers TNF-α and IFN-ɣ in a model of peritonitis [168]. In another report, ASCs at P3 downregulated the proportion of Th17 cells, in patients with active systemic lupus erythematous, and their abilities to produce IL-17, whereas ASCs at P8 had a contrasting effect [205]. hASCs at late passages had reduced levels of secreted IL-10 and HGF [168], in addition to the surface (CD200 and CD274), or intracellular (heme oxygenase 1 (HO-1)), proteins [13], and all these factors contribute to effective ASC immunomodulation[19]. Decreased gene expression of the anti-inflammatory factors (TSG6 and HLA-G) was also detected in ASCs and BMSCs over repeated passages up to P10 [169]. Regarding the expression of MHC proteins in repeatedly passaged ASCs, HLA-ABC (MHC-1) level was not affected by expansion. However, levels of HLA-DR and the surface and the intracellular HLA-G in ASCs decreased by expansion [13].

Overall, in vitro-aged hMSCs, as a result of extensive culture expansion, have been reported to show senescence

signatures, diminished immunosuppressive capacity, and weakened regenerative potential as well as pro-inflammatory features [206]. Impaired autophagy and altered epigenetics molecular mechanisms involved in MSC aging in vitro and in vivo have been reviewed [207].

In clinics, modest immunomodulatory activity of freeze-thawed, relative to fresh, MSC products has been reported [208]. Cryopreserved MSCs exhibited attenuated immunosuppressive properties in vitro as a result of heat-shock response, impaired IFN-ɣ treatment response [209], and T-cell mediated cytolysis [210]. In the context of hASCs, different cryoprotectants have been tested to include dimethyl sulfoxide (DMSO) alone or in combination with pentaisomaltose [211], intracellular delivered trehalose [212]. For clinical grade cryopreservation, xeno and/or DMSO free-cryopreservation solutions containing trehalose, dextran 40, propylene glycol, glycerol, polyvinylpyrrolidone, hydroxyethyl starch, and/or methylcellulose have been developed [213–219]. Despite the diverse protocols [214, 220], up to our knowledge, the impact of cryopreservation and freeze-thawing on the immunomodulatory properties of hASCs is still not explored. Thus, future studies, aim to investigate the inhibitory effects of long-term cryopreserved hASCs, using different cryoprotectants, on T lymphocytes and their IFN-ɣ licensing response, are recommended.

Donor-related Parameters

Age, BMI, and Sex

Increasing donor age negatively impacts the biological features of hASCs, including decreased expansion kinetics, differentiation potential [221, 222], and/or regenerative capacity [223]. Regarding the immunomodulatory properties, young rat ASCs [224] and canine ASCs [225] exhibited lower antiproliferative effects on activated T lymphocytes than old respective. Recently, an inflammatory state, characterized by elevated expression of *IL-6, IL-1β, TNF-α*, and *MCP-1*, has been detected in hASCs derived from elderly subjects (≥ 65 years) and that was associated with enhanced glycogen storage and decreased expression of sirtuin 1 and 6 [226]. Sirtuins are key metabolic sensors that links inflammation and metabolism [227]. Obesity exacerbated the inflammatory phenotype of elderly hASCs [226].

In the context BMI, numerous investigators reported the adverse effects of obesity [BMI ≥ 30] on the immunomodulatory phenotype and functions of hASCs [39, 77, 156, 226, 228–238]. ASCs isolated from patients with obesity exhibited altered glucose metabolism [226, 239, 240], reduced sirtuins expression [226, 241], pro-inflammatory phenotype [20, 156, 228, 229]. The pro-inflammatory state of hASCs from obese patients characterized by the elevated secretion of inflammatory

cytokines and chemokines including IL-6, IL-1β, TNF-α, IL-17A, IL-8 and /or MCP-1 [39, 156, 228, 229, 242]. In addition to the activation of inflammasome components, NF-Kβ [156], and mitogen-activated protein kinase (MAPK) signaling that are linked to inhibiting insulin signaling [77].

In vitro, hASCs from patients with obesity failed to inhibit T cell proliferation [156] and promoted Th17 differentiation via IL-1β [242] and/or PD-L1 [234]-mediated manner. As well obese ASCs potentiated monocyte polarization toward the inflammatory macrophages (M1) [39]. Noteworthy, obesity deteriorates the response of hASCs to IFN-ɣ -induced expression of IDO and that might explain the dysfunctional antiproliferative effect of obese ASCs on T cells [232]. A new mechanism has been recently introduced to explain the reduced therapeutic efficacy of ASCs derived from obese subjects [243]. The decrease in mitochondrial cardiolipin content in obese ASCs resulted in compromised cell potential to sequester their damaged mitochondria into LC3-dependent autophagosomes. That led to mitochondrial dysfunction, impaired intercellular mitochondrial transport, and finally attenuated therapeutic efficacy of obese ASCs. Importantly, pharmacological treatment using a compound that modulate mitophagy and/or autophagy such as pyrroloquinoline quinone rescued the mitochondria health in obese ASCs [243]. Interestingly, the addition of n-3 polyunsaturated fatty acid precursor, alpha-linolenic acid (ALA), or its derivatives, eicosapentaenoic, or docosahexaenoic acid, to co-cultures of human obese ASCs and PBMCs abrogated the immunostimulatory impact of obese ASCs. ALA inhibited obese ASC-mediated activation of Th17 cells, IL-17A secretion, Cox-2 and STAT-3 expression [244]. Another approach to rescue obese ASC biology, cytosol transfer from control ASCs derived from patients with normal weight restored the glucose metabolism in ASCs from patients with obesity via Lin28-mediated repression of let7 pathway [239].

On the other hand, limited studies illustrated maintained or slightly affected immunosuppressive potentials of ASCs from obese subjects [67, 231, 235]. The discrepancies b/w studies on the impact of obesity-associated inflammation and metabolic abnormalities on the immunosuppressive potential of hASCs could be due to differences in fat depots locations, the methods used to evaluate immunosuppressive functions in vitro, or to the use of donors with different adiposity grades, or at different stages of obesity development. Studies that investigated the effect of obesity on hASC immunomodulation are presented in Table 2.

Sex impact on the immunomodulatory properties of hASCs has been recently studied [110, 127]. Mckinnirey et al. examined the potency and functionality of both female and male ASCs in order to gain further insights into donor selection. Female ASCs, significantly suppressed activated PBMC proliferation more than male ASCs did, due to the production of higher concentrations of the anti-inflammatory

Table 2 Studies investigating the immunomodulatory properties of hASCs isolated from donors with obesity

Ref	AT site location	Age (years) (range or mean ± SEM)	Metabolic Indices (Values in range or mean ± SEM)	BMI (kg/m2) (Range or mean ± SEM)	No. of Obese Subjects	The studied immune-related properties and/or functions	The key immunomodulatory properties- relevant findings
[228]	ND	41.56 ± 3.07	Elevated glucose and total cholesterol/high-density lipoprotein (HDL) ratio as compared to non-obese individual (142.13 ± 11.95 mg/dl vs 61.54 ± 7.35 mg/dl) and (5.92 ± 0.48 vs 3.98 ± 0.38), respectively	44.44 ± 1.29	ND	Transcriptome analysis (including inflammatory genes)	From a transcriptome perspective, obese ASCs exhibited reduced stemness, adipogenic commitment, and enhanced inflammatory state ($\uparrow IL\text{-}1\beta$, $IL\text{-}8$, and $MCP\text{-}1/CCL\text{-}2$)
[242]	v. AT and sc.AT	ND	ND	ND	ND	T cells differentiation	Obese not lean ASCs promoted Th17 polarization in IL-1β, not IL-6,—dependent manner, however, they reduced Th1 cytokines. Increased IL-17A production inhibited adipogenesis and promoted insulin resistance in adipocytes
[229]	sc.AT	37.3 ± 13.4	ND	46.2 ± 5.1	$n = 12$	Cytokines released in the secretome of unprimed and LPS (0.5μg/ml) -stimulated ASCs	Enhanced inflammatory state of obese ASCs ($\uparrow$IL-6 and IL-8 in unprimed and primed obese ASCs and MCP-1 in primed obese ASCs)
[156]	v. AT and sc.AT (abdomen)	39 ± 8.9	-HOMA-IR 2.84 ± 0.2	33.1 ± 2.1	$n = 4$	-Basal expression of pro-inflammatory cytokines as IL-1β, TNF-α, MCP-1 and critical inflammasome components and anti-inflammatory cytokines as TGF-β1 and IL-10 by obese vs lean ASCs -The potential of CM of obese vs lean ASCs to suppress the proliferation of PBMCs in MLR	-Enhanced inflammatory state of obese ASCs ($\uparrow IL\text{-}1\beta$, $IL\text{-}8$, $TNF\text{-}\alpha$, and $CCL\text{-}2$) -As well, obese ASCs exhibited impaired antiproliferative effect due to inflammasome activation-mediated IL-1β elevation, however, reduced expression of TGF-β1
[230]	sc.AT (abdomen)	42.5 ± 8.9	ND	32.7 ± 3.7	$n = 6$	-Potential to suppress murine T cell proliferation The therapeutic efficacy in EAE	-Obese ASCs failed to halt the disease progression in CNS of EAE -Obese ASCs enhanced proliferation and differentiation of CD4 and CD8 T cells

Table 2 (continued)

Ref	AT site location	Age (years) (range or mean ± SEM)	Metabolic Indices (Values in range or mean ± SEM)	BMI (kg/m2) (Range or mean ± SEM)	No. of Obese Subjects	The studied immune-related properties and/or functions	The key immunomodulatory properties- relevant findings
[231]	sc.AT (abdomen)	18–62	ND	Morbidly obese, however, exact BMI is ND	n = 10	- Potential to suppress CD3 T cells proliferation Isolated from RA patient -induction of Tregs -Modulation of cytokines production	Morbidly obese ASCs were able to inhibit proliferation of CD3 T cells, derived from RA patients, in a dose-dependent fashion, however, they promoted that of CD4⁺ FOXP3⁺ T cells. They also downregulated the production of IFN-γ and TNF-α, however, upregulated IL-10 by RA-CD3 T cells
[241]	v. AT and sc.AT	40.40 ± 1.14	HbA1c (%): 5.46 ± 0.28 vs 4.70 ± 0.43 CRP: 1.23 ± 0.19 vs 0.37 ± 0.11 in obese vs non-obese individual	44.66 ± 4.64	n = 8	-Gene expression of Sirtuins 1–7 in naïve and hypoxia primed sc.ASCs ss v.ASCs	Sirtuin1–6 mRNA levels were markedly reduced in v.ASCs of obese patients. Sirtuins' expression in v.ASCs correlated negatively with BMI and CRP. Hypoxia-induced mRNA expression of all of the sirtuins only in obese v.ASCs
[20]	Sc.AT	35–45	ND	≥ 30	n = 5	-Assessment of intracellular vitamin D levels - Gene expression of pro- and anti-inflammatory mediators	Expression of IL-6, IL-8, IL-10 and MCP-1 was higher in obese ASC than in control ASCs and that was associated with reduced vitamin D levels
[233]	sc.AT (abdomen)	40.50 ± 7.46	ND	33.97 ± 3.11	n = 6 (pooled)	-The potential to modulate macrophage and microglia polarization toward anti-inflammatory phenotype -Modulation of gene expression of pro- and anti-inflammatory markers by macrophages -Modulation of NO activity and phagocytic activity of macrophages	Obese human ASCs induced polarization of murine microphages and microglia toward a pro-inflammatory phenotype

Table 2 (continued)

Ref	AT site location	Age (years) (range or mean ± SEM)	Metabolic Indices (Values in range or mean ± SEM)	BMI (kg/m2) (Range or mean ± SEM)	No. of Obese Subjects	The studied immune-related properties and/or functions	The key immunomodulatory properties- relevant findings
[234]	v. AT or sc.AT	ND	ND	> 30	ND	The contribution of PD-L1 to the obese ASC-mediated induction of Th17	Inflammation, mediated by ASCs from obese individuals, contributed to PD-L1 upregulation in cocultures with PBMCs (non-activated or PHA-activated) Blocking PD-L1 expression in cocultured cells (ASCs and PBMCs) restored TNF-α and IL-2 expression by Th1 cells and improved obese ASC-induced Th17 cell activation, without affecting pro-Inflammatory cytokine secretion by accessory cells
[235]	sc.AT	33.5–34.1	-HOMA-IR range 0.6–2.7 -Plasma adiponectin: 1310–4930	25.2–44.2	n = 5	-Immunogenic effect -Suppression of PBMC proliferation in MLR	Leaner and heavier WD donors were hypoimmunogenic, however, heavier WD donors showed superior immunosuppressive capacity
[39]	ND	57.0 ± 2.6	50% of obese subjects had hypertension, 66% had T2D, and 66% suffered from hyperlipidemia	42.9 ± 1.1	n = 6	Potential to modulate the polarization of macrophage in inflammation toward anti-iinflammatory phenotype (M2) at the expense of inflammatory phenotype M1	-ASCs isolated from obese donors had blunted immunomodulatory potential on activated macrophages - TNF-α levels were four-fold higher in CM collected from obese than from lean ASCs
[236]	v.AT	45.7 ± 6.7	ND	31.4 ± 1.8	n = 11	- Potential of the secretome of obese or lean v.ASCs to modulate macrophages in the tumor microenvironment	Only obese v.ASCs could modulate macrophage to acquire pro-tumoural phenotype, characterized by the expression of pro- and anti-inflammatory phenotype. That was mediated via internalizing the obese ASC adipokine, survivin by the macrophages, and inducing survivin expression by the tumor-associated macrophages

Table 2 (continued)

