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Comparative study of immune regulatory properties of stem cells derived from different tissues

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Abstract

Allogeneic stem cell (SC)-based therapy is a promising tool for the treatment of a range of human degenerative and inflammatory diseases. Many reports highlighted the immune modulatory properties of some SC types, such as mesenchymal stromal cells (MSCs), but a comparative study with SCs of different origin, to assess whether immune regulation is a general SC property, is still lacking. To this aim, we applied highly standardized methods employed for MSC characterization to compare the immunological properties of bone marrow (BM)-MSCs, olfactory ectomesenchymal SCs (OE-MSCs), leptomeningeal SCs (LeSCs), and three different c-Kit-positive SC types, that is, amniotic fluid SCs (AFSCs), cardiac SCs (CSCs), and lung SCs (LSCs). We found that all the analyzed human SCs share a common pattern of immunological features, in terms of expression of activation markers ICAM-1, VCAM-1, HLA-ABC, and HLA-DR, modulatory activity toward purified T, B, and NK cells, lower immunogenicity of inflammatory-primed SCs as compared to resting SCs, and indoleamine-2,3-dioxygenase (IDO)-activation as molecular inhibitory pathways, with some SC type-related peculiarities. Moreover, the SC types analyzed exert an anti-apoptotic effect toward not-activated immune effector cells (IECs). In addition, we found that the inhibitory behavior is not a constitutive property of SCs, but is acquired as a consequence of IEC activation, as previously described for MSCs. Thus, immune regulation is a general property of SCs and the characterization of this phenomenon may be useful for a proper therapeutic use of SCs.

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Abbreviations

SC	Stem Cell
ES	Embryonic Stem Cell
HSC	Hematopoietic Stem Cell
BM	Bone Marrow
HIF	Hypoxia Inducible Factor
GM-CSF	Granulocyte Colony Stimulating Factor
CAR	CXCL12 Abundant Reticular Cell
SCF	Stem Cell Factor
OE-MSC	Olfactory Ectomesenchymal Stem Cell
LeSC	Leptomeningeal Stem Cell
AFSC	Amniotic Fluid Stem cell
CSC	Cardiac Stem Cell
LSC	Lung Stem Cell
IDO	Indoleamine 2,3 Dioxygenase
IEC	Immune Effector Cell
IFN- γ	Interferon- γ
TNF- α	Tumor Necrosis Factor-α
IL	Interleukin
LPS	Lipopolysaccharide
PAMP	Pathogen Associeted Molecular Pattern
TLR	Toll Like Receptor
APC	Antigen Presenting Cells
COX-2	Cyclooxygenase-2
TGF-β	Transforming Growth Factor-β
PGE2	Prostaglandin-E2
NO	Nitric Oxide
iNOS	Inducible Nitric Oxide Synthase

T_{regs}	Regulatory T cells
EAE	Experimental Autoimmune Encephalomyelitis
GvHD	Graft Versus Host Disease
EGF	Epidermal Growth Factor
FGF	Fibroblast Growth Factor
PDGF	Platelet Derived Growth Factor
VEGF	Vascular Endothelial Growth Factor
HGF	Hepatocyte Growth Factor
IGF	Insulin-like Growth Factor
SCF	Stromal Cell-derived Factor
SCI	Spinal Cord Injury
ESC	Embryonic Stem Cell
HDACi	Histrone Deacetylase Inhibitor
VPA	Valproic Acid
iPS	Induced Pluripotent Stem Cell
OCT	Octamer-binding Transcription Factor
SOX2	Sex-determining-Region Y–Box 2
KLF	Kruppel Like Factor
α-MEM	Alpha Minimal Essential Medium
FBS	Fetal Bovine Serum
DMEM	Dulbecco Modified Eagle Medium
RPMI	Roswell Park Memorial Istitute
IMDM	Iscove Modified Dulbecco Medium
ISCT	International Society for Cellular Therapy
DC	Dendritic Cells

Publications

The data contained in this thesis have been utilized to published two different papers:

Comparative study of immune regulatory properties of stem cells derived from different tissues

Di Trapani M, Bassi G, Ricciardi M, Fontana E, Bifari F, Pacelli L, Giacomello L, Pozzobon M, Féron F, De Coppi P, Anversa P, Fumagalli G, Decimo I, Menard C, Tarte K, Krampera M.

Stem Cells Dev. 2013 Nov15;22(22):2990-3002.

Immune Regulatory Properties of CD117(pos) Amniotic Fluid Stem Cells Vary According to Gestational Age

Di Trapani M, Bassi G, Fontana E, Giacomello L, Pozzobon M, Guillot PV, De Coppi P, Krampera M.

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Chapter 1

Introduction

1.1 Stem cells and their microenvironment

The possibility to use mammalian stem cells (SCs) as novel, accessible and versatile tools for clinical applications has raised much interest in the recent years. This possibility is related to their *"plasticity"*, which makes SCs effectors of therapeutic tissue regeneration. The "*stemness"* of SCs reside in their undifferentiated state, that is the ability at single cell level to self-renew and generate multiple differentiated cell types[1]. The differentiation potential is the main feature of SCs, which depends on their origin and by their capacity to generate specific cellular lineages. SCs can be classified on the basis of their potential capabilities. The categories into which SCs fall include: (1) totipotent SCs that produce all embryonic and extra-embryonic cell lineages; (2) pluripotent SCs, also known as embryonic stem cells (ESC), isolated for the first time from the inner cell mass of blastocyst and having the capabilities to generate all cells of the embryo proper[2,3]; (3) multipotent SCs that have the ability to differentiate only a subset of cell lineages; (4) oligopotent SCs, which have a minor differentiation potential than multipotent SCs and finally (5) unipotent SCs that may generate only one cell type.

