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The immune plasticity of mesenchymal stromal cells from mice and men: concordances and discrepancies

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TABLE OF CONTENTS

1. ABSTRACT

During the last decade, mesenchymal stromal cells (MSCs) have generated numbers of clinical trials to address inflammatory diseases such as GVHD, Crohn's disease and lupus. Animal models and therapeutic protocols in patients have demonstrated their anti-inflammatory and immunosuppressive properties towards adaptive immune cells. However, the basis of their immune suppression remains hotly debated. In the present review, we discuss the comparative isolation of human and rodent MSCs, their respective immune properties, whether constitutive or licensed by inflammatory or immune reactions, as well as differential efficacy as observed in GVHD clinical trials and related mouse models. Rodent MSCs display a number of immune differences with human MSCs regarding to ease of isolation, licensing pathways resulting in immunosuppression, and expression of immune mediators. These observations urge for caution when translating results generated in murine models into clinical settings.

2. INTRODUCTION

Mesenchymal stem cells, also called mesenchymal stromal cells (MSCs), are described as adult stem cells since they show *in vitro* a potential of self-renewal and differentiation into the mesenchymal lineage, including bone, cartilage, tendon, and adipose tissues. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cell Therapy has suggested four minimal criteria for the definition of MSCs: 1) Plastic adherence in standard tissue culture conditions; 2) Expression of CD73 (ecto-5'-nucleotidase), CD90, and CD105 (endoglin); 3) No expression of CD11b, CD14, CD19, CD34, CD45, CD79a, or of MHC class II (HLA-DR); and 4) Differentiation *in vitro* to osteoblasts, adipocytes, and chondroblasts. In addition to their differentiation properties, MSCs produce growth factors and cytokines that play a major role in the function of HSC niches and hematopoiesis in the bone marrow and that support tissue healing and regeneration, as observed in the

lung, kidneys, heart, gut, liver, and the skin. The features of the different MSC populations in the body, the mechanisms of their mobilization into different organs and sites of injury, as well as the gene pathways and factors regulating their stemness and differentiation are under intense investigation. Lastly, MSCs are deemed strongly immunosuppressive since they produce several anti-inflammatory and immune-suppressive factors, especially after exposure to an inflammatory or Th1-oriented immune environment.

As a consequence of these properties, clinical applications of MSCs encompass mesenchymal tissue regeneration, wound healing, resolution of acute tissue injury, drug delivery cell system, organ transplantation, and treatment of inflammatory and/or autoimmune diseases. The last decade has seen a sharp increase in the number of clinical trials using MSCs (www.clinicaltrial.gov) and the complete analysis of the first phase III placebo-controlled clinical trial using MSCs in the context of GVHD is under review. Numerous studies have demonstrated that MSCs inhibit the proliferation and/or effector functions of conventional T cells, B cells, NK cells, and macrophages, the differentiation dendritic cells (DCs) into antigen presenting cells (APCs), while MSCs support the generation of regulatory T cells (Treg) (reviewed in (1)).

Although it is clear that MSCs display strong immune regulatory properties, the fine analysis of these properties is rendered complex by the diverse and sometime conflicting reports generated from this hotly pursued field of investigation. *In vitro* analyses of effects of MSCs on immune cells are dependent on many experimental parameters, such as the composition of MSCs and immune cell co-cultures (e.g. T cells, monocytes), their purity, and mode of activation. *In vivo* data may vary considerably between animal model of diseases performed in controlled conditions and time course in inbred and pathogen-free laboratory animals compared to clinical studies in patient cohorts with heterogeneous genetic background and medical history. Interpretations of these results are also critically dependent on the awareness of the intrinsic differences of MSCs activation pathways and immune cell responses in rodents and humans (reviewed in (2)), as well as rodent strain- or donor-related variations. In the present review we discuss the comparative isolation and immune properties of human versus mouse MSCs, and their relative effectiveness *in vivo* as observed in different diseases and animal models, in particular GVHD. This may be of importance for future clinical utilization of MSC such as generated from ongoing studies in rheumatoid arthritis mouse models.

3. HUMAN VERSUS MOUSE MSCs: ISOLATION AND COMPARISON WITH FIBROBLASTS

3.1. Isolation of human MSCs

In humans, the estimated frequency of MSCs in bone mononuclear cells is 1:100 000 to 1: 24 000 (3). Human MSCs can be facilely isolated by processing of bone marrow biopsies on a Ficoll-gradient and plastic adherence in the absence of growth factors for one or two passages to remove other non-adherent and/or growth factor-dependent bone marrow cells. Under microscopic observation, human MSCs appear morphologically heterogeneous and contain fast-replicating spindle- or round-shaped cells and slow-replicating large cells. As a whole, human MSCs cultures do not require growth factors and when plated at low density (50 cells/cm^2), the total cell number can increase by at least 10 fold over a week. In our experience, typically > 5x10^6 adherent cells are obtained at passage 1 from a 20 ml bone marrow aspirate and a yield > 5x10^8 of MSCs per donor can be expected reasonably at passage 3. MSC-like cells can be isolated from many tissues; however, most animal and clinical studies have been performed so far with bone marrow-derived MSCs. Because bone marrow aspiration is an invasive procedure, the clinical use of alternate source of universal donor MSCs is being considered. Of particular interest are MSCs derived from placental tissues or umbilical cord matrix/Wharton's Jelly (UC-MSCs). UC-MSCs and bone marrow-derived MSCs appear to share many tissue regenerative and immune properties. Human UC-MSCs suppress the activation of mitogen-activated PBMC (4), do not induce allogeneic immune response *in vivo* (5), and were effective for the reduction of symptoms in patients with severe and refractory systemic lupus erythematosus (6).