Ref	AT site location	Age (years) (range or mean ± SEM)	Metabolic Indices (Values in range or mean ± SEM)	BMI (kg/m2) (Range or mean ± SEM)	No. of Obese Subjects	The studied immune-related properties and/or functions	The key immunomodulatory properties- relevant findings
[226]	sc.AT	Adult group (48.7 ± 8.5) Elderly group 72 ± 4.7	Obese and lean groups exhibited comparable glucose levels and lipid profile	Adult group 34.3 ± 4.5 Elderly group 33.3 ± 3.5	Adult group n = 30 Elderly group n = 8	- Multiplexed cytokine array (62 cytokines) - Gene expression analysis of inflammatory markers and genes involved in glucose metabolism - Lactate secretion and glycogen content analysis - SIRT1 and SIRT6 protein expression	-Aging significantly promoted the inflammatory state of sc.ASCs as characterized by elevated expression of IL-6, IL-1β, TNF-α, and MCP-1 -Aging significantly enhanced glucose turnover, lactate secretion, and glycogen storage -Obesity exacerbated the inflammatory state of ASCs -Reduced expression of SIRT1 and SIRT6 mediated the adverse effects of aging and obesity on the metabolism and the phenotype of sc.ASCs
[237]	ND	39.43 ± 6.97	All the tested biochemical parameters were comparable between the obese and lean subjects, except for a higher cholesterol/HDL ratio in the patients with obesity	44.14 ± 5.46	n = 7	- Secreted cytokines profiling	Significant low expression of pro-inflammatory (MIP3a, IL-8, and TNFα) and regulatory (GM-CSF, Fractalkine, IL-6, IL-7, and IL-21) cytokines and chemokines in ASCs derived from obese patients, as compared to those of ASCs derived from healthy donors with normal weight

AT adipose tissue, *BMI* body mass index, *CCL-2* C–C motif chemokine ligand 2, *CNS* central nervous system, *CRP* C—reactive protein, *CM* conditioned medium, *EAE* experimental autoimmune encephalomyelitis, *FOXP3* forkhead box P3, *GM-CSF* granulocyte–macrophage colony-stimulating factor, *HbA1c* glycated hemoglobin, *HDL* high-density lipoprotein cholesterol, *HOMA-IR* homeostatic model assessment for insulin resistance, *IL-6* interleukin 6, *IL-1β* interleukin 1 beta, *IL-17A* interleukin 17A, *IL-10* interleukin 10, *IL-8* interleukin 8, *IL-7* interleukin 7, *IL-21* interleukin 21, *MCP-1* monocyte chemotactic protein 1, *MLR* mixed lymphocyte reaction, *mRNA* messenger ribonucleic acid, *MIP3a* macrophage inflammatory protein 3 alpha, *ND* not defined, *NO* Nitric oxide, *PD-L1* programmed death ligand 1, *PBMCs* peripheral blood mononuclear cells, *RA* rheumatoid arthritis, *SEM* standard error means, *s.AT* subcutaneous adipose tissue, *SIRT1* sirtuin 1, *SIRT6* sirtuin 6, *Tregs* T cell with immune regulatory functions, *TNF-α* tumor necrosis factor-alpha, *TGF-β1* transforming growth factor beta 1, *Th17* CD4 T helper 17 cells, *Th1* CD4 T helper 1 cells, *T2D* type 2 diabetes mellitus, *v.AT* visceral adipose tissue, *WD* weight- discordant

factors; IDO1, IL-1RA, and PGE-2 and the prolonged expression of VCAM-1 post-activation [110]. In another report, inter-individual variability and/or possible sex differences exist in ASCs' response to LPS treatment and the potential of LPS-ASC CM to regulate CD14 expression in THP-1 human monocytes have been reported [127]. Variables, including age, sex, and biological sources of MSC, that can guide the important choice of "universal" or "personalized" MSC therapy for autoimmune diseases have been recently reviewed [245, 246].

Presence of Inflammatory Disease

The impact of inflammatory diseases on the immunosuppressive properties of hASCs has been extensively investigated to include RD [46, 47, 96, 112, 231, 247–257], T2D [67, 109, 156, 258], sepsis [259], Crohn's disease [260–263], ulcerative colitis [264], breast cancer [265–269], osteoporosis [270], psoriasis vulgaris [140], Parkinson's disease [271], and atherosclerosis [272]. The results are contradictory from diseased ASCs having intact immunomodulatory functions to impaired immunosuppressive effects.

Effect of RD

The RD include a wide range of auto-immune and inflammatory disorders that affect bone, tendon, ligaments and/or muscles. Examples of RD forms are rheumatoid arthritis (RA), OA, systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and ankylosing spondylitis (AS) [46, 47, 96, 253, 254]. hASCs inhibited the proliferation and the production of inflammatory cytokines (IFN-ɣ, TNF-α, and IL-17) by collagen II -activated CD4$^+$ and CD8$^+$ T cells from patients with RA. hASCs also stimulated the generation of CD4$^+$CD25$^+$FOXP3$^+$ Tregs, with capacity to suppress collagen-specific T cell responses. Finally, hASCs downregulated the inflammatory response of synovial cells isolated from patients with RA, by downmodulating the production of matrix-degrading enzymes [247]. hASCs were able to modulate Th17 responses by inhibiting the gene expression and/or secretion of IL-17, IL-21, and/or IL-6 by PHA-activated PBMCs isolated from RA vs. healthy donors, with more intense suppressive effects in the RA group due to their priming by the patients' inflammation. Noteworthy, hASCs remarkably induced TGF-β1 expression in healthy PBMCs [249]. Not only the cells are effective, but also the secretome of healthy hASCs were able to downmodulate Th17 cells and significantly increase Tregs in coculture with PBMCs from RA patients [257].

Regarding the immunosuppressive potential of ASCs isolated from patients with RD, controversial data have been reported from mostly intact [47, 112, 254, 273], to affected [250], ASC immunosuppressive functions. RD/ASCs (from patients with SLE, SSc, or AS) were characterized by low basal levels of CD90 and ICAM-1 expression, upregulated secretion of IL-1Ra, TSG-6 and sHLA-G, but impaired release of kynurenines and galectin-3 [252].

Despite the altered immunomodulatory phenotype, intact immunosuppressive effects of hASCs derived from patients with SLE, SSc, or AS have been reported by the same research group [47]. Comparable to healthy ASCs, RD/ASCs were able to modulate the activation of allogeneic CD4$^+$ and CD8$^+$ T lymphocytes in direct and transwell coculture settings. RD/ and healthy ASCs attenuated the expression of CD25 and HLA-DR on T lymphocytes, however, upregulated the CD69 level [47].. Recently, ASCs from patients with RA or OA exhibited intact lymphocytes antiproliferative potential via mostly the induced release of IL-10 and PGE2 and the enhanced activity of IDO [254]. In another report, hASCs derived from healthy donors and SSc patients with extracutaneous manifestations presented the comparable potential to inhibit the PHA-activated PBMCs proliferation in direct contact settings [273].

On the contrary, Skalska and Kontny revealed that the immunosuppressive and anti-inflammatory functions of ASCs derived from inflammatory rheumatoid joints of patients with RA or OA are impaired [250]. ILl-17A is one of the crucial mediators in the development of RA [274]. The enhanced release of this cytokine by activated PBMCs after contact with rheumatoid ASCs may recommend their involvement in disease progression by promoting pro-inflammatory activity [250, 251]. Treatment of RA-ASCs with high/moderate molecular weight adiponectin only was found to considerably upregulate the secretion of the soluble factors IL-1RA, PGE2, TGF-β, IL-6, IL-8, and VEGF, however, it did not greatly impact the weak immunosuppressive effects of RA-ASCs on PHA-activated PBMCs [250].

Effect of Inflammatory Bowel Diseases

Inflammatory bowel disease (IBD) primarily comprises Crohn's disease (CD) and ulcerative colitis (UC) [264]. The available studies that addressed the immunomodulatory properties of ASCs from patients with active IBD illustrate that such cells have blunted immunosuppressive functions and so they are not suitable for autologous therapy [261–264]. Serena et al. investigated the altered immune profile of hASCs derived from mesenteric or subcutaneous fat depots from CD patients. Mesenteric creeping fat hASCs, of CD patients, exhibited exacerbated migration capacity and elevated *IL-1β* expression. Also, CM from active and inactive CD subcutaneous ASCs failed to inhibit the proliferation of stimulated T and B cells and to promote the M2 polarization. That was attributed to inflammasome activation and inflammatory markers elevation, as represented by

upregulated gene levels of *IL1β, IL6, TNF-αA,* and *CCL2*, however, reduced expression and production of the ASC immunomodulator; TGF-β [261]. In a more recent report, the authors demonstrated that sc. ASCs from active CD patients exhibited distinct epigenetic DNA methylation patterns associated with differential expression of immune system- related genes [262]. With except of *TNF-α*, in hASCs isolated from patients with inactive disease, almost the expression levels of all those genes were comparable to the control level, indicating that immune system genes affected by methylation marks were partially restored in patients during episodes of remission [262]. Importantly, cigarette smoking favored the pro-inflammatory epigenetic changes and the blunted immunosuppressive functions of hASCs from patients with active CD [263].

The first report to investigate the immunosuppressive potential of hASCs from UC patients, with different disease degrees has been recently published [264]. UC-ASCs exhibited a diminished ability to inhibit stimulated PBMC proliferation, suppress CD25 and CD69 activation marker expression, decrease the production of IFN-ɣ and TNF-α, and reduce their cytotoxic effect on A549 cells. On inflammatory priming with a mix of IFN-ɣ and TNF-α, UC-ASCs secreted lower levels of PGE2, IDO, and TSG-6, which mediated their blunted immunopotency. Moreover, UC-ASCs induced weaker therapeutic effects than healthy ASCs, in experimental UC. These findings indicate that the immunosuppressive properties of ASCs from patients with UC (mild, moderate or severe) are affected [264].

Effect of Breast Cancer

Numerous studies demonstrate that resident ASCs in breast cancer(BC) tissue are greatly affected by the tumor microenvironment [265–269]. In one study, IL-10 and TGF-β1 mRNAs were significantly higher in ASCs isolated from patients with BC (pathological stage II/III) than those from normal individuals. Moreover, the CM of ASCs isolated from patients with BC (stage III) upregulated the expression levels of the regulatory molecules genes; *IL-4, TGF-b1, IL-10, CCR4 and CD25* and increased the frequency of CD4+ CD25+ FOXP3+ Tregs in peripheral blood lymphocytes (PBLs) [265]. In a more recent report, ASCs from BC patients significantly directed naïve CD4 T lymphocytes toward Tregs with different phenotypes. They significantly induced the expansion of the CD4+CD25+Foxp3+CD45RA+, CD4+CD25+ FOXP3+Helios+, CD4+CD25− FOXP3+Helios+, and CD25+ FOXP3+CD73+CD39+ Tregs and that was associated with enhanced production of IL-10 and TGF-β1 by the generated T regs [269].

Another immunomodulatory role seems to be exerted by hASCs in the tumor microenvironment is IFN-ɣ mediated-elevated expression of major histocompatibility complex class I polypeptide-related sequence B (MIC B). MIC B is a ligand of Natural-killer Group 2, member D (NKG2D) receptor to protect the cancer cells from NK cells. Frequent stimulation of NKG2D receptor by MIC B can result in downmodulation of this receptor and the impairment of NK cells activation in invasive ductal breast carcinoma [268]. Another report demonstrated a significant decrease in the percentage of CD3− CD16+ CD56+bright and CD3− CD16+ CD56+dim NK cell subsets after exposure of PBLs to ASCs either from normal donors or patients with BC (pathological stage II/III). A considerable reduction in NK cell activating receptors as NKG2D and the CD69 among the cocultured PBLs was also observed. However, no significant difference was observed b/w cancerous vs. normal breast ASCs in the NK cell suppressive effects. However, cancerous ASCs had significantly higher *IDO1, IDO2,* and *HLA-G5* mRNAs [266].

In the context of B cells, ASCs from normal donors and patients with invasive ductal BC (stage II/III) were cocultured with B cells derived from breast tumor draining lymph nodes in direct and transwell systems [267]. ASCs from normal donors, not from patients with BC, were able to inhibit proliferation of in direct contact only. However, cancer ASCs induced higher frequency of IL-10 regulatory B cells than normal ASCs did [267]. All available studies recommend that ASCs may have crucial roles in breast tumor growth and progression by inducing regulatory molecules and promoting anti-inflammatory reactions within the tumor microenvironment.

Effect of Miscellaneous Inflammatory Diseases

Other different inflammatory or autoimmune diseases may affect the immunosuppressive phenotype and/or functions of ASCs. Under Th17 polarization conditions, hASCs from healthy donors inhibited the differentiation of CD4+ T cells, from patients with Parkinson's disease, into Th17 cells, however, they induced functional Tregs producing IL-10Such ASC immunosuppressive findings in Parkinson's disease was attributed to the release of LIF by hASCs [271]. In the context of atherosclerosis (ATH) alone or with T2D, ASCs from patients had compromised ability to suppress the proliferation of activated allogeneic CD4 + T and the effect was more profound in the presence of T2D [272].

In the context of sepsis, three monocyte subsets (CD14++CD16+, CD14+CD16++, and CD14++CD16−) were isolated from patients in the early phase of severe sepsis or septic shock [259].The levels of CD14++CD16+ monocytes (pro-inflammatory phenotype) were positively correlated with the disease severity scores. hASCs were able in coculture to switch monocytes from CD14++CD16+ to CD14++CD16−and modulate the production of inflammatory cytokines toward anti-inflammatory phenotype (increased

IL-10 secretion) via PGE2/EP4-dependent mechanism. Additionally, ASCs modified frequenciesof monocyte phenotypes in experimentalsepsis [259]. A research indicates that ASCs, from osteoporotic donors, exhibit superior anti-inflammatory effects, over osteoporotic BMSCs in vitro and in a model of osteoporosis due to their potential to maintain stemness, energy metabolism and anti-oxidative capacity [270].

Conclusion & Perspectives

In the inflammatory microenvironment, the immunoregulatory potential of MSCs is susceptible to various factors, which leads them either to promote or reduce inflammation. From clinical perspectives, numerous recent review articles presented the effective potential of hASCs in treating of autoimmune/inflammatory or degenerative disorders [1, 4, 19, 275, 276]. Due to their great contribution to hASC therapeutic effectiveness, we discussed the experimental and the donor-related parameters that may affect hASC immunomodulatory functions in vitro. However, contradictory results about the adjustments of most of those determinants have been reported.

It is commonly thought that the younger the donor is, the less the passage of MSCs, the higher their stemness. From multiomics perspective, it has been found that even in ASCs from an elderly subject (≥ 60 years), when the round of cell passages is early, the stemness is high, indicating that ASC passage has a greater impact on the stemness and characteristics of hASCs than donor age [222]. Regarding the experimental setting, direct contact with properly activated immune cells at high cell ratio may ensure effective immunosuppressive potential of hASCs. Regulatory-complaint xeno-free media are promising alternatives to preserve or even enhance the ASC immunomodulatory functions in vitro [67, 173]. However, comparative standardized research focus on profiling the changes in ASC immunomodulation in association with different culture medium compositions, is still needed for efficient FBS alternatives development and safe clinical translation.