Intrinsic tissue regeneration is a feature shown by the majority of metazoan, and this mechanism has been attributed to resident SCs, which maintain the balance between proliferation and the generation of differentiated offspring. This balance is obtained thanks to the ability of SCs to divide into a new SC and a progenitor. Thus, the tissue homeostasis is maintained by the self-renewal of SCs, which can be regulated through an extrinsic or an intrinsic mechanism. The intrinsic mechanism occurs during cell mitosis, in which the regulators of self-renewal are distributed asymmetrically into the two daughter cells. This phenomenon happens because during interphase SCs can set up an axis of polarity, distributing differently the protein determinants in the

cytoplasm[4,5]. The extrinsic mechanism depends on microenvironment in which the specific SC resides, which is known as "SC niche". In this mechanism, the SC that are in close contact with SC niche, orients its mitotic spindle perpendicularly to the niche surface and, at the end of cell division, the daughter cell that maintains the contact with niche, retain the ability of self-renewal [5,6] (**Figure 1**). Occasionally the division axis can be orientated parallel to the niche, determining an increase the number of SCs and in some cases balancing the loss SC pool. Normally, the extrinsic mechanism is more common in adult SCs respect to the intrinsic mechanism, which occurs during development[5].

SC niche is a complex structure in which different cell types, soluble factors and physical cues, such as oxygen tension or temperature, orchestrate the balance between quiescence, proliferation and commitment of SCs. The influence that the microenvironmental has on SC homeostasis must not be underestimated, because all components within the niche are responsible of the physiological funtion of SCs.

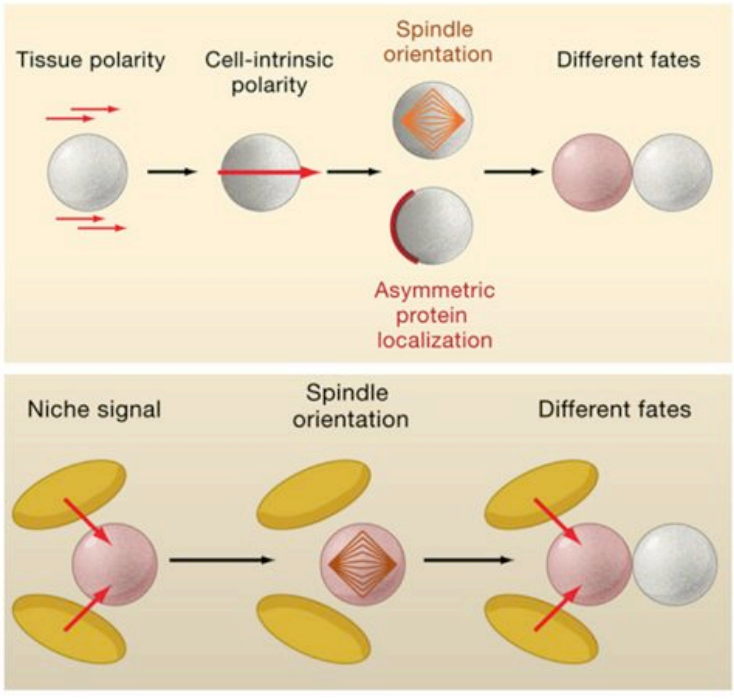

Figure 1. Extrinsic and Intrinsic Mechanism of Stem Cell Self- Renewal. In the upper panel is shown the intrinsic mechanism in which SCs can set up an axis of polarity during interphase and use it to localize cell fate determinants asymmetrically in mitosis. Orientation of the mitotic spindle along the same polarity axis ensures the asymmetric segregation of determinants into only one of the two daughter cells. In the lower panel is shown the extrinsic mechanism in which SCs are influenced surrounding niche for self-renewal. By orienting their mitotic spindle perpendicularly to the niche surface, they ensure that only one of the two daughter cells continues to receive this signal and maintains the ability to self-renew. (modified from[5]).

An example of SC niche is the bone marrow (BM), which constitutes the microenvironmental that regulates the hematopoietic stem cell (HSC) homeostasis[7]. In 1978 Schofield proposed for the first time the concept of BM niche, in which HSCs were closely associated with BM microenvironment. Today, HSCs are the best-characterized multipotent SCs. These SCs have been isolated for the first time from mouse BM about 15 years ago[8]. Today, it is widely known that multipotency, self-renewal capacity and the quiescence of HSCs are regulated by different factors inside BM. These signals can be generated by different BM cell populations, but also by physical factors. The latter are mainly related to the concentration of oxygen. In fact, when HSCs are into the endosteal region, where the low levels of oxygen[9-11], maintain their self-renewal capabilities[12]. It has been demonstrated that the disruption of hypoxia-inducible factor (HIF)-1α resulted in loss of HSC quiescence, while the presence of this molecules induced quiescence and increased repopulating activity[13,14].

On the other hand, inside BM niche there are various populations of stromal cells, which support the hematopoiesis (**Figure 2**). These SC populations include: osteolineage cells, implicated in HSC regulation through the release several cytokines, such as granulocyte colony-stimulating factor (GM- CSF)[15], thrombopoietin[16], and CXCL12[17]; perivascular CXCL12-expressing stromal cells, which include CXCL12-abundant reticular (CAR) cells, nestin positive stromal cells and leptin receptor positive stromal cells, and MSCs[18-20] (**Figure 2**).

Thus, the study of SCs must not only be focus on cell population of interest but, defining niche components and how they work in concert to regulate SC homeostasis could provide the opportunity to improve regeneration following injury and understand how disordered niche function could contribute to disease.