The question of spontaneous immortalization versus senescence and exhaustion of human MSCs during long-term culture is a hotly debated subject. Several teams reported that the long-term culture of human MSCs is associated with genetic instability, however, reports showing that approximately half of the cells could turn cancerous were recently invalidated since subsequent cell authentications suggested that cultures were cross-contaminated with sarcoma cancer cell lines such as HT1080 or U2-OS (7, 8).

3.2. Isolation of mouse MSCs

The exact frequency of mouse MSCs is not known but is estimated at approximately 1 in 10^6 bone marrow nucleated cells. However, their isolation is knowingly more challenging (9). We and others (10) have noticed that in basal medium (DMEM supplemented with 10% FBS) mouse MSCs populations hardly proliferate *in vitro*, and the vast majority of adherent MSC-like cells die within day 5 to 10 *in vitro* in basic media supplemented with bovine serum. In addition, many of the MSCs clones that eventually arise are immortalized cells with chromosomal aberrations and often engraft to form osteosarcoma-like tumors when implanted in syngeneic recipients (reviewed in (9)). For culture expansion, murine MSCs require interaction with other bone marrow cells at least during the initial passage. Their subsequent growth is dependent on growth factor stimulation (Coutu D.L., François M. and Galipeau J., submitted, (10)). Moreover, murine MSCs appear to be enriched in the bone/periosteal compartment as opposed to bone marrow. Extensive *in vitro* characterization of primary versus immortalized C57BL/6 MSCs demonstrated that multilineage differentiation was maintained in primary murine MSCs whereas immortalized cells tend to be biased toward the

osteoblast lineage, although this is quite variable from one population to another. Expression of MSC markers also vary in immortalized MSCs, for instance these cells usually lose expression of CD105 and CD34 expression (which are typically present on primary C57BL/6 murine MSCs). The capacity to present antigens to T lymphocytes has been observed comparable in primary and immortalized MSCs (François M. and Galipeau J., unpublished observations), however some immunological characteristics varied considerably. For instance, we observed that unstimulated immortalized mouse MSCs produced three times the levels of IL-6 compared to primary cells.

3.2. Comparison of MSCs and fibroblasts

Human and mouse MSCs and skin-derived fibroblasts appear morphologically similar and grow quite well *in vitro*. Expression of CD44, CD73, CD90, and CD105 (11) is present on both cell types, while some markers such as CD10 (12) and CD106 (13) are differentially expressed by fibroblasts and MSCs, respectively. Some authors have suggested that MSCs and fibroblasts are lineage linked. MSCs were shown to differentiate to stromal fibroblasts, including endometrial stromal fibroblasts. While fibroblasts cannot dedifferentiate into MSCs, fibroblasts were observed to give rise to adipocytes or osteoblasts when cultured in appropriated medium (11), an observation that was eventually contradicted (14). *In vitro*, the immune profiles of MSCs and fibroblasts appear close. Dermal fibroblasts and MSCs from healthy human donors (11, 14) or dogs (15) exert comparable levels of suppression of T cell proliferation *in vitro*. *In vivo*, however, differences were reported. Intravenous injection of 10^7 GFP-labeled MSCs led to significant amelioration of behaviour in a rat parkinsonian model, while the effects of injecting primary dermal fibroblasts were not different than injection of PBS (16). Another study reported that syngeneic or allogeneic MSCs favored wound healing compared to dermal fibroblasts, an effect that correlated with enhanced engraftment and decreased inflammation in MSCs-treated wounds. MSCs secreted significantly higher levels of numerous factors, such as EGF, KGF, IGF-1, PDGF-BB, and under hypoxic treatment VEGF-1, Ang-1 and EPO were also higher compared to dermal fibroblasts, but MSCs had lower levels of IL-6 and osteoprotegerin (17). Human dermal fibroblasts were also significantly less efficient than adipose-derived MSCs in suppressing experimental arthritis (18). These reports suggested that although MSCs and fibroblasts display comparable immune suppressive properties *in vitro*, MSCs are more effective *in vivo* for the reduction of inflammation and increased tissue healing or regeneration. Further studies are required to identify molecules differentially expressed by MSCs and fibroblasts and that may explain observations from *in vivo* experiments.

4. IMMUNE PROFILE OF NAÏVE HUMAN AND MOUSE MSCs

4.1. Expression of molecules involved in antigen presentation

Naive mouse and human MSCs constitutively express MHC class I molecules and are able to present MHC class I-restricted epitopes from transfected tumor antigens (19) or virally-introduced antigens to CD8+ T lymphocytes (20). One study has suggested that non-activated mouse MSCs are capable of low basal cross-presentation of soluble antigens (21) and effective antigenic presentation to naïve CD8+ T cells. It is not clear what co-stimulatory molecules support MHC class I-driven CD8+ T cell activation by antigen-presenting MSCs. Expression of CD80, CD86, CD28, ICOSL, and 41BBL co-stimulatory molecules was not observed on mouse and human MSCs (22). Recently however, it has been reported that mouse and human MSCs express low levels of CD54/ICAM-1 or CD106/VCAM-1 (23, 24), both of which could play a role in T cell co-stimulation.

4.2. Expression of chemokines, cytokines, and prostaglandin E2

Human and mouse MSCs were shown to produce basal levels of biologically active chemokines, cytokines, and inflammatory mediators, in particular CXCL-12/SDF-1, IL-6, prostaglandin E2 (PGE2), and TGF-□. Compared to primary macrophages, basal production of IL-6 and PGE2 is substantial in MSCs ((25) and François M and Galipeau J, unpublished data). For instance, primary unstimulated human macrophages produced less than 1 ng/48 h /106 cells of IL-6, while human MSCs produced 5 to 30 ng/48 h/106 cells of IL-6. Levels of IL-6 and CXCL-12 production in primary C57BL/6 mouse MSCs before 5 passages were approximately 10 to 20 ng/24 h/106 cells, in addition these cells expressed approximately twice the level of CXCL-12 encoding mRNA compared to non-fractioned bone marrow cells. Extensive gene expression analyses performed in our laboratory suggested that levels of CXCL-12- and TGF-□-encoding mRNA did not vary significantly in IFN-□ and/or TNF-α primed human MSCs (François M and Galipeau J, unpublished data). In contrast, production of PGE2 and IL-6 by MSCs is increased upon exposure to inflammatory products, such as TNF-α or TLR ligands.