ASC priming strategies to enhance their immunosuppressive efficacy are diverse and IFN-ɣ treatment of hASC spheroids seems to be a promising approach. Noteworthy, IFN-ɣ stimulation increased IDO expression noticeably in ASCs over BMSCs and umbilical cord blood-MSCs, however, it did not increase ASC immunosuppressive functions [22]. The latter findings might recommend assessing the IDO activity instead of expression to predict the ASC line immunosuppressive functions. Other licensing conditions can also be applied such as hypoxia, TLR stimulation and pharmacological manipulation. More attention needs to be paid to the affected molecular and signaling mechanisms, in primed ASCs, to personalize the therapeutic outcome

and improve the ASC dysfunctionality in disease. Better knowledge of the differences b/w the immunomodulatory potentials of ASCs derived from various fat depots would be of great interest for a better source selection for ASC immunotherapeutic targets. As well, the effect of physical cues such as substrate stiffness and fluid forces in 3D platform on the immunomodulatory capacity of ASCs requires further research in the near future [200].

In the context of the impact of pathological conditions on the immunomodulatory properties of hASCs, limited ability to use hASCs from patients with obesity, IBD and/or cancer in an autologous ASC based therapy is recommended. That may be due to the repeatedly reported blunted immunosuppressive capacity of ASCs from those patients. In the context of other inflammatory and autoimmune diseases, such as RD, osteoporosis, and diabetes mellitus, contradictory results from being unaffected to slightly or severely affected ASC immunoregulatory capacity from patients with one of these diseases. To minimize discordant outcomes, the future studies need to take into account stratifying patients depending on patient-related parameters (such as age, body mass index, and genetic background) and disease-related parameters (such as the exact pathogenic cause, disease stage, duration). Importantly, the contradictory results can also be attributed to lack of standardized MSC immunopotency assays on both the whole PBMCs and a purified disease-relevant immune cell type [277]. Importantly, transciptomic and proteomic analyses of ASC/ immune cell cocultures with different incubation times will improve our understanding and knowledge for ASC immunomodulatory effects in vitro and factors mediating their actions over time in culture. Such recommended research would help to get data that can be systemically compared from different laboratories to draw solid conclusions.

ASCs may exert immunosuppressive potential in vitro; however, the real functionality should be ascertained in a disease model due to the significant differences b/w in vitro and in vivo conditions. Thus, there remains a need for complementary preclinical then clinical studies to identify different conditions affecting ASC immunomodulatory effects in vivo. In summary, the hASC immunomodulatory capacities in vitro widely vary depending on experimental setup, as well as donor-related factors. The above-mentioned microenvironemental determinants are recommended to be standardized by experts in the field to establish an effective hASCs immunosuppressive coculture setting.

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Declarations

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References

1. Bunnell, B. A. (2021). Adipose tissue-derived mesenchymal stem cells. *Cells, 10*, 3433.
2. Bourin, P., Bunnell, B. A., Casteilla, L., Dominici, M., Katz, A. J., March, K. L., et al. (2013). Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: A joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy, 15*, 641–648.
3. Dubey, N. K., Mishra, V. K., Dubey, R., Deng, Y.-H., Tsai, F.-C., & Deng, W.-P. (2018). Revisiting the advances in isolation, characterization and secretome of adipose-derived stromal/stem cells. *International Journal of Molecular Sciences, 19*, 2200.
4. Patrikoski, M., Mannerström, B., & Miettinen, S. (2019). Perspectives for clinical translation of adipose stromal/stem cells. *Stem Cells International*, 1–21.
5. Burrow, K. L., Hoyland, J. A., & Richardson, S. M. (2017). Human adipose-derived stem cells exhibit enhanced proliferative capacity and retain multipotency longer than donor-matched bone marrow mesenchymal stem cells during expansion in vitro. S*tem Cells Int, 2541275.* https://doi.org/10.1155/2017/2541275
6. Melief, S. M., Zwaginga, J. J., Fibbe, W. E., & Roelofs, H. (2013). Adipose tissue-derived multipotent stromal cells have a higher immunomodulatory capacity than their bone marrow-derived counterparts. *Stem Cells Translational Medicine, 2*, 455–463.
7. Wu, H., Li, J., Xie, B., Tian, H., Fang, S., Jiang, S., et al. (2018). Lower senescence of adipose-derived stem cells than donor-matched bone marrow stem cells for surgical ventricular restoration. *Stem Cells and Development, 27*, 612–623.
8. Al-Ghadban, S., Artiles, M., & Bunnell, B. A. (2022). Adipose stem cells in regenerative medicine: Looking forward. *Frontiers in Bioengineering and Biotechnology, 9*, 837464.
9. Pinheiro-Machado, E., Getova, V. E., Harmsen, M. C., Burgess, J. K., & Smink, A. M. (2023). Towards standardization of human adipose-derived stromal cells secretomes. *Stem Cell Reviews and Reports, 19*(7), 2131–2140. https://doi.org/10.1007/s12015-023-10567-5. [cited 2023 Jul 3].
10. McIntosh, K., Zvonic, S., Garrett, S., Mitchell, J. B., Floyd, Z. E., Hammill, L., et al. (2006). The immunogenicity of human adipose-derived cells: Temporal changes in vitro. *STEM CELLS, 24*, 1246–1253.
11. Cui, L., Yin, S., Liu, W., Li, N., Zhang, W., & Cao, Y. (2007). Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E2. *Tissue Engineering, 13*, 1185–1195.
12. Menard, C., Pacelli, L., Bassi, G., Dulong, J., Bifari, F., Bezier, I., et al. (2013). Clinical-grade mesenchymal stromal cells produced under various good manufacturing practice processes differ in their immunomodulatory properties: Standardization of immune quality controls. *Stem Cells and Development, 22*, 1789–1801.
13. Buyl, K., Merimi, M., Rodrigues, R. M., Moussa Agha, D., Melki, R., Vanhaecke, T., et al. (2020). The impact of cell-expansion and inflammation on the immune-biology of human adipose tissue-derived mesenchymal stromal cells. *Journal of Clinical Medicine, 9*, 696.
14. Crop, M. J., Baan, C. C., Korevaar, S. S., IJzermans, J. N. M., Pescatori, M., Stubbs, A. P., et al. (2010). Inflammatory conditions affect gene expression and function of human adipose tissue-derived mesenchymal stem cells. *Clinical and Experimental Immunology, 162*, 474–86.
15. Frazier, T. P., Gimble, J. M., Kheterpal, I., & Rowan, B. G. (2013). Impact of low oxygen on the secretome of human adipose-derived stromal/stem cell primary cultures. *Biochimie, 95*, 2286–2296.
16. Roemeling-van Rhijn, M., Reinders, M. E., Franquesa, M., Engela, A. U., Korevaar, S. S., Roelofs, H., Genever, P. G., Ijzermans, J. N., Betjes, M. G., Baan, C. C., Weimar, W., & Hoogduijn, M. J. (2013). Human alogeneic bone marrow and adipose tissue derived mesenchymal stromal cells induce CD8+ Cytotoxic T cell reactivity. *J Stem Cell Res Ther, 3*(Suppl 6), 004. https://doi.org/10.4172/2157-7633.S6-004
17. Chang, S.-H., Kim, H. J., & Park, C.-G. (2020). Allogeneic ADSCs induce the production of alloreactive memory-CD8 T cells through HLA-ABC antigens. *Cells, 9*, 1246.
18. Ankrum, J. A., Ong, J. F., & Karp, J. M. (2014). Mesenchymal stem cells: Immune evasive, not immune privileged. *Nature Biotechnology, 32*, 252–260.
19. Ceccarelli, S., Pontecorvi, P., Anastasiadou, E., Napoli, C., & Marchese, C. (2020). Immunomodulatory effect of adipose-derived stem cells: The cutting edge of clinical application. *Frontiers in Cell and Developmental Biology, 8*, 236.
20. Pérez, L. M., de Lucas, B., Lunyak, V. V., & Gálvez, B. G. (2017). Adipose stem cells from obese patients show specific differences in the metabolic regulators vitamin D and Gas5. *Molecular Genetics and Metabolism Reports., 12*, 51–56.
21. Fiori, A., Uhlig, S., Klüter, H., & Bieback, K. (2021). Human adipose tissue-derived mesenchymal stromal cells inhibit CD4+ T cell proliferation and induce regulatory T cells as well as CD127 expression on CD4+CD25+ T cells. *Cells, 10*, 58.
22. Torres Crigna, A., Uhlig, S., Elvers-Hornung, S., Klüter, H., & Bieback, K. (2020). Human adipose tissue-derived stromal cells suppress human, but not murine lymphocyte proliferation, via indoleamine 2,3-dioxygenase activity. *Cells, 9*, 2419.
23. DelaRosa, O., Lombardo, E., Beraza, A., Mancheño-Corvo, P., Ramirez, C., Menta, R., et al. (2009). Requirement of

IFN-γ–mediated indoleamine 2,3-dioxygenase expression in the modulation of lymphocyte proliferation by human adipose-derived stem cells. *Tissue Engineering Part A, 15*, 2795–2806.

24. Najar, M., Raicevic, G., Boufker, H. I., Kazan, H. F., Bruyn, C. D., Meuleman, N., et al. (2010). Mesenchymal stromal cells use PGE2 to modulate activation and proliferation of lymphocyte subsets: Combined comparison of adipose tissue, Wharton's Jelly and bone marrow sources. *Cellular Immunology, 264*, 171–179.

25. Najar, M., Raicevic, G., Boufker, H. I., Fayyad-Kazan, H., De Bruyn, C., Meuleman, N., et al. (2010). Adipose-tissue-derived and Wharton's Jelly-derived mesenchymal stromal cells suppress lymphocyte responses by secreting leukemia inhibitory factor. *Tissue Engineering Part A, 16*, 3537–3546.

26. Song, W.-J., Li, Q., Ryu, M.-O., Ahn, J.-O., Ha Bhang, D., Chan Jung, Y., et al. (2017). TSG-6 secreted by human adipose tissue-derived mesenchymal stem cells ameliorates DSS-induced colitis by inducing M2 macrophage polarization in mice. *Scientific Reports, 7*, 1–14.

27. Rodriguez, L. A., Mohammadipoor, A., Alvarado, L., Kamucheka, R. M., Asher, A. M., Cancio, L. C., et al. (2019). Preconditioning in an inflammatory milieu augments the immunotherapeutic function of mesenchymal stromal cells. *Cells, 8*, 462.

28. Huang, Y., Wu, Q., & Tam, P. K. H. (2022). Immunomodulatory mechanisms of mesenchymal stem cells and their potential clinical applications. *International Journal of Molecular Sciences., 23*, 10023.

29. Ivanova-Todorova, E., Bochev, I., Dimitrov, R., Belemezova, K., Mourdjeva, M., Kyurkchiev, S., Kinov, P., Altankova, I., & Kyurkchiev, D. (2012). Conditioned medium from adipose tissue-derived mesenchymal stem cells induces CD4+FOXP3+ cells and increases IL-10 secretion. *J Biomed Biotechnol*, 295167. https://doi.org/10.1155/2012/295167

30. Engela, A. U., Hoogduijn, M. J., Boer, K., Litjens, N. H. R., Betjes, M. G. H., Weimar, W., et al. (2013). Human adipose-tissue derived mesenchymal stem cells induce functional *de-novo* regulatory T cells with methylated FOXP3 gene DNA. *Clinical and Experimental Immunology, 173*, 343–354.

31. Shi, D., Liao, L., Zhang, B., Liu, R., Dou, X., Li, J., et al. (2011). Human adipose tissue–derived mesenchymal stem cells facilitate the immunosuppressive effect of cyclosporin A on T lymphocytes through Jagged-1–mediated inhibition of NF-κB signaling. *Experimental Hematology, 39*, 214-224.e1.

32. Rubtsov, Y., Goryunov, K, Romanov, A., Suzdaltseva, Y., Sharonov, G., & Tkachuk, V. (2017). Molecular mechanisms of immunomodulation properties of mesenchymal stromal cells: A new insight into the role of ICAM-1. *Stem Cells International, 2017*, 1–15.

33. Zhou, K., Guo, S., Tong, S., Sun, Q., Li, F., Zhang, X., et al. (2018). Immunosuppression of human adipose-derived stem cells on T cell subsets via the reduction of NF-kappaB activation mediated by PD-L1/PD-1 and Gal-9/TIM-3 pathways. *Stem Cells and Development., 27*, 1191–1202.

34. Franquesa, M., Mensah, F. K., Huizinga, R., Strini, T., Boon, L., Lombardo, E., et al. (2015). Human adipose tissue-derived mesenchymal stem cells abrogate plasmablast formation and induce regulatory B cells independently of T helper cells. *Stem Cells., 33*, 880–891.

35. Ivanova-Todorova, E., Bochev, I., Mourdjeva, M., Dimitrov, R., Bukarev, D., Kyurkchiev, S., et al. (2009). Adipose tissue-derived mesenchymal stem cells are more potent suppressors of dendritic cells differentiation compared to bone marrow-derived mesenchymal stem cells. *Immunology Letters, 126*, 37–42.

36. DelaRosa, O., Sánchez-Correa, B., Morgado, S., Ramírez, C., del Río, B., Menta, R., et al. (2012). Human adipose-derived stem cells impair natural killer cell function and exhibit low

37. susceptibility to natural killer-mediated lysis. *Stem Cells and Development, 21*, 1333–1343.

37. Ribeiro, A., Laranjeira, P., Mendes, S., Velada, I., Leite, C., Andrade, P., et al. (2013). Mesenchymal stem cells from umbilical cord matrix, adipose tissue and bone marrow exhibit different capability to suppress peripheral blood B, natural killer and T cells. *Stem Cell Research & Therapy, 4*, 125.

38. Wang, Z., Li, S., Wang, Y., Zhang, X., Chen, L., & Sun, D. (2019). GDNF enhances the anti-inflammatory effect of human adipose-derived mesenchymal stem cell-based therapy in renal interstitial fibrosis. *Stem Cell Research, 41*, 101605.

39. Zhu, X., Klomjit, N., Conley, S. M., Ostlie, M. M., Jordan, K. L., Lerman, A., et al. (2021). Impaired immunomodulatory capacity in adipose tissue-derived mesenchymal stem/stromal cells isolated from obese patients. *Journal of Cellular and Molecular Medicine, 25*, 9051–9059.