1.2 Mesenchymal Stromal Cells (MSCs)

MSCs are a population of multipotent SCs identified for the first time between the 1960s and 1970s. At that time, Friedenstein and his colleagues studied a subpopulation of BM cells with osteogenic capabilities, which were confirmed through heterotopic transplantation[21]. In fact, when this BM cells were transplanted into subcutaneous space or in kidney, they were able to form ectopic marrow, including trabecular bone, adipocytes and myelosupportive stroma.

Successively, the same group demonstrated that these BM cells were able to adhere to tissue culture plastic, generate colony forming unit (CFU), showing a fibroblast-like morphology[22].

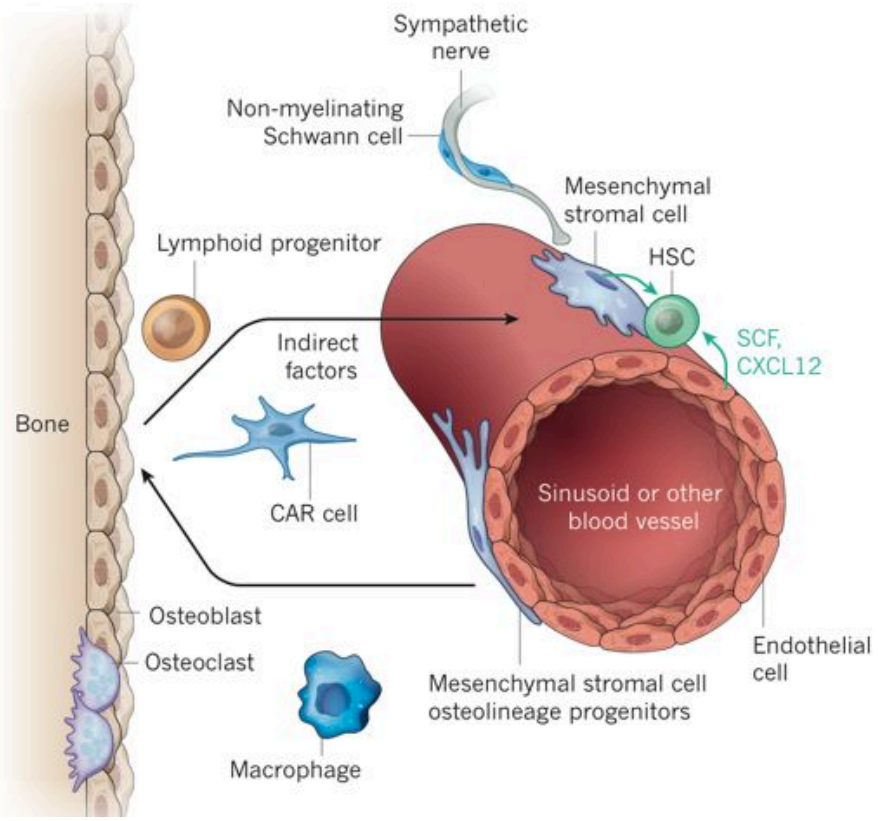

Figure 2. **Haematopoietic stem cells (HSCs) niche.** HSCs are localized near to sinusoids in the BM, where endothelial cells and MSCs regulate HSC homeostasis by release of stem cell factor (SCF), CXCL12 and other factors. Also other cells are involved in the HSC maintenance such as perivascular stromal cells, including CAR cells, but also sympathetic nerves, non-myelinating Schwann cells, macrophages and osteoclasts. (modified from[7]).

A few years later, other research groups have been determined that BM stromal cells can be expanded *in vitro* for long term culture, and supported the haematopoiesis[23]. In the same period, Caplan described these BM cells as mesenchymal "stem" cells, for their ability to generate different cells of mesodermal lineage[24]. However, the MSC differentiation potential toward adipose tissue, bone and cartilage was later shown[25].
Today, several research groups are focusing the attention on the study the mesenchymal stromal/stem cells (MSCs) not exclusively for their differential capabilities, but also for their ability to support haematopoiesis and regulate the immune response.
MSCs can be also isolated from other tissues, including adipose tissue[26], placenta[27], amniotic fluid[28], Wharton's jelly[29] and dental pulp[30]. The growing interest in the use of MSCs for clinical applications has led to an increase of publications in the last decade. Currently, PubMED identify about 33.000 references for mesenchymal stem cells and over 20.000 for mesenchymal stromal cells. The continuous amount of publications led to growing insights on MSCs, but also to an increase of heterogeneous data. In fact, there are different terminologies to identified MSCs, but also different methods of isolation and approaches to expand and characterize the cells.
To overcome these issues, the committee of International Society for Cellular Therapy (ISCT) has proposed the minimal criteria to define human MSCs[31,32]. First of all, the Authors defined the fibroblast-like plastic-adherent cells as "Mesenchymal Stromal Cells" regardless of the tissue from which they are isolated to overcome the inconsistency between nomenclatures and facilitate exchange of knowledge among biomedical investigators[31]. However, given that not all these cells are SCs, the term mesenchymal stem cells was specifically used to identify the subpopulation of cells that showed stem cell features, i.e. a long-term self-renewal and the capability to differentiate into different specific lineage *in vivo*. For example, the cells isolated from BM through plastic adherence are not uniform cell population, and this aspect is proven by the relative small fraction of these cells to produce fibroblast colonies (CFU-F), even if all these cells showed multipotentialy. Thus, since it is difficult to discriminate the different subpopulation, the Authors suggested the term mesechymal stromal cells to avoid misunderstanding.
Thereafter, ISCT Committee proposed the minimal criteria to define MSCs[32]. They suggested three criteria that are: (1) the adherence to plastic, (2) the specific surface

antigen expression, and (3) the multipotent differentiation potential (**Table 1**). Specifically, to identify a MSC, at least the 95% of population must express CD105 (endoglin, SH2), CD90 (Thy1) and CD73 (ecto-5'-nucleotidase) defined mesenchymal markers, as measured by flow cytometry. Furthermore, the MSC population must lack expression (≤2% positive) of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA class II. The multipotent differentiation potential was instead defined by their ability to differentiate toward osteoblasts, adipocytes and chondroblasts under specific stimuli *in vitro*.