CXCL-12 is a small chemotactic cytokine that is often found in inflammatory sites and is an important chemoattractannt for a variety of cells, in particular hematopoietic stem/progenitor cells. It supports the function of MSCs in HSC niches and tissue regeneration. The role of CXCL-12 in MSCs immune properties has not been fully investigated; however, while this cytokine has been described to mediate attraction and proliferation of lymphocytes, it may also favour the establishment of immune tolerance *in vivo*. Recently, it was reported that injection of CXCL-12 in a mouse model of EAE results in remission of the disease and switch of antigen-specific Th17 and Th1 cells into IL-10-producing T cells (26).

Interleukin-6 is a well-characterized cytokine that functions in inflammation, lymphocyte proliferation, and B cell maturation. Accordingly, inhibition of IL-6 produced by MSCs results in enhanced suppression of proliferation of activated lymphocytes *in vitro*. Interleukin-6 was shown to be responsible of neutrophil protection from apoptosis in cultures with low numbers of human MSCs (27). Interleukin 6 produced by MSCs has been suggested to play a role in the blocking of monocyte differentiation into DCs (28).

MSC-derived TGF-β was shown to play a role on the inhibition of NK cells *in vitro* (29). TGF-β is the dominant cytokine that drives the polarization of $FoxP3^-$ $CD4^+$ T cells to $FoxP3^+$ Treg cells. Accordingly, production of TGF-β by MSCs favours the induction of Treg cells *in vivo* (30).

Prostaglandin E_2 is a metabolite product of arachidonic acid conversion by the enzyme COX-1 and COX-2 that signals through binding to four subtypes of G protein-coupled receptors (EP1, EP2, EP3, and EP4). PGE_2 is produced by many cells of the body and regulates the inflammatory response as a key mediator of pyrexia, hyperalgesia, and arterial vasodilatation, which increase blood flow to inflamed tissues and, in combination with enhanced microvascular permeability, results in edema. The involvement of PGE_2 in inflammation is supported by the effectiveness of COX inhibitors, such as aspirin and other nonsteroidal anti-inflammatory drugs, to suppress inflammation. Human and mouse MSCs express constitutively COX-2 and secrete low levels of PGE_2. Expression of COX-2 and secretion of PGE_2 are considerably increased upon encounter with an inflammatory signal such as IFN-γ, TNF-α, or LPS. In addition to its role in the inflammatory reaction, PGE_2 is described as an essential mediator in T cell immunosuppression mediated by human and mouse MSCs, as detailed in the following section.

4.3. Other immune molecules expressed by MSCs

Human and mouse MSCs are identified by their expression of CD73, CD90, and CD105. The immune role of these molecules in MSCs has not been investigated yet; however, CD73, an ecto-enzyme that catalyzes the dephosphorylation of adenosine monophosphates into adenosine, is known to be overexpressed on breast-cancer cells where it plays a role in tumor metastasis and suppression of adaptive immune responses (31). Mouse MSCs were reported to constitutively produce an antagonistic form of CCL2, produced by MMP-mediated cleavage, which was shown to inhibit Th17 cell activation and to confer protection against autoimmunity in a mouse model of EAE (32). One study demonstrated that mouse as well as a subpopulation of human MSCs express the IL-1 receptor antagonistic (IL-1RN) that is involved in the inhibition of TNF-α production by activated macrophages (33). Expression of several molecules known to play an important role in fetomaternal tolerance was reported in human MSCs. Human MSCs display a constitutive expression of leukemia inhibitory factor (LIF) (34), accumulation of intracellular HLA-G as well as soluble and cell-surface-associated galectins, in particular galectin-1, -3 and -8 (35-39). Exposure of MSCs to IFN-γ increases the expression of these factors that mediate inhibitory action on T cell and NK activities.

4.4. *In vivo* effects of MSCs in the absence of an ongoing immune or inflammatory response

It is difficult to assess *in vivo* the physiological importance of each of the immune mediators that are constitutively expressed by MSCs. Nevertheless, several observations give hints on the overall effect of injected MSCs *in vivo* in the absence of an inflammatory context in immunocompetent hosts. Several studies have suggested that naïve MSCs are ignored by the immune system and/or weakly immuno-suppressive. For instance, it was reported the persistence of fetal MSCs into mothers' bone marrow decades after pregnancies (40). Baboons that received allogeneic MSCs injected at high doses (5.10^6 MSCs/kg) first I.V. and then I.M. developed alloantigen-specific antibodies but had reduced alloantigen-induced PBMC proliferation compared to naïve controls, and persistence of donor MSCs could be observed 4 weeks later (41). Other studies have suggested that MSCs can be fully recognized by the immune system and/or support immune cell activation in an antigen-independent fashion. In mice (42) and pigs (43), allogeneic MSCs injected S.C. induced both alloantigen-specific T and B cell responses. Naïve non-activated syngeneic or allogeneic mouse MSCs expressing a weakly antigenic antigen, such as the Her-2/neu tumor antigen (MSC/Neu), could induce Her-2/neu-specific immune responses after S.C. injections, leading to the reject of transplantated neu-expressing tumors (19). These properties were lost upon priming of MSCs/Neu with a combination of IFN-γ and TNF-α prior to their injection, in spite of increased cell surface MHC class I-mediated antigenic presentation induced by IFN-γ and TNF-α. In a rat model of transplantation, allogeneic heart transplants were rejected earlier if recipients were previously sensitized to donor MSCs (44). In human MSCs, detailed analyses showed that in certain experimental conditions MSCs can support *in vitro* allogeneic T cell proliferation, LPS- or antigen-induced IgG secretion by spleen B cells, or suppress neutrophil apopoptosis. These conditions were obtained at a low lymphocyte:MSC or neutrophil:MSC ratio (27, 45) or following a weak immune stimulation of T or B lymphocytes (44, 46). Hence, it is assumed that if MSCs do not encounter strong inflammatory or immune responses their production of immunosuppressive factors likely remains low, which can result in pro-inflammatory effects of MSCs on T and B lymphocyte proliferation and maturation. These events could be initiated through direct antigenic recognition of MSCs and soluble factors produced by MSCs, in particular IL-6. This information is of considerable importance for selecting the appropriate mode and timing of MSCs delivery for the treatment of inflammatory diseases, which should be performed optimally I.V. at the peak of systemic inflammation or directly into localized inflammation sites.