40. Giacomini, C., Granéli, C., Hicks, R., & Dazzi, F. (2023). The critical role of apoptosis in mesenchymal stromal cell therapeutics and implications in homeostasis and normal tissue repair. *Cellular & Molecular Immunology, 20*, 570–582.

41. Nasef, A., Chapel, A., & Fouillard, L. (2020). Effect of culture environement on mesenchymal stem cell immunomodulatory ability. *Studies on Stem Cells Research and Therapy, 6*, 009–015.

42. Leto Barone, A. A., Khalifian, S., Lee, W. P., & Brandacher, G. (2013). Immunomodulatory effects of adipose-derived stem cells: Fact or fiction? *BioMed Research International, 2013*, 383685.

43. Rossignol, J., Boyer, C., Thinard, R., Remy, S., Dugast, A., Dubayle, D., et al. (2009). Mesenchymal stem cells induce a weak immune response in the rat striatum after Allo or xenotransplantation. *J Cellular Molecular Medi., 13*, 2547–2558.

44. Griffin, M. D., Ryan, A. E., Alagesan, S., Lohan, P., Treacy, O., & Ritter, T. (2013). Anti-donor immune responses elicited by allogeneic mesenchymal stem cells: What have we learned so far? *Immunology and Cell Biology, 91*, 40–51.

45. Gornostaeva, A., Andreeva, E., & Buravkova, L. (2016). Factors governing the immunosuppressive effects of multipotent mesenchymal stromal cells in vitro. *Cytotechnology, 68*, 565–577.

46. Kuca-Warnawin, E., Plebańczyk, M., Bonek, K., & Kontny, E. (2021). Inhibition of allogeneic and autologous T cell proliferation by adipose-derived mesenchymal stem cells of ankylosing spondylitis patients. *Stem Cells International, 2021*, 1–17. Frith J, editor.

47. Kuca-Warnawin, E., Janicka, I., Szczęsny, P., Olesińska, M., Bonek, K., Głuszko, P., et al. (2020). Modulation of T-cell activation markers expression by the adipose tissue-derived mesenchymal stem cells of patients with rheumatic diseases. *Cell Transplantation, 29*, 096368972094568.

48. Elshahat, M. M., Abu-Shahba, N. M., Nour El-Din, G. M., Ghorab, R. M., El-Erian, A. M., Amr, K. S., et al. (2023). Immunomodulatory properties of human adipose mesenchymal stromal/stem cell in type 2 diabetes milieu. *Azhar International Journal of Pharmaceutical and Medical Sciences., 3*, 144–155.

49. Thakkar, U. G., Trivedi, H. L., Vanikar, A. V., & Dave, S. D. (2015). Insulin-secreting adipose-derived mesenchymal stromal cells with bone marrow–derived hematopoietic stem cells from autologous and allogenic sources for type 1 diabetes mellitus. *Cytotherapy, 17*, 940–947.

50. Li, C., Zhao, H., Cheng, L., & Wang, B. (2021). Allogeneic vs. autologous mesenchymal stem/stromal cells in their medication practice. *Cell & Bioscience, 11*, 187.

51. Puissant, B., Barreau, C., Bourin, P., Clavel, C., Corre, J., Bousquet, C., et al. (2005). Immunomodulatory effect of human adipose tissue-derived adult stem cells: Comparison with bone marrow mesenchymal stem cells. *British Journal of Haematology, 129*, 118–129.

52. Wolbank, S., Peterbauer, A., Fahrner, M., Hennerbichler, S., Van Griensven, M., Stadler, G., et al. (2007). Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: A comparison with human mesenchymal stem cells from adipose tissue. *Tissue Engineering, 13*, 1173–1183.

53. Patrikoski, M., Sivula, J., Huhtala, H., Helminen, M., Salo, F., Mannerström, B., et al. (2014). Different culture conditions modulate the immunological properties of adipose stem cells. *Stem Cells Translational Medicine, 3*, 1220–1230.

54. Pourgholaminejad, A., Aghdami, N., Baharvand, H., & Moazzeni, S. M. (2016). The effect of pro-inflammatory cytokines on immunophenotype, differentiation capacity and immunomodulatory functions of human mesenchymal stem cells. *Cytokine, 85*, 51–60.

55. Ayatollahi, M., Talaei-Khozani, T., & Razmkhah, M. (2016). Growth suppression effect of human mesenchymal stem cells from bone marrow, adipose tissue, and Wharton's jelly of umbilical cord on PBMCs. *Iranian Journal of Basic Medical Sciences, 19*, 145.

56. Quaedackers, M. E., Baan, C. C., Weimar, W., & Hoogduijn, M. J. (2009). Cell contact interaction between adipose-derived stromal cells and Allo-activated T lymphocytes. *European Journal of Immunology, 39*, 3436–3446.

57. Liu, W., Putnam, A. L., Xu-Yu, Z., Szot, G. L., Lee, M. R., Zhu, S., et al. (2006). CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. *The Journal of experimental medicine., 203*, 1701–1711.

58. Gornostaeva, A. N., Bobyleva, P. I., Andreeva, E. R., Yakubets, D. A., & Buravkova, L. B. (2020). Adipose-derived stromal cell immunosuppression of T cells is enhanced under "physiological" hypoxia. *Tissue and Cell, 63*, 101320.

59. Toia, F., Lo Presti, E., Di Stefano, A. B., Di Simone, M., Trapani, M., Corsale, A. M., et al. (2023). An analysis of the immunomodulatory properties of human spheroids from adipose-derived stem cells. *Life Sciences, 321*, 121610.

60. Yañez, R., Lamana, M. L., García-Castro, J., Colmenero, I., Ramírez, M., & Bueren, J. A. (2006). Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. *Stem Cells, 24*, 2582–2591.

61. Kronsteiner, B., Wolbank, S., Peterbauer, A., Hackl, C., Redl, H., van Griensven, M., et al. (2011). Human mesenchymal stem cells from adipose tissue and amnion influence T-cells depending on stimulation method and presence of other immune cells. *Stem Cells and Development, 20*, 2115–2126.

62. Anderson, P., Gonzalez-Rey, E., O'Valle, F., Martin, F., Oliver, F. J., & Delgado, M. (2017). Allogeneic adipose-derived mesenchymal stromal cells ameliorate experimental autoimmune encephalomyelitis by regulating self-reactive T cell responses and dendritic cell function. *Stem Cells International, 2017*, 1–15.

63. Xishan, Z., Baoxin, H., Xinna, Z., & Jun, R. (2013). Comparison of the effects of human adipose and bone marrow mesenchymal stem cells on T lymphocytes. *Cell Biology International, 37*, 11–18.

64. Valencia, J., Blanco, B., Yáñez, R., Vázquez, M., Sánchez, C. H., Fernández-García, M., et al. (2016). Comparative analysis of the immunomodulatory capacities of human bone marrow–and adipose tissue–derived mesenchymal stromal cells from the same donor. *Cytotherapy, 18*, 1297–1311.

65. Waldner, M., Zhang, W., James, I. B., Allbright, K., Havis, E., Bliley, J. M., et al. (2018). Characteristics and immunomodulating functions of adipose-derived and bone marrow-derived mesenchymal stem cells across defined human leukocyte antigen barriers. *Frontiers in Immunology, 9*, 1642.

66. Özdemir, R. B. Ö., Özdemir, A. T., Sarıboyacı, A. E., Uysal, O., Tuğlu, M. İ, & Kırmaz, C. (2019). The investigation of immunomodulatory effects of adipose tissue mesenchymal stem cell educated macrophages on the CD4 T cells. *Immunobiology, 224*, 585–594.

67. Mahmoud, M., Juntunen, M., Adnan, A., Kummola, L., Junttila, I. S., Kelloniemi, M., et al. (2023). Immunomodulatory functions of adipose mesenchymal stromal/stem cell derived from donors with type 2 diabetes and obesity on CD4 T cells. *Stem Cells, 41*, 505–519.

68. Buravkova, L. B., Grigorieva, O. G., Andreeva, E. R., Andrianova, I. V., & Rylova, Yu. V. (2011). Subpopulation composition and activation of T lymphocytes during coculturing with mesenchymal stromal cells in medium with different O2 content. *Bulletin of Experimental Biology and Medicine, 151*, 344–6.

69. Gornostaeva, A. N., Andreeva, E. R., Andrianova, I. V., & Buravkova, L. B. (2011). Immunosuppressive effects of multipotent mesenchymal stromal cells in cultures with different O2 content in the medium. *Bulletin of Experimental Biology and Medicine, 151*, 526–529.

70. Gornostaeva, A. N., Andreeva, E. R., & Buravkova, L. B. (2013). Human MMSC immunosuppressive activity at low oxygen tension: Direct cell-to-cell contacts and paracrine regulation. *Human Physiology, 39*, 136–146.

71. Roemeling-van Rhijn, M., Mensah, F. K. F., Korevaar, S. S., Leijs, M. J., van Osch, G. J. V. M., IJzermans, J. N. M., et al. (2013). Effects of Hypoxia on the Immunomodulatory Properties of Adipose Tissue-Derived Mesenchymal Stem cells. *Frontiers in Immunology, 4*. [cited 2023 Jan 8]. http://journal.frontiersin.org/article/10.3389/fimmu.2013.00203/abstract

72. Bobyleva, P. I., Andreeva, E. R., Gornostaeva, A. N., & Buravkova, L. B. (2016). Tissue-related hypoxia attenuates proinflammatory effects of allogeneic PBMCs on adipose-derived stromal cells *In Vitro*. *Stem Cells International, 2016*, 1–13.

73. Gornostaeva, A. N., Andreeva, E. R., Bobyleva, P. I., & Buravkova, L. B. (2018). Interaction of allogeneic adipose tissue-derived stromal cells and unstimulated immune cells in vitro: The impact of cell-to-cell contact and hypoxia in the local milieu. *Cytotechnology, 70*, 299–312.

74. Cheng, T., Shen, H., Rodrigues, N., Stier, S., & Scadden, D. T. (2001). Transforming growth factor β1 mediates cell-cycle arrest of primitive hematopoietic cells independent of p21Cip1/Waf1or p27Kip1. *Blood, 98*, 3643–3649.

75. Augello, A., Tasso, R., Negrini, S., Amateis, A., Indiveri, F., Cancedda, R., et al. (2005). Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. *European Journal of Immunology, 35*, 1482–1490.

76. Waterman, R. S., Tomchuck, S. L., Henkle, S. L., & Betancourt, A. M. (2010). A New Mesenchymal Stem Cell (MSC) paradigm: Polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. *PLoS ONE, 5*, e10088. Unutmaz D, editor.

77. Pestel, J., Blangero, F., & Eljaafari, A. (2023). Pathogenic role of adipose tissue-derived mesenchymal stem cells in obesity and obesity-related inflammatory diseases. *Cells, 12*, 348.

78. Mancheño-Corvo, P., Menta, R., del Río, B., Franquesa, M., Ramírez, C., Hoogduijn, M. J., et al. (2015). T lymphocyte prestimulation impairs in a time-dependent manner the capacity of adipose mesenchymal stem cells to inhibit proliferation: Role of interferon γ, poly I:C, and tryptophan metabolism in restoring adipose mesenchymal stem cell inhibitory effect. *Stem Cells and Development., 24*, 2158–2170.

79. Niemeyer, P., Kornacker, M., Mehlhorn, A., Seckinger, A., Vohrer, J., Schmal, H., et al. (2007). Comparison of immunological

properties of bone marrow stromal cells and adipose tissue-derived stem cells before and after osteogenic differentiation *In Vitro*. *Tissue Engineering, 13*, 111–121.

80. Bochev, I., Elmadjian, G., Kyurkchiev, D., Tzvetanov, L., Altankova, I., Tivchev, P., et al. (2008). Mesenchymal stem cells from human bone marrow or adipose tissue differently modulate mitogen-stimulated B-cell immunoglobulin production in vitro. *Cell Biology International, 32*, 384–393.

81. Yoo, K. H., Jang, I. K., Lee, M. W., Kim, H. E., Yang, M. S., Eom, Y., et al. (2009). Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. *Cellular Immunology, 259*, 150–156.

82. Yañez, R., Oviedo, A., Aldea, M., Bueren, J. A., & Lamana, M. L. (2010). Prostaglandin E2 plays a key role in the immunosuppressive properties of adipose and bone marrow tissue-derived mesenchymal stromal cells. *Experimental Cell Research, 316*, 3109–3123.

83. Najar, M., Raicevic, G., Fayyad-Kazan, H., De Bruyn, C., Bron, D., Toungouz, M., et al. (2013). Impact of different mesenchymal stromal cell types on T-cell activation, proliferation and migration. *International Immunopharmacology, 15*, 693–702.

84. Jang, I. K., Yoon, H. H., Yang, M. S., Lee, J. E., Lee, D.-H., Lee, M. W., et al. (2014). B7–H1 inhibits T cell proliferation through MHC class II in human mesenchymal stem cells. *Transplantation Proceedings, 46*, 1638–1641.

85. Kim, D. S., Jang, I. K., Lee, M. W., Ko, Y. J., Lee, D.-H., Lee, J. W., et al. (2018). Enhanced immunosuppressive properties of human mesenchymal stem cells primed by interferon-γ. *eBioMedicine, 28*, 261–273.

86. Regmi, S., Pathak, S., Thanh, T. P., Nguyen, T. T., Sung, J.-H., Yook, S., et al. (2019). Intraportally delivered stem cell spheroids localize in the liver and protect hepatocytes against GALN/LPS-induced fulminant hepatic toxicity. *Stem Cell Research & Therapy, 10*, 230.

87. Tran, T. D. X., Pham, V. Q., Tran, N.N.-T., Dang, H. C. N., Tran, N. T. A., Vu, N. B., et al. (2022). *Stromal vascular fraction and mesenchymal stem cells from human adipose tissue: A comparison of immune modulation and angiogenic potential*. Cham: Springer International Publishing. https://doi.org/10.1007/5584_2022_708 [cited 2023 Jan 8].

88. Wolbank, S., Stadler, G., Peterbauer, A., Gillich, A., Karbiener, M., Streubel, B., et al. (2009). Telomerase immortalized human amnion-and adipose-derived mesenchymal stem cells: Maintenance of differentiation and immunomodulatory characteristics. *Tissue Engineering Part A, 15*, 1843–1854.