Many studies have reported that MSCs can also trans-differentiate into multiple cell types of mesodermal and non-mesodermal origin, such as endothelial cells[33], cardiomyocytes[34], hepatocytes[35] and neural cells[36], although this MSC feature in not universally accepted.

Despite, the identification of MSCs by use of specific markers is an simple procedure, the same is not so easy *in vivo*[37]. However, the use of some markers, such as nestin, leptin receptor, Gli1, and FAP has followed the identification of tissue resident MSCs, and the study of migration and function of injected MSCs, after population expansion *in vitro*[38-40]. Mendez-Ferrer and colleagues have been identified a population nestin-expressing cells into endosteal niche[41]. These cells showed MSC features, as defined by their ability to form colony-forming unit fibroblast. They have been shown for the first time that MSCs are the progenitors of mature osteochondral cells in BM, and that these cells are essential for the maintenance of the niche by supporting haematopoiesis SCs function[41].

Although the identification of physiologic function of resident MSCs needs to be clarified, the effects of *in vitro* expanded MSCs has provided important clues about their roles *in vivo*, especially in disease models. When MSCs are injected in *vivo*, they are able to migrate toward the damage tissues, such as infarcted myocardium, traumatic brain injury or fibrotic liver where they play an important role in tissue repair[42]. MSCs are able to migrate toward damage tissue, where they exert beneficial effects both by the replacement of damage cells that by release of soluble factors. Particularly, in presence of inflammatory stimuli, such as TNF-α, lipopolusaccharide or hypoxia, MSCs release various growth factors, including epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF-b), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF)-1, angiopoietin-1

and stromal cell-derived factor (SDF)-1, which promote the tissue repair[43,44]. Moreover, these molecules can activate resident cells, such as endothelial cells and fibroblast, which are important for the angiogenesis and remodelling of the extracellular matrix respectively, and resident progenitor cells that can be induced to differentiate in specialized tissue cells (**Figure 3**).

1. Plastic adherence		
2. Phenotype	Positive (≥95% +)	Negative (≤2% +)
	CD105	CD45
	CD90	CD34
	CD73	CD14 or CD11b CD79α or CD19 HLA class II
3. In vitro capability to differentiate to osteoblasts, adipocytes and chondroblasts.		

Table 1. Minimal criteria to define Mesenchymal Stromal Cells (MSCs).

1.2.1 MSCs, Inflammation and Immunomodulation

Several Authors have been shown the efficacy of MSCs in preclinical and clinical studies, but this capability has not always been confirmed. In fact, most of injected MSCs previously expanded *in vitro* remain trapped in the lungs. On the other hand, the MSCs that reach the damaged tissues have a poor rate of engraftment, and remain active for short period. This aspect suggests that the effects of MSCs are not limited to replacement of these cells into the site of injury, but probably molecule released by MSCs can play a crucial role in this scenario[45,46]. For example, it has demonstrated that the use of supernatants of MSCs, including molecules such as TSG6 and HGF, can improve inflammatory diseases, improving cardiac function during myocardial infarction and promote the recovery in EAE, respectively[47,48].

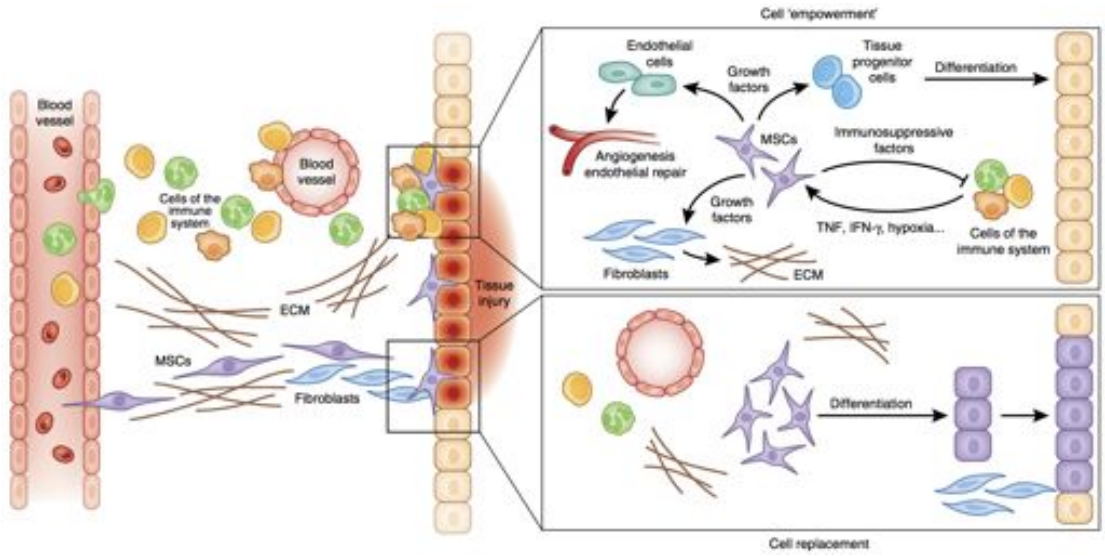

Figure 3. Effect of MSCs in tissue regeneration and immune regulation. MSCs are able to reach the tissue damaged when injected *in vivo*, where they can differentiate into functional cells to replace damaged cells. Alternatively, MSCs by realising of growth factors can stimulate resident cells, including endothelial cells, fibroblasts and, most importantly, tissue progenitor cells, which help the tissue repair through angiogenesis, remodeling of the extracellular matrix (ECM) and the differentiation of tissue progenitor cells. Moreover, the inflammatory microenvironmental induce MSC switch toward an immunosuppressive status and in turn to modulate the inflammatory response. (Modified from[49])

The therapeutic effects of MSCs are not only associated to their ability to regulate the tissue remodelling by secretion of different cytokines and growth factors, but also to their ability to regulate the immune response (**Figure 3**). The immunomodulation is a peculiar feature of MSCs, with which these cells influence both adoptive and innate immunity.