5. LICENSING OF MSCs BY INFLAMMATORY SIGNALS

5.1. Chemoattraction and adhesion to immune cells

Human and mouse MSCs express various receptors for inflammatory signals, such as receptors for chemokines, type I and II IFNs, IL-1, and TNF-α, as well as TLRs that bind to pathogen-associated conserved motifs. Whereas it is known that most cells of the body express one or two TLRs, the exact expression profile of TLRs in MSCs is controversial. Protein expression of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, and TLR9 has been reported in human and mouse MSCs (reviewed in (47)), however in humans we have observed that only TLR3 and

TLR4 were expressed at levels comparable to either primary macrophages or unfractioned PBMC (25). In fact, most studies investigating effects of TLR ligands on MSCs have used poly I:C and/or LPS that are TLR3 and TLR4 ligands, respectively.

MSCs were suggested to home preferentially in inflammatory sites through the expression of chemokine receptors (reviewed in (1)). In addition, MSCs respond to inflammatory mediators present at these sites such as cytokines or TLR ligands by the production of a broad array of chemokines and immune adhesion molecules. *In vitro*, chemoattraction of T cells by human or mouse MSCs occurs concomitant to T cell activation, such as in the presence of anti-CD3 (48). Optimal induction of granulocyte and lymphocyte-specific chemokines such as CXCL9, CXCL10, and CXCL11 was observed dependent on the combinatorial action of IFN-γ and TNF-α (23, 49) or IFN-γ and TLR ligands (25) in human and mouse MSCs. Adhesion molecules such as CD54/ICAM-1 and CD106/VCAM-1 are also synergistically upregulated by IFN-γ and TNF-α, which was suggested by some authors as being critical (48) or not (38) for subsequent interaction with immune cells and immunosuppression.

5.2. Antigen processing

Human and mouse MSCs activated with IFN-γ upregulate MHC class I molecule expression and MHC class I-mediated antigen presentation of endogenously expressed viral proteins to CTL lines (20). They can also cross-present soluble exogenous antigens to naïve CD8+ T cells (21). Expression of MHC class II molecules and antigenic presentation to $CD4^+$ T cells was observed to be induced by IFN-γ (50), and depended *in vitro* on cell density (51) and IFN-γ concentration (52). Antigenic presentation by mouse or human MSCs to $CD8^+$ or $CD4^+$ T lymphocytes induced IFN-γ and IL-2 production by T lymphocytes and T lymphocytes proliferation. It has also been suggested that human MSCs are refractory to CTL lysis, an effect mediated by soluble HLA-G release by MSCs (20). We have observed that antigen processing was not affected by the sole treatment with TNF-α, TLR3 or TLR4 ligands.

5.3. Immunosuppression

In addition to increasing antigen processing and chemoattraction of innate and adaptive immune cells, ongoing immune responses are critical for the licensing of immunosuppressive functions in MSCs. Mediators produced by fully activated immune cells upregulate the expression of immunosuppressive factors by MSCs, which in turn dampens the immune and/or inflammatory response (44). Numerous *in vitro* T cell-based assays have reported that allogeneic or CD3/CD28-induced T cell activation and proliferation is inhibited after several days in co-cultures with MSCs, and the early neutralization of IFN-γ produced by T cells abrogates the immunosuppression by MSCs. Accordingly, *in vivo* experiments in the GVHD model demonstrated that MSCs were not effective at controlling GVHD if mice were transplanted with T cells defective for IFN-γ production and MSCs pre-treated with IFN-γ were more powerful than non-treated MSCs at inhibiting GVHD (53).

The response to TLR ligands in human and mouse MSCs appears quite different compared to macrophages. When activated, both cell types secrete chemokines, PGE_2, and IL-6, however, only macrophages produce IL-10, TNF-α, and, following IFN-γ priming, IL-12. In addition, factors secreted by TLR-activated MSCs, in particular PGE_2, act *in vitro* and *in vivo* on surrounding macrophages resulting in increased production of IL-10 by the latter and protection against septic shock (54). Most authors have nevertheless observed that TLR activation either does not affect or decreases the suppressive potential of MSCs on T cell proliferation induced by CD3/CD28 agonists *in vitro* (reviewed in (47)).

6. IMMUNOSUPPRESSIVE MOLECULES PRODUCED BY IMMUNE REACTION-LICENSED MSCs : SPECIES DIFFERENCES OR VARIATIONS WITH METHODOLOGY?