89. Perea-Gil, I., Monguió-Tortajada, M., Gálvez-Montón, C., Bayes-Genis, A., Borràs, F. E., & Roura, S. (2015). Preclinical evaluation of the immunomodulatory properties of cardiac adipose tissue progenitor cells using umbilical cord blood mesenchymal stem cells: a direct comparative study. *BioMed Research International, 2015*, 1–9.

90. Menta, R., Mancheño-Corvo, P., Del Río, B., Ramírez, C., DelaRosa, O., Dalemans, W., et al. (2014). Tryptophan concentration is the main mediator of the capacity of adipose mesenchymal stromal cells to inhibit T-lymphocyte proliferation in vitro. *Cytotherapy, 16*, 1679–1691.

91. Matula, Z., Németh, A., Lőrincz, P., Szepesi, Á., Brózik, A., Buzás, E. I., et al. (2016). The role of extracellular vesicle and tunneling nanotube-mediated intercellular cross-talk between mesenchymal stem cells and human peripheral T cells. *Stem Cells and Development, 25*, 1818–1832.

92. Dang, S., Yu, Z., Zhang, C., Zheng, J., Li, K., Wu, Y., et al. (2015). Autophagy promotes apoptosis of mesenchymal stem cells under inflammatory microenvironment. *Stem Cell Research & Therapy, 6*, 247.

93. Najar, M., Lombard, C. A., Fayyad-Kazan, H., Faour, W. H., Merimi, M., Sokal, E. M., et al. (2019). Th17 immune response to adipose tissue-derived mesenchymal stromal cells. *Journal Cellular Physiology., 234*, 21145–21152.

94. Roberts, C. A., Dickinson, A. K., & Taams, L. S. (2015) The interplay between monocytes/macrophages and CD4+ T cell subsets in rheumatoid arthritis. *Frontiers in Immunology, 6*. [cited 2023 Jan 8]. http://journal.frontiersin.org/Article/10.3389/fimmu.2015.00571/abstract

95. Karaöz, E., Çetinalp Demircan, P., Erman, G., Güngörürler, E., & Eker Sarıboyacı, A. (2016). Comparative analyses of immune-suppressive characteristics of bone-marrow, Wharton's Jelly a nd adipose-tissue derived human MSCs. *Turkish Journal of Hematology*. [cited 2023 Jan 8]. http://www.tjh.com.tr/jvi.aspx?pdir=tjh&plng=eng&un=TJH-23911&look4=

96. Kuca-Warnawin, E., Olesińska, M., Szczęsny, P., & Kontny, E. (2022). Impact and possible mechanism(s) of adipose tissue-derived mesenchymal stem cells on T-cell proliferation in patients with rheumatic disease. *Frontiers in Physiology, 12*, 749481.

97. Cuerquis, J., Romieu-Mourez, R., François, M., Routy, J.-P., Young, Y. K., Zhao, J., et al. (2014). Human mesenchymal stromal cells transiently increase cytokine production by activated T cells before suppressing T-cell proliferation: Effect of interferon-γ and tumor necrosis factor-α stimulation. *Cytotherapy, 16*, 191–202.

98. Wang, Y., Chen, X., Cao, W., & Shi, Y. (2014). Plasticity of mesenchymal stem cells in immunomodulation: Pathological and therapeutic implications. *Nature Immunology, 15*, 1009–1016.

99. Bobyleva, P., Gornostaeva, A., Andreeva, E., Ezdakova, M., Gogiya, B., & Buravkova, L. (2019). Reciprocal modulation of cell functions upon direct interaction of adipose mesenchymal stromal and activated immune cells. *Cell Biochemistry and Function, 37*, 228–238.

100. Ceruso, A., Gonzalez-Pujana, A., Igartua, M., Santos-Vizcaino, E., & Hernandez, R. M. (2021). Latest advances to enhance the therapeutic potential of mesenchymal stromal cells for the treatment of immune-mediated diseases. *Drug Delivery and Translational Research, 11*, 498–514.

101. Seo, Y., Shin, T.-H., & Kim, H.-S. (2019). Current strategies to enhance adipose stem cell function: An update. *IJMS., 20*, 3827.

102. Lee, M. J., Kim, J., Kim, M. Y., Bae, Y.-S., Ryu, S. H., Lee, T. G., et al. (2010). Proteomic analysis of tumor necrosis factor-α-induced secretome of human adipose tissue-derived mesenchymal stem cells. *Journal of Proteome Research, 9*, 1754–1762.

103. Najar, M., Raicevic, G., Kazan, H. F., De Bruyn, C., Bron, D., Toungouz, M., et al. (2012). Immune-related antigens, surface molecules and regulatory factors in human-derived mesenchymal stromal cells: The expression and impact of inflammatory priming. *Stem Cell Reviews and Reports, 8*, 1188–1198.

104. Khairoun, M., & Korevaar, S. S. (2013). Human bone marrow- and adipose tissue-derived mesenchymal stromal cells are immunosuppressive in vitro and in a humanized allograft rejection model. *Journal of Stem Cell Research & Therapy*. [cited 2023 Jan 12]. https://www.omicsonline.org/human-bone-marrow-adipose-tissue-derived-mesenchymal-stromal-cells-2157-7633.S6-001.php?aid=20780

105. Roemeling-van Rhijn, M., Khairoun, M., Korevaar, S. S., Lievers, E., Leuning, D. G., IJzermans, J. N., et al. (2013). Human bone marrow- and adipose tissue-derived mesenchymal stromal cells are immunosuppressive in vitro and in a humanized allograft rejection model. *Journal of Stem Cell Research & Theraphy, Suppl 6*, 20780.

106. Domenis, R., Cifù, A., Quaglia, S., Pistis, C., Moretti, M., Vicario, A., et al. (2018). Pro inflammatory stimuli enhance the immunosuppressive functions of adipose mesenchymal stem cells-derived exosomes. *Science and Reports, 8*, 13325.

107 Ragni, De Luca, Orfei, P., Colombini, Viganò, Lugano, et al. (2019). Insights into inflammatory priming of adipose-derived mesenchymal stem cells: Validation of extracellular vesicles-embedded miRNA reference genes as a crucial step for donor selection. *Cells, 8*, 369.

108 Serejo, T. R. T., Silva-Carvalho, A. É., Braga, L. D. de C. F., Neves, F. de A. R., Pereira, R. W., de Carvalho, J. L., et al. (2019). Assessment of the immunosuppressive potential of INF-γ licensed adipose mesenchymal stem cells, their secretome and extracellular vesicles. *Cells, 8*, 22.

109 Abu-Shahba, N., Mahmoud, M., El-Erian, A. M., Husseiny, M. I., Nour-Eldeen, G., Helwa, I., et al. (2021). Impact of type 2 diabetes mellitus on the immunoregulatory characteristics of adipose tissue-derived mesenchymal stem cells. *The International Journal of Biochemistry & Cell Biology, 140*, 106072.

110 Mckinnirey, F., Herbert, B., Vesey, G., & McCracken, S. (2021). Immune modulation via adipose derived mesenchymal stem cells is driven by donor sex in vitro. *Science and Reports, 11*, 12454.

111 Ting, H.-K., Chen, C.-L., Meng, E., Cherng, J.-H., Chang, S.-J., Kao, C.-C., et al. (2021). Inflammatory regulation by TNF-α-activated adipose-derived stem cells in the human bladder cancer microenvironment. *International Journal of Molecular Sciences, 22*, 3987.

112 Colombini, A., Libonati, F., Cangelosi, D., Lopa, S., De Luca, P., Coviello, D. A., et al. (2022). Inflammatory priming with IL-1β promotes the immunomodulatory behavior of adipose derived stem cells. *Frontiers in Bioengineering and Biotechnology, 10*, 1000879.

113 Sarsenova, M., Kim, Y., Raziyeva, K., Kazybay, B., Ogay, V., & Saparov, A. (2022). Recent advances to enhance the immunomodulatory potential of mesenchymal stem cells. *Frontiers in Immunology, 13*, 1010399.

114 Yao, M., Chen, Z., He, X., Long, J., Xia, X., Li, Z., et al. (2022). Cross talk between glucose metabolism and immunosuppression in IFN-γ–primed mesenchymal stem cells. *Life Science Alliance, 5*, e202201493.

115 Jitschin, R., Böttcher, M., Saul, D., Lukassen, S., Bruns, H., Loschinski, R., et al. (2019). Inflammation-induced glycolytic switch controls suppressivity of mesenchymal stem cells via STAT1 glycosylation. *Leukemia, 33*, 1783–1796.

116 Fayyad-Kazan, M., Najar, M., Fayyad-Kazan, H., Raicevic, G., & Lagneaux, L. (2017). Identification and evaluation of new immunoregulatory genes in mesenchymal stromal cells of different origins: Comparison of Normal and inflammatory conditions. *Medical Science Monitor Basic Research, 23*, 87–96.

117 Fuentes-Julián, S., Arnalich-Montiel, F., Jaumandreu, L., Leal, M., Casado, A., García-Tuñón, I., et al. (2015). Adipose-derived mesenchymal stem cell administration does not improve corneal graft survival outcome. *PLoS ONE, 10*, e0117945. Beltrami AP, editor.

118 Müller, M., Carter, S., Hofer, M. J., & Campbell, I. L. (2010). Review: The chemokine receptor CXCR3 and its ligands CXCL9, CXCL10 and CXCL11 in neuroimmunity - a tale of conflict and conundrum: CXCR3 and its ligands in CNS inflammation. *Neuropathology and Applied Neurobiology, 36*, 368–387.

119 Yamamoto, M., Ota, A., Hori, T., Imai, S., Sohma, H., Suzuki, N., et al. (2011). Early expression of plasma CCL8 closely correlates with survival rate of acute graft-vs.-host disease in mice. *Experimental Hematology, 39*, 1101–12.

120 Ren, G., Zhang, L., Zhao, X., Xu, G., Zhang, Y., Roberts, A. I., et al. (2008). Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell, 2*, 141–150.

121 Lee, Y., Park, Y. S., Choi, N. Y., Kim, Y. I., & Koh, Y.-G. (2021). Proteomic analysis reveals commonly secreted proteins of mesenchymal stem cells derived from bone marrow, adipose tissue, and synovial membrane to show potential for cartilage regeneration in knee osteoarthritis. *Stem Cells International, 2021*, 1–18.

122 Lombardo, E., DelaRosa, O., Mancheño-Corvo, P., Menta, R., Ramírez, C., & Büscher, D. (2009). Toll-like receptor-mediated signaling in human adipose-derived stem cells: Implications for immunogenicity and immunosuppressive potential. *Tissue Engineering Part A, 15*, 1579–1589.

123 Baer, Koch, Hickmann, Schubert, Cinatl, Hauser, et al. (2019). Isolation, characterization, differentiation and immunomodulatory capacity of mesenchymal stromal/stem cells from human perirenal adipose tissue. *Cells, 8*, 1346.

124 Liu, G.-Y., Liu, Y., Lu, Y., Qin, Y.-R., Di, G.-H., Lei, Y.-H., et al. (2016). Short-term memory of danger signals or environmental stimuli in mesenchymal stem cells: Implications for therapeutic potential. *Cellular & Molecular Immunology, 13*, 369–378.

125 Wu, J., Niu, P., Zhao, Y., Cheng, Y., Chen, W., Lin, L., et al. (2019). Impact of miR-223–3p and miR-2909 on inflammatory factors IL-6, IL-1ß, and TNF-α, and the TLR4/TLR2/NF-κB/STAT3 signaling pathway induced by lipopolysaccharide in human adipose stem cells. *PLoS ONE, 14*, e0212063. Ahmad A, editor.

126 Jafari, M., Asghari, A., Delbandi, A.-A., Jalessi, M., Jazayeri, M. H., Samarei, R., et al. (2020). Priming TLR3 and TLR4 in human adipose- and olfactory mucosa-derived mesenchymal stromal cells and comparison of their cytokine secretions. *Cytotechnology, 72*, 57–68.

127 Yasmeen, R., Pham, Q., Fukagawa, N. K., & Wang, T. T. Y. (2022). Individual variabilities in adipose stem cell proliferation, gene expression and responses to lipopolysaccharide stimulation. *International Journal of Molecular Sciences, 23*, 12534.

128 Bidkhori, H. R., Farshchian, M., Kazemi Noughabi, M., Hassanzadeh, H., & RafatPanah, H. (2023). Alteration of immunoregulatory genes expression in mesenchymal stromal cells upon priming with B18R as an interferon binding protein. *Iranian Journal of Basic Medical Sciences, 26*. [cited 2023 Jul 4]. https://doi.org/10.22038/ijbms.2022.67353.14771

129 Rivera-Cruz, C. M., & Figueiredo, M. L. (2023). Evaluation of human adipose-derived mesenchymal stromal cell Toll-like receptor priming and effects on interaction with prostate cancer cells. *Cytotherapy, 25*, 33–45.

130 Wobma, H. M., Kanai, M., Ma, S. P., Shih, Y., Li, H. W., Duran-Struuck, R., et al. (2018). Dual IFN-γ/hypoxia priming enhances immunosuppression of mesenchymal stromal cells through regulatory proteins and metabolic mechanisms. *Journal of Immunology and Regenerative Medicine, 1*, 45–56.

131 Kurte, M., Vega-Letter, A. M., Luz-Crawford, P., Djouad, F., Noël, D., Khoury, M., et al. (2020). Time-dependent LPS exposure commands MSC immunoplasticity through TLR4 activation leading to opposite therapeutic outcome in EAE. *Stem Cell Research & Therapy, 11*, 416.

132 Zhao, X., Liu, D., Gong, W., Zhao, G., Liu, L., Yang, L., et al. (2014). The Toll-like receptor 3 ligand, Poly(I:C), improves immunosuppressive function and therapeutic effect of mesenchymal stem cells on sepsis via inhibiting MiR-143. *Stem Cells, 32*, 521–533.

133 Ryu, D.-B., Lim, J.-Y., Lee, S.-E., Park, G., & Min, C.-K. (2016). Induction of indoleamine 2,3-dioxygenase by pre-treatment with Poly(I:C) may enhance the efficacy of MSC treatment in DSS-induced colitis. *Immune Network, 16*, 358.

134 Rashedi, I., Gómez-Aristizábal, A., Wang, X.-H., Viswanathan, S., & Keating, A. (2017). TLR3 or TLR4 activation enhances mesenchymal stromal cell-mediated treg induction via notch signaling. *Stem Cells, 35*, 265–275.