MSCs carry out a double function, by switching from an anti-inflammatory to proinflammatory status, interacting with different components of immune system[50,51]. The immunomodulatory effects of MSCs have been displayed for different IECs of acquired immunity, such as T- and B-cells, but also for cells of innate immunity, including NK-cells, monocytes, macrophages and neutrophilis. Several reports have been shown the ability of MSCs to modulate the immune system both *in vitro* that *in vivo*, sometimes with conflicting data. However, they have provided the basis for the MSC clinical application for disorders resulting from autogenic or allogeneic immune response, such as Graft-versus-Host Disease (GvHD), multiple

sclerosis, and other autoimmune disorders[50,52].

Actually, MSCs are not constitutively immunosuppressive cells. During physiological conditions they show anti-apoptotic and supportive properties toward different cell types, such as HSCs, IECs, plasma and neoplastic cells[50]. The immune modulatory properties of MSCs are associated to the inflammatory microenvironment. In presence of specific cytokines, mainly interferon (IFN)-γ, tumor necrosis factor-α (TNF-α) and interleukin (IL)-1α/β, MSCs can switch toward a proinflammatory or anti-inflammatory status, for this reason this SCs have been described as "sensor of inflammation"[51]. This double function is related to the levels of these inflammatory cytokines. For instance, during the early phase of inflammation, when the levels of cytokines are low, MSCs adopt a proinflammatory phenotype, supporting the development of inflammatory response. Conversely, during the late phase of inflammation, the levels of cytokines are higher due to the constant release of cytokines by IECs in the microenvironment, and the MSCs differentiate toward the anti-inflammatory phenotype.

Thus, the MSCs are regulators of immunity response and modulate the balance between the activation and suppression of inflammation. However, to better understand how MSCs suppress the IECs is important to focus the attention on the impact of the inflammatory microenvironment on MSCs.

Inflammation is a protective response trigger by infection or tissue injury, which helps to eradicate pathogens and restored host integrity. During the onset of inflammation, the first cells implicated in the inflammatory response are the innate effector cells, mainly phagocytes, such as macrophages and neutrophils[53]. These cells express on their plasmatic membrane specific receptors, such as the molecules belonging to the family of Toll-like receptors (TLRs), which are activated by molecules associated with pathogens or cell stress. These ligands are known also as pathogen-associeted molecular patterns (PAMPs), and they are evolutionarily conserved in the different species. PAMPs include the endotoxin bacterial lipopolysaccharide (LPS), recognized by TLR4, the flagellin, recognized by TLR5, and the PAMPs normally associated with virus, that is double-stranded RNA (dsRNA), which act on TLR3[54].

TLRs are not exclusively express by phagocytes but also by MSCs[54,55]. It has been demonstrated that the expression of TLRs on MSCs contributes to their polarization. For instance, the activation of TLR4 on MSCs, induces the change toward the proinflammatory phenotype, while the activation of TLR3 polarize the MSCs toward

the anti-inflammatory status[56]. Thus, the MSC polarization should be seen how a synergic collaboration between the inflammatory cytokines and the TLR-activation. In fact, during the onset of inflammation, the secretion of inflammatory cytokines, induce an overexpression of TLRs on MSCs, which become more responsive to inflammatory microenvironment. On the contrary the high concentration of cytokines and the protracted stimulation of MSCs, induce a downregulation of TLR2 and TLR4, which are mainly involved in proinflammatory response, and consequently cause a suppressive polarization of MSCs mainly driven by TLR3[56].

Similarly to MSCs, monocytes can differentiate into macrophages with a proinflammatory (M1) or an anti-inflammatory (M2) function, and this polarization can be influenced by MSCs. In fact, the production of IL-6 by MSCs induce a polarization of monocytes toward the anti-inflammatory status, on the contrary the lack IL-6 release allows the switch toward the proinflammatory phenotype[51].

The anti-inflammatory M2 cells are able to secrete cytokines including IL-10, TGF- β and low levels of IL-1, IL-6, IFN-γ and TNF-α[51].

In addition to monocytes, MSCs interact with other components of innate immunity. For instance, the secretion of IL-6, Il-8, GM-CSF and macrophage migration inhibitory factor by MSCs induce the recruitment of neutrophils, which collaborate with other cells into the site of inflammation to increase the immune response[57,58]. In this scenario, the relationship of MSCs with effector cells of innate immunity is essential to maintain the correct balance between activation and inhibition of inflammatory response.

The recruiting activity of MSCs involved also other IECs. In particular, after inflammatory cytokine stimulation, MSCs secrete molecules belonging to the family of (C-X-C motif) ligand, such as CXCL-9, CXCL-10 and CXCL-11, and to (C-C motif) ligand, such as CCL2, CCL3 and CCL12, which are involved in the recruitment of different components of innate immunity, as monocytes, macrophages but also of different type of lymphocytes[52].

All these mechanisms generate a loop of signals that lead to a correct development of an inflammatory response, which induce the immunosuppressive phenotype of MSCs.