6.1. Characteristics of MSCs and immune cell co-cultures

Functional immune suppressive effector mechanisms exerted by MSCs are classically investigated *in vitro* in MSC and immune cell co-cultures. Most human studies have used either bulk PBMC or purified T cells as responders, live or irradiated MSCs as inhibitors, and different immune stimulation signals: alloantigens (live or irradiated allogeneic PBMC, DCs, or B-EBV cells), CD3 stimulation in combination with IL-2 or CD28, or mitogens such as PHA (Table 1). It is difficult to assess the effect of irradiation of MSCs or PBMC on immediate and late cell secretome, responsiveness, and viability, and one can assume that live as well as irradiated PBMC or DCs can participate in the overall response to MSCs when the purified T cells are used as responder cells. Suppression of T cell proliferation by MSCs could be observed in the sole presence of purified CD3-activated T cells (55), however, we observed that MSCs had a reduced inhibitory action on T cells in the absence of monocytes (François M and Galipeau J, manuscript in preparation). The diversity of theses observations could be related to inter-laboratory discrepancies but also suggests that multiple factors play a role in MSC-mediated immunosuppression. While some of these factors are common to mouse and human MSCs, others are different and regulated in a different manner in human versus mouse MSCs (see below, Table 2). In addition, more studies are clearly needed to standardize the screening of MSC donors prior to their use in clinical trials.

6.2. PGE_2 is upregulated by IFN-γ and/or TNF-α in human and mouse MSCs

PGE_2 is known to have diverse inhibitory actions on T cells, depending on their maturation and activation state. Early studies on antigen or PMA-activated human Th clones reported that exogenous PGE_2 suppressed the production of IFN-γ and increased the production of IL-4, especially in the presence of IL-2 (56). The hypothesis that PGE_2 has Th1-inhibiting properties was recently challenged by the finding that PGE_2-EP4 pathway promoted

Table 1. Immune suppression mechanisms exerted by human MSCs as observed in MSC:immune cell co-cultures

Co-culture	Activation	Read-out	Effector mechanism	References
CD2 T cells + irradiated MSC	Allogeneic irradiated DC	⇓T cell proliferation	TGF-β and HGF Not IL-6	(89)
T cells + live MSC	Allogeneic mitomycin C-treated PBMC	⇓T cell proliferation	IDO	(64)
PBMC + irradiated MSC	Allogeneic irradiated PBMC	⇑T cell apoptosis	IDO	(65)
PBMC + Irradiated MSC	PHA	⇓T cell proliferation	PGE_2	(62)
PBMC + Irradiated MSC	Irradiated allogeneic PBL	⇓T cell proliferation	Not PGE_2	(62)
PBMC + live MSC	PHA	⇓T cell proliferation	PGE_2	(60)
CD4 T cells + live MSC	Irradiated allogeneic T cell-depleted PBMC	⇓T cell proliferation	IDO	(66)
PBMC + live MSC	Anti-CD3 (OKT3) and IL-2	⇓T cell proliferation	IDO, IGF-1	(67)
PBMC + live MSC	Allogeneic live PBMC	⇓T cell proliferation	IL-10, TGF-β, IGF-1, PGE_2, IDO	(68)
PBMC + live MSC	Allogeneic irradiated B-EBV	⇓T cell proliferation ⇑Treg cells ⇑IL-10	HLA-G5	(36)
CD3 T cells + live MSC	Live allogeneic PBMC	⇓T cell proliferation ⇑Treg cells	LIF	(34)
PBMC + live MSC	Live allogeneic PBMC	⇓T cell proliferation ⇑Treg cells	Not LIF	(34)
PBMC or T cell blasts + live MSC	Anti-CD3 and –CD28	⇓T cell proliferation	IDO Not PGE_2, TGF-β, IL-10, NO	(49)
CD3 T cells + irradiated MSC	Allogeneic irradiated PBMC	⇓T cell proliferation	PGE_2	(55)
PBMC + irradiated MSC	Allogeneic live PBMC	⇓T cell proliferation	Galectin-3	(37)
T cells + live MSC	Anti-CD3 and –CD28 or allogeneic PBMC	⇓T cell proliferation	Galectin-1	(38)
PBMC or CD4 T cells or CD8 T cells + live MSC	Anti-CD3 (OKT3) and IL-2 or irradiated allogeneic PBMC	⇓T cell proliferation	Galectin-1	(39)

These results were observed at high ratio MSC:T lymphocytes (usually 1:10 to 1:100). At low ratios, MSCs were rather shown to support T cell proliferation.

Table 2. Identified immune features of mouse and human MSCs

Phenotype	Human MSCs	Mouse MSCs
Constitutively expressed immune receptors	Cytokine receptors (e.g. IFN-γ, IL-1, TNF-α, TGF-β), chemokine receptors, TLRs	Cytokine receptors (e.g. IFN-γ, IL-1, TNF-α, TGF-β), chemokine receptors, TLRs
Constitutively expressed immune mediators	CXCL-12, IL-6, TGF-β, PGE_2, LIF, HLA-G, galectins, low levels of chemokines, IL-1RN in some cells	CXCL-12, IL-6, TGF-β, PGE_2, IL-1RN, aCCL2, low levels of chemokines
IFN-γ-induced MHC class II expression	Dependent on low cell density and IFN-γ concentration	Dependent on high cell density
IFN-γ-induced APC features	MHC class I-mediated antigen presentation Low/No expression of co-stimulatory molecules	MHC class I-mediated antigen presentation and cross-presentation MHC class II-mediated antigen presentation Low/No expression of co-stimulatory molecules
Injected doses (GVHD)	0.4 to 9.10^6 cells / kg	0.1 to 4.10^6 cells / mouse i.e. approximately 5 to 200.10^6 cells / kg
Immune mediators upregulated by IFN-γ	Chemokines, HLA-G, galectins, PGE_2, IDO	Chemokines, IL-6, galectins, PGE_2
Immune mediators upregulated by TNF-α	Chemokines, IL-6, PGE_2	Chemokines, IL-6, PGE_2
Immune mediators synergistically upregulated by IFN-γ and TNF-α	Chemokines	Chemokines, NO
Others	Low/No constitutive or induced expression of IL-12 or IL-10 compared to DCs or macrophages	Low/No constitutive or induced expression of IL-12 or IL-10 compared to DCs or macrophages

inflammation mediated by Th1 and Th17 cells *in vivo* (57). In mice, Th17 cells are polarized by IL-6 and TGF-β and thereafter in an autocrine manner IL-21 via the STAT3 pathway and the major nuclear orphan receptor RORγ. The resultant polarization increases their susceptibility for further polarization by IL-23, a member of the IL-6 family of cytokines. In humans, IL-1β, IL-6, and IL-23 appear sufficient to induce Th17 cell differentiation. In this context, PGE_2 appears to play a role in the generation of human and mouse Th17 cells. PGE_2 in combination with IL-23 and/or IL-1β significantly enhanced IL-17 production by human naïve CD4+ T cells activated by TcR cross-link (58) and PGE_2 exacerbates various experimental autoimmune diseases in mice through the IL-23-IL-17 pathway (57, 59).