135. Kalinina, N., Kharlampieva, D., Loguinova, M., Butenko, I., Pobeguts, O., Efimenko, A., et al. (2015). Characterization of secretomes provides evidence for adipose-derived mesenchymal stromal cells subtypes. *Stem Cell Research & Therapy, 6*, 221.

136. Yousefi, F., Lavi Arab, F., Jaafari, M. R., Rastin, M., Tabasi, N., Hatamipour, M., et al. (2019). Immunoregulatory, proliferative and anti-oxidant effects of nanocurcuminoids on adipose-derived mesenchymal stem cells. *EXCLI Journal, 18*:Doc405; ISSN 1611-2156 [Internet]. [cited 2023 Jan 13]. https://www.excli.de/vol18/Mahmoudi_17062019_proof.pdf

137. Li, C., Huang, J., Zhu, H., Shi, Q., Li, D., & Ju, X. (2019). Pyridoxal-5′-phosphate promotes immunomodulatory function of adipose-derived mesenchymal stem cells through indoleamine 2,3-Dioxygenase-1 and TLR4/NF-κ B pathway. *Stem Cells International, 2019*, 1–15.

138. Teklemariam, T., Purandare, B., Zhao, L., & Hantash, B. M. (2014). Inhibition of DNA methylation enhances HLA-G expression in human mesenchymal stem cells. *Biochemical and Biophysical Research Communications, 452*, 753–759.

139. Oses, C., Olivares, B., Ezquer, M., Acosta, C., Bosch, P., Donoso, M., et al. (2017). Preconditioning of adipose tissue-derived mesenchymal stem cells with deferoxamine increases the production of pro-angiogenic, neuroprotective and anti-inflammatory factors: Potential application in the treatment of diabetic neuropathy. *PLoS ONE, 12*, e0178011. Kirchmair R, editor.

140. Xiuping, Y., Xiaotong, Z., Rongjia, Z., & Ping, S. (2022). Effect of astragaloside IV on the immunoregulatory function of adipose-derived mesenchymal stem cells from patients with psoriasis vulgaris. *Journal of Traditional Chinese Medicine, 42*, 513.

141. Kazemi, R., Mohammadi, M., Salimiyan, S., Aliakbari, S., Ahmadi, M., & Rahmani, M. (2023). Long-term effects of low-dose naltrexone on immunomodulatory properties of human adipose-derived mesenchymal stem cells. Iranian Journal of Immunology [Internet]. [cited 2023 Jul 4]. https://doi.org/10.22034/iji.2023.95659.2385

142. Azam, M., Ghufran, H., Tasneem, S., Mehmood, A., Malik, K., Yousaf, M. A., et al. (2023). Priming of adipose-derived stem cells with curcumin prior to cryopreservation preserves their functional potency: Towards an 'off-the-shelf' therapy for burns. *Cryobiology, 110*, 69–78.

143. Kowalczuk, A., Marycz, K., Kornicka-Garbowska, K., Kornicka, J., Bujalska-Zadrożny, M., & Groborz, S. (2022). Cannabidiol (CBD) protects adipose-derived mesenchymal stem cells (ASCs) against endoplasmic reticulum stress development and its complications. *International Journal of Environmental Research and Public Health, 19*, 10864.

144. Kowalczuk, A., Marycz, K., Kornicka, J., Groborz, S., Meissner, J., & Mularczyk, M. (2023). Tetrahydrocannabivarin (THCV) protects adipose-derived mesenchymal stem cells (ASC) against endoplasmic reticulum stress development and reduces inflammation during adipogenesis. *International Journal of Molecular Sciences, 24*, 7120.

145. Payne, N. L., Dantanarayana, A., Sun, G., Moussa, L., Caine, S., McDonald, C., et al. (2012). Early intervention with gene-modified mesenchymal stem cells overexpressing interleukin-4 enhances anti-inflammatory responses and functional recovery in experimental autoimmune demyelination. *Cell Adhesion & Migration, 6*, 179–189.

146. Choi, E. W., Shin, I. S., Song, J. W., Lee, M., Yun, T. W., Yang, J., et al. (2016). Effects of transplantation of CTLA4Ig-overexpressing adipose tissue-derived mesenchymal stem cells in mice with sustained severe rheumatoid arthritis. *Cell Transplantation, 25*, 243–259.

147. Li, R., Wang, R., Zhong, S., Asghar, F., Li, T., Zhu, L., et al. (2021). TGF-β1-overexpressing mesenchymal stem cells reciprocally regulate Th17/Treg cells by regulating the expression of IFN-γ. *Open Life Sciences, 16*, 1193–1202.

148. Yang, H.-M., Sung, J.-H., Choi, Y.-S., Lee, H.-J., Roh, C.-R., Kim, J., et al. (2012). Enhancement of the immunosuppressive effect of human adipose tissue-derived mesenchymal stromal cells through HLA-G1 expression. *Cytotherapy, 14*, 70–79.

149. Tran, T. T., & Kahn, C. R. (2010). Transplantation of adipose tissue and stem cells: Role in metabolism and disease. *Nature Reviews Endocrinology, 6*, 195–213.

150. Pan, Z., Zhou, Z., Zhang, H., Zhao, H., Song, P., Wang, D., et al. (2019). CD90 serves as differential modulator of subcutaneous and visceral adipose-derived stem cells by regulating AKT activation that influences adipose tissue and metabolic homeostasis. *Stem Cell Research & Therapy, 10*, 355.

151. Badimon, L., & Cubedo, J. (2017). Adipose tissue depots and inflammation: Effects on plasticity and resident mesenchymal stem cell function. *Cardiovascular Research, 113*, 1064–1073.

152. Tokunaga, M., Inoue, M., Jiang, Y., Barnes, R. H., Buchner, D. A., & Chun, T.-H. (2014). Fat depot-specific gene signature and ECM remodeling of Sca1high adipose-derived stem cells. *Matrix Biology, 36*, 28–38.

153. Tsekouras, A., Mantas, D., Tsilimigras, D. I., Moris, D., Kontos, M., & Zografos, G. C. (2017). Comparison of the viability and yield of adipose-derived stem cells (ASCs) from different donor areas. *In Vivo, 31*, 1229–34.

154. Abu-Shahba, N., Mahmoud, M., Abdel-Rasheed, M., Darwish, Y., AbdelKhaliq, A., Mohammed, E., et al. (2020). Immunomodulatory and antioxidative potentials of adipose-derived mesenchymal stem cells isolated from breast versus abdominal tissue: A comparative study. *Cell Regeneration, 9*, 1–14.

155. Posada-González, M., Villagrasa, A., García-Arranz, M., Vorwald, P., Olivera, R., Olmedillas-López, S., et al. (2022). Comparative analysis between mesenchymal stem cells from subcutaneous adipose tissue and omentum in three types of patients: cancer, morbid obese and healthy control. *Surgical Innovation, 29*, 9–21.

156. Serena, C., Keiran, N., Ceperuelo-Mallafre, V., Ejarque, M., Fradera, R., Roche, K., et al. (2016). Obesity and type 2 diabetes alters the immune properties of human adipose derived stem cells. *Stem Cells, 34*, 2559–2573.

157. Ishiuchi, N., Nakashima, A., Maeda, S., Miura, Y., Miyasako, K., Sasaki, K., et al. (2023). Comparison of therapeutic effects of mesenchymal stem cells derived from superficial and deep subcutaneous adipose tissues. *Stem Cell Research & Therapy, 14*, 121.

158. Thejaswi, K., Amarnath, M., Srinivas, G., Jerald, M., Raj, T. A., & Singh, S. (2012). Immune modulatory responses of mesenchymal stem cells from different sources in cultures and in vivo. *Cell & Tissue Transplantation & Therapy, 4*, 1–13. https://doi.org/10.4137/CTTT.S9812

159. Montespan, F., Deschaseaux, F., Sensébé, L., Carosella, E. D., & Rouas-Freiss, N. (2014). Osteodifferentiated mesenchymal stem cells from bone marrow and adipose tissue express HLA-G and display immunomodulatory properties in HLA-mismatched settings: Implications in bone repair therapy. *Journal of Immunology Research, 2014*, 1–10.

160. Du, W.-J., Reppel, L., Leger, L., Schenowitz, C., Huselstein, C., Bensoussan, D., et al. (2016). Mesenchymal stem cells derived from human bone marrow and adipose tissue maintain their immunosuppressive properties after chondrogenic differentiation: Role of HLA-G. *Stem Cells and Development, 25*, 1454–1469.

161. Xishan, Z., Bin, Z., Haiyue, Z., Xiaowei, D., Jingwen, B., & Guojun, Z. (2015). Jagged-2 enhances immunomodulatory activity in adipose derived mesenchymal stem cells. *Scientific Reports, 5*, 1–11.

162. Munir, H., Ward, L. S. C., Sheriff, L., Kemble, S., Nayar, S., Barone, F., et al. (2017). Adipogenic differentiation of mesenchymal

stem cells alters their immunomodulatory properties in a tissue-specific manner. *Stem Cells, 35*, 1636–1646.

163. Chehimi, M., Robert, M., Bechwaty, M. E., Vial, G., Rieusset, J., Vidal, H., et al. (2016). Adipocytes, like their progenitors, contribute to inflammation of adipose tissues through promotion of Th-17 cells and activation of monocytes, in obese subjects. *Adipocyte, 5*, 275–282.

164. Ménard, C., & Tarte, K. (2013). Immunoregulatory properties of clinical grade mesenchymal stromal cells: Evidence, uncertainties, and clinical application. *Stem Cell Research & Therapy, 4*, 64.

165. Silva-Carvalho, A. É., Neves, F. A. R., & Saldanha-Araujo, F. (2020). The immunosuppressive mechanisms of mesenchymal stem cells are differentially regulated by platelet poor plasma and fetal bovine serum supplemented media. *International Immunopharmacology, 79*, 106172.

166. Di Stefano, A. B., Grisafi, F., Perez-Alea, M., Castiglia, M., Di Simone, M., Meraviglia, S., et al. (2021). Cell quality evaluation with gene expression analysis of spheroids (3D) and adherent (2D) adipose stem cells. *Gene, 768*, 145269.

167. Purandare, B., Teklemariam, T., Zhao, L., & Hantash, B. M. (2014). Temporal HLA profiling and immunomodulatory effects of human adult bone marrow- and adipose-derived mesenchymal stem cells. *Regenerative Medicine, 9*, 67–79.

168. Wang, X., Liu, C., Li, S., Xu, Y., Chen, P., Liu, Y., et al. (2015). Effects of continuous passage on immunomodulatory properties of human adipose-derived stem cells. *Cell and Tissue Banking, 16*, 143–150.

169. Mun, C. H., Kang, M.-I., Shin, Y. D., Kim, Y., & Park, Y.-B. (2018). The expression of immunomodulation-related cytokines and genes of adipose- and bone marrow-derived human mesenchymal stromal cells from early to late passages. *Tissue Engineering and Regenerative Medicine, 15*, 771–779.

170. Peng, Q., Alipour, H., Porsborg, S., Fink, T., & Zachar, V. (2020). Evolution of ASC immunophenotypical subsets during expansion in vitro. *International Journal of Molecular Sciences, 21*, 1408.

171. Kouroupis, D., & Correa, D. (2021). Increased mesenchymal stem cell functionalization in three-dimensional manufacturing settings for enhanced therapeutic applications. *Frontiers in Bioengineering and Biotechnology, 9*, 621748.

172. Witzeneder, K., Lindenmair, A., Gabriel, C., Höller, K., Theiß, D., Redl, H., et al. (2013). Human-derived alternatives to fetal bovine serum in cell culture. *Transfusion Medicine and Hemotherapy, 40*, 417–423.

173. Kouroupis, D., Bowles, A. C., Best, T. M., Kaplan, L. D., & Correa, D. (2020). CD10/Neprilysin enrichment in infrapatellar fat pad-derived mesenchymal stem cells under regulatory-compliant conditions: Implications for efficient synovitis and fat pad fibrosis reversal. *American Journal of Sports Medicine, 48*, 2013–2027.

174. Escobar, C. H., & Chaparro, O. (2016). Xeno-free extraction, culture, and cryopreservation of human adipose-derived mesenchymal stem cells. *Stem Cells Translational Medicine, 5*, 358–365.

175. Li, C., Wu, X., Tong, J., Yang, X., Zhao, J., Zheng, Q., et al. (2015). Comparative analysis of human mesenchymal stem cells from bone marrow and adipose tissue under xeno-free conditions for cell therapy. *Stem Cell Research & Therapy, 6*, 55.

176. Lee, R. H., Pulin, A. A., Seo, M. J., Kota, D. J., Ylostalo, J., Larson, B. L., et al. (2009). Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell, 5*, 54–63.

177. Cheng, N.-C., Tu, Y.-K., Lee, N.-H., & Young, T.-H. (2020). Influence of human platelet lysate on extracellular matrix deposition and cellular characteristics in adipose-derived stem cell sheets. *Frontiers in Cell and Developmental Biology, 8*, 558354.

178. Follin, B., Juhl, M., Cohen, S., Pedersen, A. E., Kastrup, J., & Ekblond, A. (2016). Increased paracrine immunomodulatory potential of mesenchymal stromal cells in three-dimensional culture. *Tissue Engineering Part B: Reviews, 22*, 322–329.

179. Yen, B. L., Hsieh, C.-C., Hsu, P.-J., Chang, C.-C., Wang, L.-T., & Yen, M.-L. (2023). Three-dimensional spheroid culture of human mesenchymal stem cells: offering therapeutic advantages and in vitro glimpses of the in vivo state. *Stem Cells Translational Medicine, 12*, 235–244.

180. Ezquerra, S., Zuleta, A., Arancibia, R., Estay, J., Aulestia, F., & Carrion, F. (2021). Functional properties of human-derived mesenchymal stem cell spheroids: A meta-analysis and systematic review. *Stem Cells International, 2021*, 1–12. Najimi M, editor.

181. Amos, P. J., Kapur, S. K., Stapor, P. C., Shang, H., Bekiranov, S., Khurgel, M., et al. (2010). Human adipose-derived stromal cells accelerate diabetic wound healing: impact of cell formulation and delivery. *Tissue Engineering Part A, 16*, 1595–1606.

182. Emmert, M. Y., Wolint, P., Winklhofer, S., Stolzmann, P., Cesarovic, N., Fleischmann, T., et al. (2013). Transcatheter based electromechanical mapping guided intramyocardial transplantation and in vivo tracking of human stem cell based three dimensional microtissues in the porcine heart. *Biomaterials, 34*, 2428–2441.