1.2.2 MSC-mediated Immunosuppression

The progression of inflammation together with the recruiting of different IECs into the inflammatory site, result in an amount of inflammatory cytokines, which are responsible of MSC differentiation toward the immunosuppressive status. This process, known as priming or lincesing of MSCs, is mainly induced by the presence of IFN-γ and by the activation of its receptor IFN-γR1[50]. It has been demonstrated that the blockage of IFN-γ or its receptor IFN-γR1 by use of specific antibodies, reverted the MSC-mediated immunosuppression[59]. Also the other inflammatory cytokines, including TNF-α and IL1α/β, taking active part in this mechanism but are primarily involved in the enhancing of MSC licensing[50].

The next question that needs to be discussed is how primed MSCs inhibit the immune response.

Different reports have been shown a large number of MSC immunosuppressive molecules, including interferon-γ (IFN-γ)[59,60], interleukin-1β (IL-1β)[61], transforming growth factor-β[62-64], indoleamine-2,3-dioxygenase (IDO)[59,65], IL-6[66,67], IL-10[68], prostaglandin-E2 (PGE2)[44], hepatocyte growth factor[62], TNF-α[69-71], nitric oxide (NO)[72], heme oxygenase-1 (HO-1)[73], HLA-G5[74,75], and many other factors, some of which are still unidentified.

The MSC immunosuppression is a conserved and species-specific mechanism[76]. It has been shown that BM-MSCs derived from human, monkey and pig use mainly IDO to inhibit IEC proliferation, whereas BM-MSCs from mouse, rat, hamster and rabbit generally utilized iNOS expression.

This change in the immunosuppression mechanism is not related to the lack of expression of these molecules in the different species, but for a singular mechanism of regulation. In fact, even if human IDO shares 62% sequence homology with the mouse IDO, only in human there is an upregulation of this molecule following IFN-γ induction[77-79].

IDO is an enzyme involved in the catabolism of the tryptophan (TRP). It acts in two different ways. The first is the production of kynurenine (KYN), which binds the aryl hydrocarbon receptor (AhR) on target cells, consequently causing suppression, anergy or cellular death. In addition, the AhR pathway can promote differentiation of forkhead-box (Fox)p3^{+} T regulatory (T_{regs}) cells, which lead to an increased of immunosuppression[80,81].

The second mechanism is related to the depletion of TRP by IDO, which in turn, acts on the cell vitality. Moreover, the TRP reduction trigger the molecular stress-response pathways on target cells, such as GCN2 kinase and the mammalian target of rapamycin (mTOR)[82] (**Figure 4**).

The role of IDO in MSC-mediated immunosuppression has been confirmed by use of L-1MT, the specific inhibitor of IDO. Similarly, the addition of tryptophan into MSC/T cell coculture restored T cell proliferation[65].

In addition to MSCs, different types of antigen-presenting (APC) cells, including dendritic cells (DCs) and macrophages utilized IDO mechanism to suppress the proliferation of T and NK cells[82].

Differently from human, mouse MSCs suppress the IEC proliferation by the expression of iNOS, which is also modulated by the presence of IFN-γ, TNF-α and IL-1-α/β. NO released by MSCs induce the activation of STAT5 pathway in T cells, which lead to the apoptosis of these IECs *in vitro*[72]. The effect of iNOS has been confirmed by use of specific inhibitors, which reverted the MSC-mediated immunosuppression both in mouse model of GvHD that after heart allograft transplantation in rat model[73,83].

Unlike IDO, NO have a short-range effect due to its low stability when released into microenvironment, thus its effect decreased as the distance from the cells secreting it[84]. However, following inflammatory priming the secretion of chemokines and the overexpression adhesion molecules by MSCs, including ICAM-1 (CD54) and VCAM-1 (CD106), lead to the recruitment and tethering of IECs in close proximity to the MSCs, where the NO concentration is higher to be efficacy[83,85].

IDO and NO are not the only molecules involved in MSC-mediated immunosuppression. It has been demonstrated that the inhibition of cyclooxygenase-2 (COX-2), the enzyme that produces PGE2, restored proliferation of T cells when cultured with MSCs[44,86].

The effects of MSC suppressive molecules have been shown for different IEC population. For example, MSCs can inhibit different subsets of proinflammatory T cells, including TH1 and TH17, but also MSCs can polarize the T cells toward a regulatory phenotype, thus improving the anti-inflammatory mechanism[87].

Similarly to the effect previously shown for KYN pathway, the production of PGE2, TGF-β or HLA-G5 by human MSCs induced a differentiation of $CD4^+$ T cells toward $CD25^+FoXP3^+$ T_{regs} when MSCs were cultured in presence of PBMCs[74,88,89].

MSC-mediated immunosuppression is not only a direct mechanism mediated by

release of molecules directly, but MSCs can induce other cells to suppress the IECs. For example, MSCs can induce the differentiation of T_{regs} also by activation of TGF-β-produced macrophages or by induction of DCs via IL-10[90,91].