MSC-derived PGE_2 has been shown to have variable effects. *In vitro*, PGE_2, possibly along with other factors produced by human MSCs, participates in the overall reduction of the T cell proliferative response as observed using T cells activated with PHA (60) or alloantigens (55). PGE_2 production by mouse MSCs induces an inhibitory IL-10-secreting macrophage phenotype in co-cultures in the presence of LPS (54, 61),

an effect correlated to PGE_2 and NO production by LPS-stimulated MSCs. PGE_2 produced by MSCs was also reported to be involved in inhibition of NK cell cytotoxicity. In addition, *in vitro* human MSCs can inhibit Th17 polarization and induce a shift into IL-10-producing cells that is mediated by PGE_2 (24). Consistently, recent phase I clinical trials of lupus patients demonstrate that injections of MSCs leads to a decrease in Th1 and Th17 cells and an increase in Treg cells (6). In contrast, Rasmusson *et al.* observed that PGE_2 plays a role in MSC-mediated immunosuppression when T cells were activated with PHA but not alloantigens (62), while other authors have also reported an absence of effect of PGE_2 when T cells are activated with anti-CD3/CD28 (49). Lastly, the possibility that increased PGE_2 production by cytokine-activated MSCs could act on inflammation and formation of edema has not been investigated *in vivo* in mouse models. Altogether, the role of PGE_2 in MSC-mediated immunosuppression and regulation of inflammation needs to be further explored.

6.3. Nitric oxide versus indoleamine 2,3-dioxygenase production by mouse and human MSCs

Independent studies reported that when MSCs are cultured with alloantigen- or CD3/CD28-activated T cells, PGE_2 inhibitors had only a marginal effect suppression of T cell proliferation mediated by human and mouse MSCs (23, 49, 62). In contrast inhibition of nitric oxide (NO) in mouse MSCs (23, 63) and indoleamine 2,3-dioxygenase (IDO) in human MSCs totally abrogated the suppression of T cell proliferation (49, 64-68). Both NO and IDO act locally, therefore explaining the need for MSCs to migrate toward and/or attract immune cells prior to immunosuppression. At high concentration NO appears to inhibit TcR-induced T cell activation in addition to its suppressive action on macrophages, leading to the production of IL-10 by macrophages. IDO is a materno-fetal tolerance molecule that catalyzes the degradation of tryptophan, which generates several catabolites including kynurenine. These catabolites were shown to suppress NK cell or T cell proliferation, induce T cell apoptosis as well as Treg differentiation. In addition, IDO-mediated tryptophan depletion by human MSCs results in a broad antimicrobal activity against clinically relevant pathogens (69).

The latter studies seem to have delineated a shift in MSC-based immune effectors depending on species, with cytokine-activated mouse and human MSCs producing NO and IDO, respectively. *In vitro* studies demonstrated that upregulation of *iNOS* occurs in mouse and human MSCs upon IFN-γ and TNF-α or LPS activation (49), however levels are higher in mouse MSCs and they accordingly secrete higher levels of NO. In fact, there has been a broad controversy as to whether human immune cells can produce significant levels of NO. Some studies suggested that in human monocytes/macrophages the upregulation of NO is not significant upon activation with IFN-γ and TNF-α or LPS, but rather relies on other signals such as type I IFNs, IL-4 plus anti-CD23 (reviewed in (70)). Several reports demonstrated that mouse MSCs fail to upregulate significantly IDO upon activation with IFN-γ and/or TNF-α *in vitro* (49, 69), however an *in vivo* study reported that wild-type but not IDO knockout primary mouse MSCs induced immune tolerance to a kidney allograft suggesting that *in vivo* secretion of IDO can be induced by an ongoing immune reaction in mouse MSC and plays a role in immunosuppression (71).

Importantly, in mouse MSCs, the dual activation with IFN-γ and TNF-α synergistically upregulate expression of *iNOS* and NO production while in human MSCs, the sole activation with IFN-γ is sufficient for the optimal upregulation of IDO (Table 2). These important differences between the mechanisms of priming and action of immunosuppression by mouse versus human MSCs may explain discrepancies observed with *in vivo* studies assessing the immunosuppressive properties of these cells in animal models or in clinical trials, as detailed below in the case of GVHD.

6.4. Other immune suppressive factors induced by IFN-γ and/or TNF-α in MSCs

As detailed above, IFN-γ especially in combination with TLR ligands, TNF-α, or IL-1 increases production of a broad array of chemokines by human and mouse MSCs, which is reflected by increased chemoattraction of innate and adaptive immune cells. In addition to IDO, several fetomaternal tolerance molecules including LIF, HLA-G, and galectin-1 and -3 are upregulated by IFN-γ in human MSCs (34-39). Expression of LIF and galectins has not been investigated in mouse MSCs.

In human and mouse MSCs, IFN-γ induces the expression of PD-L1. Engagement of its receptor, PD-1, on T cells was first suggested as an inducer of programmed cell death and later of immune exhaustion, a state in which T cells remain present but are unable to respond to antigenic signals. *In vitro*, blockade of PD-1 pathway in PHA-activated spleen cells in co-culture with allogeneic MSCs led to a partial reversion of the inhibitory actions of MSCs (72). Effect of the PD-1/PD-L1 blockade during a physiological activation of T cells, such as obtained with CD3/CD28 stimulation has not been investigated.