183. Sadeghzadeh, H., Mehdipour, A., Dianat-Moghadam, H., Salehi, R., Khoshfetrat, A. B., Hassani, A., & Mohammadnejad, D. (2022). PCL/COL I-based magnetic nanocomposite scaffold provides an osteoinductive environment for ADSCs in osteogenic cues-free media conditions. *Stem Cell Research & Therapy, 13*(1), 143.

184. Park, I. S., Rhie, J.-W., & Kim, S.-H. (2014). A novel three-dimensional adipose-derived stem cell cluster for vascular regeneration in ischemic tissue. *Cytotherapy, 16*, 508–522.

185. Follin, B., Juhl, M., Cohen, S., Pedersen, A. E., Gad, M., Kastrup, J., et al. (2015). Human adipose-derived stromal cells in a clinically applicable injectable alginate hydrogel: Phenotypic and immunomodulatory evaluation. *Cytotherapy, 17*, 1104–1118.

186. Xu, Y., Shi, T., Xu, A., & Zhang, L. (2016). 3D spheroid culture enhances survival and therapeutic capacities of MSC s injected into ischemic kidney. *Journal of Cellular and Molecular Medicine, 20*, 1203–1213.

187. Da Silva, L. P., Santos, T. C., Rodrigues, D. B., Pirraco, R. P., Cerqueira, M. T., Reis, R. L., et al. (2017). Stem cell-containing hyaluronic acid-based spongy hydrogels for integrated diabetic wound healing. *Journal of Investigative Dermatology, 137*, 1541–1551.

188. Nilforoushzadeh, M. A., Khodadadi Yazdi, M., Baradaran Ghavami, S., Farokhimanesh, S., Mohammadi Amirabad, L., Zarrintaj, P., et al. (2020). Mesenchymal stem cell spheroids embedded in an injectable thermosensitive hydrogel: An in situ drug formation platform for accelerated wound healing. *ACS Biomaterials Science & Engineering, 6*, 5096–5109.

189. Lee, S., Kim, H.-S., Min, B.-H., Kim, B. G., Kim, S. A., Nam, H., et al. (2021). Enhancement of anti-inflammatory and immunomodulatory effects of adipose-derived human mesenchymal stem cells by making uniform spheroid on the new nano-patterned plates. *Biochemical and Biophysical Research Communications, 552*, 164–169.

190. Tu, C.-C., Cheng, N.-C., Yu, J., Pan, Y.-X., Tai, W.-C., Chen, Y.-C., et al. (2023). Adipose-derived stem cell spheroid-laden microbial transglutaminase cross-linked gelatin hydrogel for treating diabetic periodontal wounds and craniofacial defects. *Stem Cell Research & Therapy, 14*, 20.

191. Mineda, K., Feng, J., Ishimine, H., Takada, H., Doi, K., Kuno, S., et al. (2015). Therapeutic potential of human adipose-derived stem/stromal cell microspheroids prepared by three-dimensional culture in non-cross-linked hyaluronic acid gel. *Stem Cells Translational Medicine, 4*, 1511–1522.

192. Bender, R., McCarthy, M., Brown, T., Bukowska, J., Smith, S., Abbott, R. D., et al. (2020). Human adipose derived cells in two- and three-dimensional cultures: functional validation of an in vitro fat construct. *Stem Cells International, 2020*, 1–14.

193. Gonzalez-Pujana, A., Vining, K. H., Zhang, D. K. Y., Santos-Vizcaino, E., Igartua, M., Hernandez, R. M., et al. (2020). Multifunctional biomimetic hydrogel systems to boost the immunomodulatory potential of mesenchymal stromal cells. *Biomaterials, 257*, 120266.

194. García, J. R., Quirós, M., Han, W. M., O'Leary, M. N., Cox, G. N., Nusrat, A., et al. (2019). IFN-γ-tethered hydrogels enhance mesenchymal stem cell-based immunomodulation and promote tissue repair. *Biomaterials, 220*, 119403.

195. Iwazawa, R., Kozakai, S., Kitahashi, T., Nakamura, K., & Hata, K. (2018). The therapeutic effects of adipose-derived stem cells and recombinant peptide pieces on mouse model of DSS colitis. *Cell Transplantation, 27*, 1390–1400.

196. Kim, W.-S., Han, J., Hwang, S.-J., & Sung, J.-H. (2014). An update on niche composition, signaling and functional regulation of the adipose-derived stem cells. *Expert Opinion on Biological Therapy, 14*, 1091–1102.

197. Borgese, M., Barone, L., Rossi, F., Raspanti, M., Papait, R., Valdatta, L., et al. (2020). Effect of nanostructured scaffold on human adipose-derived stem cells: outcome of in vitro experiments. *Nanomaterials, 10*, 1822.

198. Wan, S., Fu, X., Ji, Y., Li, M., Shi, X., & Wang, Y. (2018). FAK- and YAP/TAZ dependent mechanotransduction pathways are required for enhanced immunomodulatory properties of adipose-derived mesenchymal stem cells induced by aligned fibrous scaffolds. *Biomaterials, 171*, 107–117.

199. Gao, Q., Jia, F., Li, X., Kong, Y., Tian, Z., Bi, L., et al. (2023). Biophysical cues to improve the immunomodulatory capacity of mesenchymal stem cells: the progress and mechanisms. *Biomedicine & Pharmacotherapy, 162*, 114655.

200. Saleem, S., Li, J., Yee, S.-P., Fellows, G. F., Goodyer, C. G., & Wang, R. (2009). β1 integrin/FAK/ERK signalling pathway is essential for human fetal islet cell differentiation and survival: β1 integrin/FAK/ERK in human fetal pancreas. *The Journal of Pathology, 219*, 182–192.

201. Biggs, M. J. P., Richards, R. G., & Dalby, M. J. (2010). Nanotopographical modification: a regulator of cellular function through focal adhesions. *Nanomedicine: Nanotechnology, Biology and Medicine, 6*, 619–33.

202. Kang, Y.-G., Nam, J.-H., Kim, K.-H., & Lee, K.-S. (2010). FAK pathway regulates PGE $_2$ production in compressed periodontal ligament cells. *Journal of Dental Research, 89*, 1444–1449.

203. Liu, S., Liu, F., Zhou, Y., Jin, B., Sun, Q., & Guo, S. (2020). Immunosuppressive property of MSCs mediated by cell surface receptors. *Frontiers in Immunology, 11*, 1076.

204. Álvarez-Viejo, M. (2015). CD271 as a marker to identify mesenchymal stem cells from diverse sources before culture. *World Journal of Stem Cells, 7*, 470.

205. Lai, K., Zeng, K., Zeng, F., Wei, J., & Tan, G. (2011). Allogeneic adipose-derived stem cells suppress Th17 lymphocytes in patients with active lupus in vitro. *Acta Biochimica et Biophysica Sinica, 43*, 805–812.

206. Zhang, Y., Ravikumar, M., Ling, L., Nurcombe, V., & Cool, S. M. (2021). Age-related changes in the inflammatory status of human mesenchymal stem cells: Implications for cell therapy. *Stem Cell Reports, 16*, 694–707.

207. Neri, S., & Borzì, R. (2020). Molecular mechanisms contributing to mesenchymal stromal cell aging. *Biomolecules, 10*, 340.

208. Moll, G., Alm, J. J., Davies, L. C., Von Bahr, L., Heldring, N., Stenbeck-Funke, L., et al. (2014). Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties? *Stem Cells, 32*, 2430–2442.

209. François, M., Copland, I. B., Yuan, S., Romieu-Mourez, R., Waller, E. K., & Galipeau, J. (2012). Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-γ licensing. *Cytotherapy, 14*, 147–152.

210. Chinnadurai, R., Copland, I. B., Garcia, M. A., Petersen, C. T., Lewis, C. N., Waller, E. K., et al. (2016). Cryopreserved mesenchymal stromal cells are susceptible to T-cell mediated apoptosis which is partly rescued by IFNγ licensing. *Stem Cells, 34*, 2429–2442.

211. Svalgaard, J. D., Munthe-Fog, L., Ballesteros, O. R., Brooks, P. T., Rangatchew, F., Vester-Glowinski, P. V., et al. (2020). Cryopreservation of adipose-derived stromal/stem cells using 1–2% Me2SO (DMSO) in combination with pentaisomaltose: An effective and less toxic alternative to comparable freezing media. *Cryobiology, 96*, 207–213.

212. Crowley, C. A., Smith, W. P. W., Seah, K. T. M., Lim, S.-K., & Khan, W. S. (2021). Cryopreservation of human adipose tissues and adipose-derived stem cells with DMSO and/or trehalose: A systematic review. *Cells, 10*, 1837.

213. Park, S., Lee, D. R., Nam, J. S., Ahn, C. W., & Kim, H. (2018). Fetal bovine serum-free cryopreservation methods for clinical banking of human adipose-derived stem cells. *Cryobiology, 81*, 65–73.

214. Shaik, S., & Devireddy, R. (2018). Cryopreservation protocols for human adipose tissue derived adult stem cells. In B. A. Bunnell & J. M. Gimble (Eds.), *Adipose-Derived Stem Cells* (pp. 231–59). Springer New York. https://doi.org/10.1007/978-1-4939-7799-4_19 [cited 2023 Jul 4].

215. Gao, S., Ogawa, M., Takami, A., Takeshita, K., & Kato, H. (2020). Practical and safe method of long-term cryopreservation for clinical application of human adipose-derived mesenchymal stem cells without a programmable freezer or serum. *Cryo Letters, 41*, 337–343.

216. Zhang, T.-Y., Tan, P.-C., Xie, Y., Zhang, X.-J., Zhang, P.-Q., Gao, Y.-M., et al. (2020). The combination of trehalose and glycerol: An effective and non-toxic recipe for cryopreservation of human adipose-derived stem cells. *Stem Cell Research & Therapy, 11*, 460.

217. López, M., & Eroglu, A. (2021). Chemically defined, clinical-grade cryopreservation of human adipose stem cells. In W. F. Wolkers & H. Oldenhof (Eds.), *Cryopreservation and freeze-drying protocols* (pp. 555–67). Springer US. https://doi.org/10.1007/978-1-0716-0783-1_28 [cited 2023 Jul 4].

218. Fujita, Y., Nishimura, M., Komori, N., Sawamoto, O., & Kaneda, S. (2021). Protein-free solution containing trehalose and dextran 40 for cryopreservation of human adipose tissue-derived mesenchymal stromal cells. *Cryobiology, 100*, 46–57.

219. Fujita, Y., Nishimura, M., Wada, T., Komori, N., & Otoi, T. (2022). Dimethyl sulfoxide-free cryopreservation solution containing trehalose, dextran 40, and propylene glycol for therapy with human adipose tissue-derived mesenchymal stromal cells. *Cytotechnology, 74*, 515–529.

220. Miyagi-Shiohira, C., Kurima, K., Kobayashi, N., Saitoh, I., Watanabe, M., Noguchi, Y., et al. (2015). Cryopreservation of adipose-derived mesenchymal stem cells. *Cell Medicine, 8*, 3–7.

221. Choudhery, M. S., Badowski, M., Muise, A., Pierce, J., & Harris, D. T. (2014). Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. *Journal of Translational Medicine, 12*, 8.

222. Lu, G.-M., Rong, Y.-X., Liang, Z.-J., Hunag, D., Ma, Y.-F., Luo, Z.-Z., et al. (2020). Landscape of transcription and expression regulated by DNA methylation related to age of donor and cell

passage in adipose-derived mesenchymal stem cells. *Aging, 12*, 21186–21201.

223. Ma, N., Qiao, C., Zhang, W., Luo, H., Zhang, X., Liu, D., et al. (2017). Original research: Adipose-derived stem cells from younger donors, but not aging donors, inspire the host self-healing capability through its secreta. *Experimental Biology and Medicine (Maywood, N.J.), 242*, 68–79.

224. Wu, L. W., Wang, Y.-L., Christensen, J. M., Khalifian, S., Schneeberger, S., Raimondi, G., et al. (2014). Donor age negatively affects the immunoregulatory properties of both adipose and bone marrow derived mesenchymal stem cells. *Transplant Immunology, 30*, 122–127.

225. Taguchi, T., Borjesson, D. L., Osmond, C., & Griffon, D. J. (2019). Influence of donor's age on immunomodulatory properties of canine adipose tissue-derived mesenchymal stem cells. *Stem Cells and Development, 28*, 1562–1571.

226. Terrón-Puig, M., Huber-Ruano, I., Sabadell-Basallote, J., Ejarque, M., Núñez-Roa, C., Maymó-Masip, E., et al. (2022). Glycogen accumulation in adipocyte precursors from elderly and obese subjects triggers inflammation via SIRT1 /6 signaling. *Aging Cell, 21*. [cited 2023 Jul 4]. https://onlinelibrary.wiley.com/doi/10.1111/acel.13667

227. Fang, Y., Tang, S., & Li, X. (2019). Sirtuins in metabolic and epigenetic regulation of stem cells. *Trends in Endocrinology and Metabolism, 30*, 177–188.

228. Oñate, B., Vilahur, G., Camino-López, S., Díez-Caballero, A., Ballesta-López, C., Ybarra, J., et al. (2013). Stem cells isolated from adipose tissue of obese patients show changes in their transcriptomic profile that indicate loss in stemcellness and increased commitment to an adipocyte-like phenotype. *BMC Genomics, 14*, 625.

229. Silva, K. R., Liechocki, S., Carneiro, J. R., Claudio-da-Silva, C., Maya-Monteiro, C. M., Borojevic, R., et al. (2015). Stromalvascular fraction content and adipose stem cell behavior are altered in morbid obese and post bariatric surgery ex-obese women. *Stem Cell Research & Therapy, 6*, 72.

230. Strong, A. L., Bowles, A. C., Wise, R. M., Morand, J. P., Dutreil, M. F., Gimble, J. M., et al. (2016). Human adipose stromal/stem cells from obese donors show reduced efficacy in halting disease progression in the experimental autoimmune encephalomyelitis model of multiple sclerosis. *Stem Cells, 34*, 614–626.

231. Usha Shalini, P., Vidyasagar, J. V. S., Kona, L. K., Ponnana, M., & Chelluri, L. K. (2017). In vitro allogeneic immune cell response to mesenchymal stromal cells derived from human adipose in patients with rheumatoid arthritis. *Cellular Immunology, 314*, 18–25.