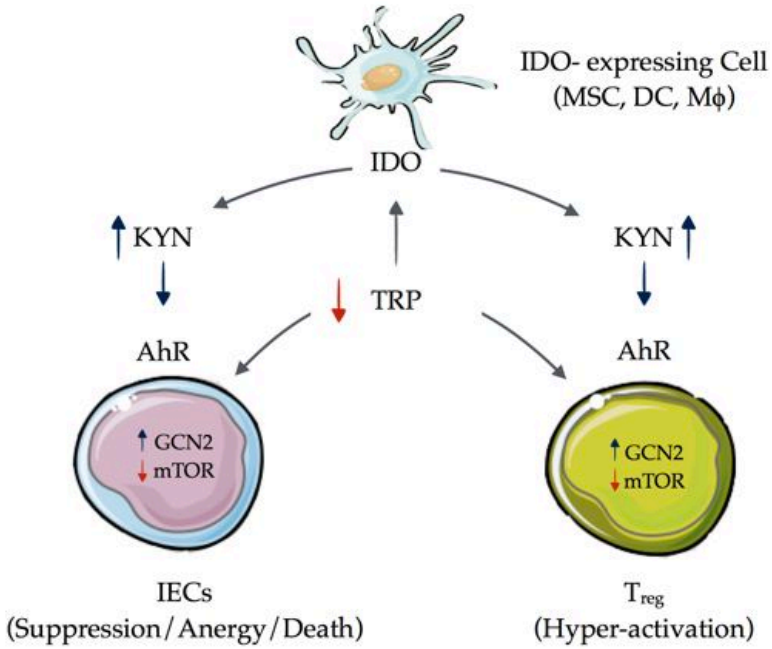

Figure 4. Mechanism of IDO. Metabolic control of T cells and T_{regs} responses via IDO. The release of KYN and TRP consumption by accessory cells expressing IDO generates signals via AhR and amino-acid sensors (GCN2, mTOR), respectively, that have profound effects on T cell and T_{regs} responses to inflammatory and antigenic signals. IDO activity in MSCs also enhances T_{regs} differentiation from naive CD4 T cells via these metabolic pathways. (modified from[82]).

On the other hand, the release of PGE2 by MSCs inhibits the differentiation, maturation and activity of DCs, which normally promote T-cell alloreactivity inducing graft rejection. Furthermore, MSCs cultured with DCs in presence of differentiation stimuli, including LPS and TNF-α, obstruct the expression of CD83, MHC class II, CD40 or CD86 and induce also indirect expansion of T_{regs}[92,93].

Another important point to discuss is the activity of MSCs on NK cells, which are effector of innate immunity with cytotoxic activity toward cells that lack the expression of MHC class I[94].

The NK cell activity depends on the balance between positive or negative signals induced by specific ligands express on target cells. Although resting MSCs express

low level of MHC class I, they are vulnerable to NK-mediated. This aspect can be explained by the presence of activating ligands on MSC surface, which move the NK cell balance toward to activate status. The NK activating ligands include the UL16-binding proteins (ULBPs), the MHC class I-related molecule-1 (MICA/B), which are recognized by NKG2D receptors on NK cells, and nectin-2 (CD112) and Poliovirus receptor (PVR or CD155), which stimulate DNAX Accessory Molecule (DNAM-1). Interestingly, the IFN-γ stimulation of MSCs induces an overexpression of all NK activating ligands, but also the MHC class I molecule. The latter is the pivotal inhibitory ligand of NK cells and its overexpression induces MSCs to become resistance to NK-cytotoxicity[94,95].

MSCs inhibit also the proliferation of NK cells and this mechanism seems related to IDO, PGE2, TGF-β and HLA-G. Moreover, these molecules seem to reduce also the NK cell cytotoxicity versus target cells[74,95,96].

In addition to the mechanism described on T and NK cell proliferation, primed MSCs are able to suppress the B cell proliferation, but how this regulation is carried out is still not clear[50,59]. MSC suppression on B cells involved also the reduction of antibody production and the release of chemokines responsible of B cell migration[97].

Finally, another important aspect to take into account in view of clinical application of MSCs is the capability of these cells to be immunoprivileged cells. In fact, MSCs cultured in presence of unstimulated T cells fails to induce lymphocyte activation[98]. This aspect can be explained by the lack of costimulatory molecules, including CD80 or CD86 (B7 superfamily) on MSC membrane, which are essential for T-cell activation. T cells need to two different signals for activation that are, the MHC molecules associated with their antigen and the costimulatory molecules, recognized by the T-cell receptor and CD28 on T cell surface respectively.

The low expression of MHC class I molecules and the lack of expression of MHC class II that CD80, CD86 or CD40 on MSCs prevent T cell activation.

Interestingly, under inflammatory priming conditions MSCs overexpress both MHC molecules, but never express the costimulatory molecules, thus also in primed condition they are not able to activate T cell proliferation[50,99].

Overall, MSCs are immunoprivileged cells that modulate the balance of inflammatory response through activation or suppression of different IECs (**Figure 5**). MSCs support the development of inflammation by activation of monocyte differentiation toward the M1 phenotype and by release of chemokines involved in the recruitment of IECs. Once

inflammatory response has developed, the amount of cytokines levels stimulates the MSCs to become immunosuppressive. Licensing MSCs overexpress different genes, which produce immunosuppressive molecules, cytokines and growth factors, allowing the reduction of immune response and the induction of tissue repair.