7. COMPARING HUMAN AND MOUSE MSCs EFFICACY IN GVHD CLINICAL TRIALS AND MOUSE MODELS

7.1. MSC-based GVHD clinical trials

GVHD is a life-threatening complication after allogeneic HSC transplantation in 20 to 70% of patients depending on the underlying disease, age, and HLA compatibility of HSC. Treatment of steroid-resistant and/or severe (grade III/IV) acute GVHD is the most challenging, and overall survival is low. Large studies suggested that the use of corticosteroids give at best a complete response (CR) rate of 35%, and in patients failing the steroid therapy, a two-year survival of less than 10%. The discovery of biological properties of MSCs and their role in the bone marrow niche prompted their testing in clinics as early as 2001, to promote hematopoietic recovery after irradiation and HSC transplantation or to treat acute and/or severe GVHD. Much has been done since the first described

Table 3. MSC therapy for treatment of acute GVHD

Indication	Number of Patients	Treatment	Outcome	Reference
Steroid-resistant acute GVHD	1 child	1 injection I.V. with 2.10^6 BM-MSC / kg from the patient's mother,	Remission	(73)
Steroid-resistant acute or chronic GVHD	8 adults and children	1 to 2 injections I.V. with 0.9 to 9.10^6 BM-MSC / kg (HLA-identical, -haploidentical, or -mismatched)	6 patients with CR, 5 patients were alive 2 years later	(90)
Steroid-resistant acute GVHD	55 adults and children	1 to 5 injections I.V. with 0.4 to 9.10^6 BM-MSC / kg (HLA-identical, -haploidentical, or -mismatched)	30 patients with CR, 53% of whom were alive 2 years later	(74)
Newly diagnosed acute GVHD	31 adults	1 injection I.V. with 2 or 8.10^6 BM-MSC / kg (HLA-mismatched) and steroids	24 patients with CR, 88% of whom were alive at day 90	(91)
Steroid-resistant acute GVHD	12 children	2 to 21 injections I.V. with 2 or 8.10^6 BM-MSC / kg (HLA -mismatched)	7 patients with CR, 5 of whom were alive at day 427-1111	(92)
Steroid-resistant acute or chronic GVHD	3 adults	1 intraarterial injection directly to the target organs with $0.025\text{-}0.8.10^6$ BM-MSC / kg (HLA-mismatched)	1 PR, 0 CR	(93)
Steroid-resistant acute GVHD Randomized trial	244 adults	8 to 12 injections with I.V. with 8.10^6 BM-MSC / kg (HLA-mismatched) or with placebo and steroids	No significant difference in overall response rates in MSC (35%) vs placebo (30%) groups Improvement of liver and gut GVHD in MSC group	(75)
Steroid-resistant acute GVHD Randomized trial	28 children	8 to 12 injections with I.V. with 8.10^6 BM-MSC / kg (HLA-mismatched) or with placebo and steroids	Significant improved complete response rates in MSC (64%) vs placebo (43%) groups	(76)

successful treatment of 9-year old boy with an acute steroid-resistant GVHD with MSCs (73). Several phase I/II clinical and multi-institutional trials have been published with encouraging results in adult or paediatric patients (Table 3). In all studies but one, MSCs were infused I.V. at a dose of 1 to $8x10^6$ MSCs/kg in subjects with acute, steroid-resistant GVHD. The I.V. route allowed the injection of several doses of fresh or frozen MSCs, and the number of injections was often increased in patients with partial responses. It is not always clear whether fresh or frozen MSCs were injected in patients, and no data indicate if the immune-suppressive functions of frozen MSCs are comparable to cultured MSCs. MSCs were from HLA-identical siblings or HLA-matched or –mismatched donors, however the response rate to MSCs infusion was not related to the degree of HLA matching. The most complete multicentre phase II experimental study was described in 2008, in which 55 adult and paediatric patients with steroid-resistant acute GVHD were treated with steroids and MSC infusions (74). Thirty patients had a CR, 53% of whom were alive 2 years later. As a result, a fast-track FDA-approved phase III randomized placebo-controlled clinical trial for steroid-refractory acute GVHD in adults or children has been completed and results presented in February 2010 at the BMT Tandem Meetings (75, 76). Six infusions of unrelated MSCs (from Prochymal, Osiris Therapeutics at a dose of $2x10^6$ MSCs/kg) were administered 6 to 8 times in addition to standard glucocorticoid therapy. The preliminary results that have been released to date demonstrate that in adults there was no statistical difference between MSCs and placebo on the overall response rate (35% versus 30%, n=244). Nevertheless, in a post-hoc analysis, MSCs significantly improved liver GVHD (day 100 response rates of 76% versus 47%) or gastrointestinal GVHD (day 100 response rates of 82% versus 68%). In pediatric patients, Prochymal showed a clearer trend of improvement in durable CR rates (64% versus 43%, n=28). These studies also reported that treatment of acute GVHD with MSCs was not associated with increased cancer relapse. In contrast, the preventive use of MSCs injected at the same time as allogeneic HSC transplantation reduced the incidence of GVHD but significantly increased the rate of tumor relapse (77).