232. Louwen, F., Ritter, A., Kreis, N. N., & Yuan, J. (2018). Insight into the development of obesity: Functional alterations of adipose-derived mesenchymal stem cells: Dysfunctional ASCs in obesity. *Obesity Reviews, 19*, 888–904.

233. Harrison, M. A. A., Wise, R. M., Benjamin, B. P., Hochreiner, E. M., Mohiuddin, O. A., & Bunnell, B. A. (2020). Adipose-derived stem cells from obese donors polarize macrophages and microglia toward a pro-inflammatory phenotype. *Cells, 10*, 26.

234. Eljaafari, A., Pestel, J., Le Magueresse-Battistoni, B., Chanon, S., Watson, J., Robert, M., et al. (2021). Adipose-tissue-derived mesenchymal stem cells mediate PD-L1 overexpression in the white adipose tissue of obese individuals, resulting in T cell dysfunction. *Cells, 10*, 2645.

235. Juntunen, M., Heinonen, S., Huhtala, H., Rissanen, A., Kaprio, J., Kuismanen, K., et al. (2021). Evaluation of the effect of donor weight on adipose stromal/stem cell characteristics by using weight-discordant monozygotic twin pairs. *Stem Cell Research & Therapy, 12*, 516.

236. Benaiges, E., Ceperuelo-Mallafré, V., Madeira, A., Bosch, R., Núñez-Roa, C., Ejarque, M., et al. (2021). Survivin drives tumor-associated macrophage reprogramming: A novel mechanism with potential impact for obesity. *Cellular Oncology, 44*, 777–792.

237. Villagrasa, A., Posada-González, M., García-Arranz, M., Zapata, A. G., Vorwald, P., Olmedillas-López, S., et al. (2022). Implication of stem cells from adipose tissue in wound healing in obese and cancer patients. *Cirugía y cirujanos, 90*, 487–496.

238. Mahmoud, M., & Abdel-Rasheed, M. (2023). Influence of type 2 diabetes and obesity on adipose mesenchymal stem/stromal cell immunoregulation. *Cell and Tissue Research, 394*(1), 33–53.

239. Pérez, L. M., Bernal, A., San Martín, N., Lorenzo, M., Fernández-Veledo, S., & Gálvez, B. G. (2013). Metabolic rescue of obese adipose-derived stem cells by Lin28/Let7 pathway. *Diabetes, 62*, 2368–2379.

240. Mastrangelo, A., Panadero, M. I., Pérez, L. M., Gálvez, B. G., García, A., Barbas, C., et al. (2016). New insight on obesity and adipose-derived stem cells using comprehensive metabolomics. *Biochemical Journal, 473*, 2187–2203.

241. Mariani, S., Di Rocco, G., Toietta, G., Russo, M. A., Petrangeli, E., & Salvatori, L. (2017). Sirtuins 1–7 expression in human adipose-derived stem cells from subcutaneous and visceral fat depots: Influence of obesity and hypoxia. *Endocrine, 57*, 455–463.

242. Eljaafari, A., Robert, M., Chehimi, M., Chanon, S., Durand, C., Vial, G., et al. (2015). Adipose tissue-derived stem cells from obese subjects contribute to inflammation and reduced insulin response in adipocytes through differential regulation of the Th1/Th17 balance and monocyte activation. *Diabetes, 64*, 2477–2488.

243. Sagar, S., Faizan, M. I., Chaudhary, N., Singh, V., Singh, P., Gheware, A., et al. (2023). Obesity impairs cardiolipin-dependent mitophagy and therapeutic intercellular mitochondrial transfer ability of mesenchymal stem cells. *Cell Death & Disease, 14*, 324.

244. Chehimi, M., Ward, R., Pestel, J., Robert, M., Pesenti, S., Bendridi, N., et al. (2019). Omega-3 polyunsaturated fatty acids inhibit IL-17A secretion through decreased ICAM-1 expression in T cells co-cultured with adipose-derived stem cells harvested from adipose tissues of obese subjects. *Molecular Nutrition & Food Research, 63*, 1801148.

245. Carp, D. M., & Liang, Y. (2022). Universal or personalized mesenchymal stem cell therapies: impact of age, sex, and biological source. *Cells, 11*, 2077.

246. Georgiev-Hristov, T., García-Arranz, M., Trébol-López, J., Barba-Recreo, P., & García-Olmo, D. (2022). Searching for the optimal donor for allogenic adipose-derived stem cells: A comprehensive review. *Pharmaceutics, 14*, 2338.

247. Gonzalez-Rey, E., Gonzalez, M. A., Varela, N., O'Valle, F., Hernandez-Cortes, P., Rico, L., et al. (2010). Human adipose-derived mesenchymal stem cells reduce inflammatory and T cell responses and induce regulatory T cells in vitro in rheumatoid arthritis. *Annals of the Rheumatic Diseases, 69*, 241–248.

248. Manferdini, C., Maumus, M., Gabusi, E., Piacentini, A., Filardo, G., Peyrafitte, J.-A., et al. (2013). Adipose-derived mesenchymal stem cells exert antiinflammatory effects on chondrocytes and synoviocytes from osteoarthritis patients through prostaglandin E $_2$. *Arthritis & Rheumatism, 65*, 1271–1281.

249. Baharlou, R., Ahmadi-Vasmehjani, A., Faraji, F., Atashzar, M. R., Khoubyari, M., Ahi, S., et al. (2017). Human adipose tissue-derived mesenchymal stem cells in rheumatoid arthritis: Regulatory effects on peripheral blood mononuclear cells activation. *International Immunopharmacology, 47*, 59–69.

250. Skalska, U., & Kontny, E. (2016). Adipose-derived mesenchymal stem cells from infrapatellar fat pad of patients with rheumatoid arthritis and osteoarthritis have comparable immunomodulatory properties. *Autoimmunity, 49*, 124–131.

251. Skalska, U., Kuca-Warnawin, E., Kornatka, A., Janicka, I., Musiałowicz, U., Burakowski, T., et al. (2017). Articular and

subcutaneous adipose tissues of rheumatoid arthritis patients represent equal sources of immunoregulatory mesenchymal stem cells. *Autoimmunity, 50*, 441–450.

252. Kuca-Warnawin, E., Skalska, U., Janicka, I., Musiałowicz, U., Bonek, K., Głuszko, P., et al. (2019). The phenotype and secretory activity of adipose-derived mesenchymal stem cells (ASCs) of patients with rheumatic diseases. *Cells, 8*, 1659.

253. Kuca-Warnawin, E., Plebańczyk, M., Ciechomska, M., Olesińska, M., Szczęsny, P., & Kontny, E. (2022). Impact of adipose-derived mesenchymal stem cells (ASCs) of rheumatic disease patients on T helper cell differentiation. *International Journal of Molecular Sciences, 23*, 5317.

254. Kuca-Warnawin, E., Kurowska, W., Plebańczyk, M., Wajda, A., Kornatka, A., Burakowski, T., et al. (2023). Basic properties of adipose-derived mesenchymal stem cells of rheumatoid arthritis and osteoarthritis patients. *Pharmaceutics, 15*, 1003.

255. Baharlou, R., Rashidi, N., Ahmadi-Vasmehjani, A., Khoubyari, M., Sheikh, M., & Erfanian, S. (2019). Immunomodulatory effects of human adipose tissue-derived mesenchymal stem cells on T cell subsets in patients with rheumatoid arthritis. *Iran J Allergy Asthma Immunol, 18*(1), 114–119.

256. Sayegh, S., El Atat, O., Diallo, K., Rauwel, B., Degboé, Y., Cavaignac, E., et al. (2019). Corrigendum: rheumatoid synovial fluids regulate the immunomodulatory potential of adipose-derived mesenchymal stem cells through a TNF/NF-κB-dependent mechanism. *Frontiers in Immunology, 10*, 1961.

257. Vasilev, G., Ivanova, M., Ivanova-Todorova, E., Tumangelova-Yuzeir, K., Krasimirova, E., Stoilov, R., et al. (2019). Secretory factors produced by adipose mesenchymal stem cells downregulate Th17 and increase Treg cells in peripheral blood mononuclear cells from rheumatoid arthritis patients. *Rheumatology International, 39*, 819–826.

258. Aliakbari, S., Mohammadi, M., Rezaee, M. A., Amini, A. A., Fakhari, S., & Rahmani, M. R. (2019). Impaired immunomodulatory ability of type 2 diabetic adipose-derived mesenchymal stem cells in regulation of inflammatory condition in mixed leukocyte reaction. *EXCLI Journal, 18*:Doc852; ISSN 1611-2156 [Internet]. [cited 2023 Jan 14]. https://www.excli.de/vol18/Rahmani_23092 019_proof.pdf

259. Qiu, G., Zheng, G., Ge, M., Huang, L., Tong, H., Chen, P., et al. (2017). Adipose-derived mesenchymal stem cells modulate CD14++CD16+ expression on monocytes from sepsis patients in vitro via prostaglandin E2. *Stem Cell Research & Therapy, 8*, 97.

260. Forte, D., Ciciarello, M., Valerii, M. C., De Fazio, L., Cavazza, E., Giordano, R., et al. (2015). Human cord blood-derived platelet lysate enhances the therapeutic activity of adipose-derived mesenchymal stromal cells isolated from Crohn's disease patients in a mouse model of colitis. *Stem Cell Research & Therapy, 6*, 170.

261. Serena, C., Keiran, N., Madeira, A., Maymó-Masip, E., Ejarque, M., Terrón-Puig, M., et al. (2017). Crohn's disease disturbs the immune properties of human adipose-derived stem cells related to inflammasome activation. *Stem Cell Reports, 9*, 1109–1123.

262. Serena, C., Millan, M., Ejarque, M., Saera-Vila, A., Maymó-Masip, E., Núñez-Roa, C., et al. (2020). Adipose stem cells from patients with Crohn's disease show a distinctive DNA methylation pattern. *Clinical Epigenetics, 12*, 53.

263. Boronat-Toscano, A., Vañó, I., Monfort-Ferré, D., Menacho, M., Valldosera, G., Caro, A., et al. (2023). Smoking suppresses the therapeutic potential of adipose stem cells in Crohn's disease patients through epigenetic changes. *Cells, 12*, 1021.

264. Wu, X., Mu, Y., Yao, J., Lin, F., Wu, D., & Ma, Z. (2022). Adipose-derived stem cells from patients with ulcerative colitis exhibit impaired immunosuppressive function. *Frontiers in Cell and Developmental Biology, 10*, 822772.

265. Razmkhah, M., Jaberipour, M., Erfani, N., Habibagahi, M., Talei, A., & Ghaderi, A. (2011). Adipose derived stem cells (ASCs) isolated from breast cancer tissue express IL-4, IL-10 and TGF-β1 and upregulate expression of regulatory molecules on T cells: Do they protect breast cancer cells from the immune response? *Cellular Immunology, 266*, 116–122.

266. Bahrami, B., Hosseini, A., Talei, A.-R., Ghaderi, A., & Razmkhah, M. (2017). Adipose derived stem cells exert immunomodulatory effects on natural killer cells in breast cancer. *Cell Journal (Yakhteh), 19*, 137.

267. Mehdipour, F., Razmkhah, M., Rezaeifard, S., Bagheri, M., Talei, A.-R., Khalatbari, B., et al. (2018). Mesenchymal stem cells induced anti-inflammatory features in B cells from breast tumor draining lymph nodes: MSCs-B cells interaction in breast cancer. *Cell Biology International, 42*, 1658–1669.

268. Mansourabadi, Z., Razmkhah, M., Mohtasebi, M., Talei, A.-R., & Ghaderi, A. (2019). Expression of major histocompatibility complex class I polypeptide-related sequence B in adipose-derived stem cells from breast cancer patients and normal individuals. *Journal of Cancer Research and Therapeutics, 15*, 1067.

269. Fakhimi, M., Talei, A.-R., Ghaderi, A., Habibagahi, M., & Razmkhah, M. (2019). Helios, CD73 and CD39 induction in regulatory T cells exposed to adipose derived mesenchymal stem cells. *Cell Journal, 22*. [cited 2023 Apr 8]. https://doi.org/10.22074/cellj. 2020.6313

270. Zheng, C.-X., Sui, B.-D., Liu, N., Hu, C.-H., He, T., Zhang, X.-Y., et al. (2018). Adipose mesenchymal stem cells from osteoporotic donors preserve functionality and modulate systemic inflammatory microenvironment in osteoporotic cytotherapy. *Science and Reports, 8*, 5215.

271. Bi, Y., Lin, X., Liang, H., Yang, D., Zhang, X., Ke, J., et al. (2020). Human adipose tissue-derived mesenchymal stem cells in parkinson's disease: inhibition of T helper 17 cell differentiation and regulation of immune balance towards a regulatory T cell phenotype. *Clinical Interventions in Aging, 15*, 1383–1391.

272. Mancini, O. K., Shum-Tim, D., Stochaj, U., Correa, J. A., & Colmegna, I. (2017). Erratum to: Age, atherosclerosis and type 2 diabetes reduce human mesenchymal stromal cell-mediated T-cell suppression. *Stem Cell Research & Therapy, 8*, 35.

273. Capelli, C., Zaccara, E., Cipriani, P., Di Benedetto, P., Maglione, W., Andracco, R., et al. (2017). Phenotypical and functional characteristics of in vitro-expanded adipose-derived mesenchymal stromal cells from patients with systematic sclerosis. *Cell Transplantation, 26*, 841–854.

274. Azizi, G., Jadidi-Niaragh, F., & Mirshafiey, A. (2013). Th17 cells in immunopathogenesis and treatment of rheumatoid arthritis. *International Journal of Rheumatic Diseases, 16*, 243–253.

275. Miklosz, A., & Chabowski, A. (2023). Adipose-derived mesenchymal stem cells therapy as a new treatment option for diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism, 108*(8), 1889–1897.

276. Muthu, S., Patil, S. C., Jeyaraman, N., Jeyaraman, M., Gangadaran, P., Rajendran, R. L., et al. (2023). Comparative effectiveness of adipose-derived mesenchymal stromal cells in the management of knee osteoarthritis: A meta-analysis. *World Journal of Orthopedics, 14*(1), 23–41.

277. Mahmoud, M., Abu-Shahba, N., Azmy, O., & El-Badri, N. (2019). Impact of diabetes mellitus on human mesenchymal stromal cell biology and functionality: Implications for autologous transplantation. *Stem Cell Reviews and Reports, 15*, 194–217.