7.2. Efficacy of MSCs in mouse models of GVHD

In mice, experimental disease is induced by total body irradiation and bone marrow reconstitution with allogeneic HSCs mixed with donor $CD3^+$ T cells, which mediate GVHD. In general, fresh but not frozen donor or recipient MSCs were injected once I.V., at the same time as or after bone marrow transplantation. Doses of MSCs injected per weight were significantly higher in mouse models ($0.1x10^6$ to $4x10^6$ MSCs/mouse i.e. approximately $5x10^6$ to $200x10^6$ MSCs/kg) than in human clinical trials (0.4 to 9.10^6 MSCs/kg). Mouse studies reported that $0.10x10^6$ BALB/c-derived MSCs were effective at inhibiting GVHD in irradiated recipient C57BL/6 mice but only when injected after allogeneic HSCs and $CD3^+$ T cells (53). When injected at the same time as transplantation of HSC and $CD3^+$ T cells, low doses of the C3H/he mouse strain-derived C3H10T1/2 MSC cell line ($0.5x10^6$ cells/mouse) did not reduce GVHD-induced death in BALB/c mice, however higher doses (1 to $2x10^6$ MSCs/mouse) significantly inhibited GVHD (78). This contrasts with other reports showing that injection of 1 to $4x10^6$ C57BL-6-derived MSCs at the same time as or 1 to 5 days after HSCs and $CD3^+$ T cells transplantation had no effect on the course of GVHD in BALB/c or B6D2F1 mice, respectively (79, 80). Taken together, these results may suggest that mouse MSCs are less effective than human MSCs in inhibiting or preventing GVHD. This could be due to i) the use of mouse MSC cell lines or transformed MSCs that for instance produce higher levels of IL-6 than their non-transformed counterparts, ii) the dependency on mouse MSCs on IFN-γ and TNF-α or IL-1 for the full upregulation of immunosuppressive properties such as the production of NO, in the absence of production of IDO, and iii) the fact that mouse MSCs were not always injected at the peak of acute GVHD as in humans, which may result in a lack of full licensing of immunosuppressive functions of MSCs.

8. FROM MOUSE MODELS TO HUMAN STUDIES: MSC EFFECTS IN RHEUMATOID ARTHRITIS?

Rheumatoid arthritis (RA) is one of the most common immune-mediated inflammatory diseases in humans. RA affects the synovial joints of all extremities and is characterized by the progressive destruction of the cartilage and subchondral bone. In humans, the initiation of the disease has been suggested to be T cell-dependent while its chronicity is favored by autoreactive B cells directed at a broad range of autoantigens in the context of an exacerbated inflammatory response. Currently, the disease in most patients is controlled by the use of non-steroidal anti-inflammatory drugs, glucocorticoids, and nonbiologic (traditional small molecule or synthetic) or biologic disease-modifying antirheumatic drugs (DMARDs). Biologic DMARDS target cytokines or their receptors (TNF-α, IL-1, and IL-6), and in patients who have had an inadequate response to these agents, other biologic response modifiers of immune responses such as the CTLA4-Ig and the anti-CD20 B-cell depleting monoclonal antibody.

To date, studies assessing the potential role of MSCs to address RA had been done only in animal models. Small animal models include collagen-induced arthritis (CIA) or adjuvant arthritis (AA) to study early autoimmune events of RA, as well as mice knock-out for the endogenous gene encoding IL-1RN (gene: il1rn) or transgenic mice that express TNF-α in the joints, which both develop spontaneous RA to study the clinical consequences of a chronic pathological process in the joints. It was suggested that RA in $il1rn^{-/-}$ mice is associated with modifications in the bone marrow environment resulting in a decreased differentiation capacity of MSCs and possibly osteoporosis (81). Several studies have reported the potential use of MSCs using the CIA model. One to $2x10^6$ syngeneic or allogeneic mouse MSCs (82, 83) or human adipose-derived MSCs (18) administered I.V., I.P., or intraarticularly at the time of immunization or at the onset of the disease were shown to significantly reduce inflammation and the Th1/Th17 response, induce Treg cells, and prevent severe damage to the bone. In contrast, other reports showed that 1 to $4x10^6$ C3 MSCs cell line or syngeneic MSCs administered I.V. or I.P. at had either no effect (84) or aggravating effects (85-87) on the course of CIA. Interleukin 6 levels were increased in MSC-treated mice and was likely to play a role in the aggravation of the disease since this cytokine is known to favor B cell survival and is an essential factor for Th17 cells.

Studies in the context of osteoarthritis (OA) suggested that MSCs may play a significant regenerative role in the articulate surface of the joint. In a goat OA model induced by medial meniscus and resection of the anterior cruciate ligament, the intraarticular injection of $10x10^6$ autologous MSCs in hyaluronan favored the meniscal repair and limited the bone destruction compared to hyaluronan treatment alone (88). Many investigators have reported that implantation of MSCs in scaffolds led to increased subchondral bone and to a lesser extent cartilage formation, especially if MSCs were pre-differentiated *in vitro* into chondrocytes or if scaffolds were loaded with TGF-β, a pro-chondrogenic and osteogenic factor (reviewed in (86)). In contrast, it is not known if MSCs could exert similar regenerative functions in RA. Further studies in the CIA and TNF-α transgenic small rodent models are required to assess the effect of allogeneic or syngeneic MSCs embedded or not in scaffolds on the early RA autoimmune responses in the CIA model, as well as on the inflammation-associated chondral and bone destruction. From an immunological standing, the exact role of several mouse MSC-specific immune factors such as NO, as well as factors commonly produced by mouse and human MSCs in particular PGE_2 and IL-6, needs to be addressed for their involvement in the inflammatory process and formation of oedema and immunosuppression. Furthermore, one has to keep in mind that the generation of Th17 from naïve T cells may follow different requirements in humans and mice. *In vitro*, in mice commitment to the Th17 lineage is dependent on TGF-β and IL-6 that are both produced by MSCs, whereas in humans IL-1β, IL-6 and IL-23 but not TGF-β appear to be required.

9. CONCLUSION

In conclusion, the study of human MSC immune mediators is restricted to fairly rudimentary *in vitro* assays susceptible to important inter-laboratory discrepancies on the basis of cell culture conditions and bioassay systems. In contrast, mouse MSC immunology allows for *in vivo* validation of complex interplays between veto MSCs and immune effector cells in the context of disease models. However, the recognition of similarities and differences between simian, including human, and rodent immunobiology needs to be taken in to account when extrapolating findings between species.

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