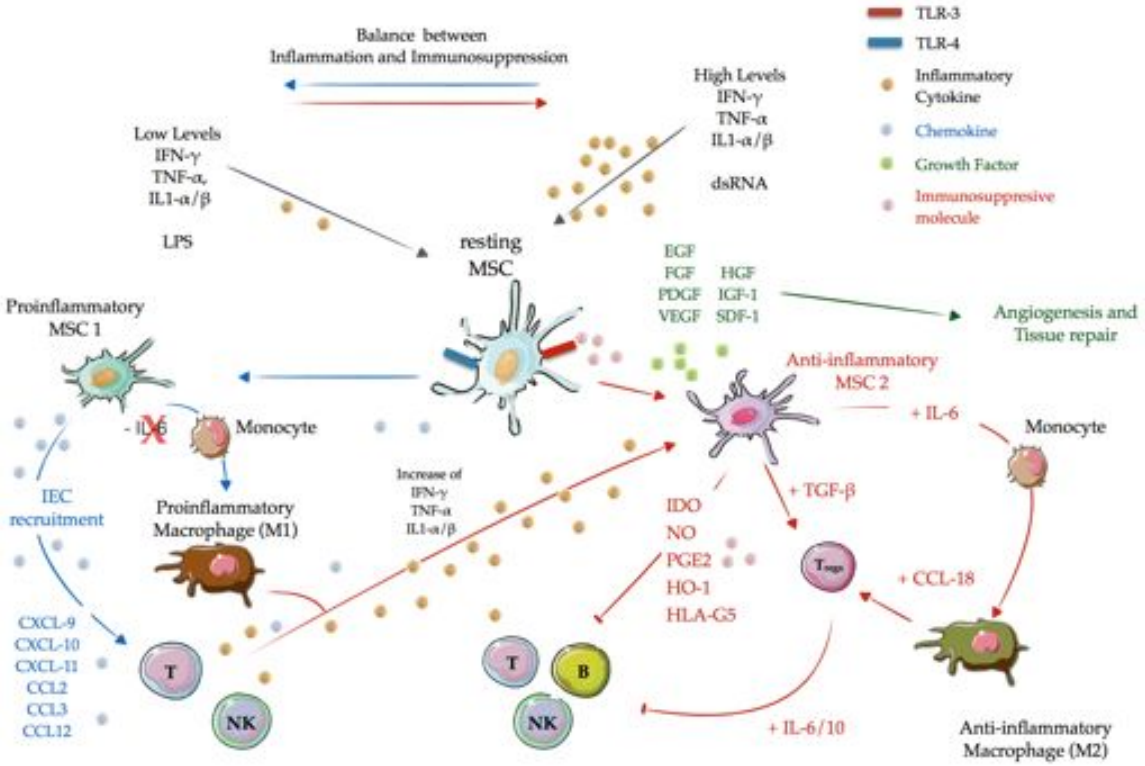

Figure 5. Role of inflammatory microenvironmental on MSC polarization. Representation of the capabilities of MSCs to differentiate toward proinflammatory or anti-inflammatory cells. As described in the text, low level of inflammatory cytokines, but also the activation of TLR-4, induce a switch toward the proinflammatory phenotype (MSC 1), which release chemokines involved in the recruitment of IECs and induce monocytes to differentiate into macrophages M1. Conversely, the higher concentration of cytokines, increased also by the continuous recruiting of IECs, together with the activation of TLR-3, induces MSCs to become immunosuppressive cells (MSC 2). Primed MSCs release different immunosuppressive molecules that regulate the IEC proliferation, induce monocyte via IL-6 to differentiate into macrophages (M2) and produce various growth factor involved in the angiogenesis and tissue repair.

1.2.3 Potential Application of MSCs

The immunosuppressive and differentiation capabilities of MSCs, together with the ease isolation procedures of these SCs from different tissues, makes MSCs promising tools for the treatment of different degenerative and inflammatory diseases.

The immunomodulatory effects of MSCs have been largely confirmed in different

disease models, including GvHD, experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis (CIA), inflammatory bowel disease (IBD), type 1 diabetes, and systemic lupus erythematosus (SLE)[100-105] and currently there are over 390 active MSC-based trials worldwide.

The first success of MSC therapy has been shown by using third-party haploidentical BM-MSCs to treat patients with cyclosporine and steroid resistant grade IV acute GvHD[106-108]. Similar effects have been demonstrated in other inflammatory diseases, including systemic lupus erythematosus and Crohn's disease[107,108].

However, many other studies have been shown their inefficacy. For example, in a mouse model of CIA, the intravenous administration of MSCs did not display any effect, while beneficial effects were showed after intraperitoneal injection of MSCs in a collagen-induced arthritis models[109,110].

Similarly, conflicting data have been reported in mouse model of SLE. Allogenic MSC treatment in increased the progression of disease in lupus mice model (NZB/W mice), in contrast to the beneficial effect showed in MRL/*lpr* mice and in human clinical trials[100,111].

These failures seem related to the poor rate of MSC engraftment but also to the timing and the site of MSC administration. For instance, the effects of MSCs in acute GvHD model were ineffective when these cells were administrated at the same time of the GvHD onset. By contrast, multiple administrations of MSCs at weekly intervals efficiently prevented the development of the disease[112,113]. The beneficial effect seems related to the levels of cytokines, mainly IFN-γ, which induce a correct MSC licensing. In fact, the pretreatment of MSCs with inflammatory cytokines before their administration, led to a significantly improvement in GvHD mice model but also on concanavalin A-induced mice hepatitis[101,114].

Similar results in patients have been also reported. In fact, MSC administration did not shown any advantage when injected at the same time of HSC transplantation[115].

Comparably, BM-MSCs were effective in EAE models only when they are administered at disease onset (days 3 and 8), but they are completely useless after disease stabilization (days 10 and 15)[116].

In future, it could be important to develop animal models that will make easier to understand the complex relationship between MSCs and host microenvironment, developing approaches for monitoring the inflammatory status of patients at the time of MSC infusion. Moreover, further studies will be important to understand the best

source and the passage in culture of MSCs, but also to clarify the differences between the application of autologous or allogeneic MSCs. Moreover, in depth analysis will be carry out to assess the MSC immunomodulation, increasing the experience in their clinical application, in order to provide reproducible data to their future clinical application.

1.3 Olfactory Ectomesenchymal Stem Cells (OE-MSCs)

OE-MSCs are a subpopulation of SCs residing within the nasal olfactory mucosa[117,118]. The olfactory mucosa is a double-stratum organ separated by a basement membrane, including neuroephitelium that is located in front of the lumen, and the lamina propria, which is made up of Bowman glands that produce mucus, endothelian cells, ensheathing cells and SCs. Inside the olfactory mucosa there are two different types of SCs, that are neuroepithelian SCs and lamina propria SCs. The identity of neuroepithelian SCs has been debated for long time[119], moreover it has been demonstrated that these SCs have a relative poor proliferating rate, and a limited differential potential[120]. On the contrary, it has been shown that olfactory lamina propria SCs have a high proliferation rate and differentiate into neural and non-neural cell types both *in vitro* that *in vivo*. For instance, it has been demonstrated that OE-MSCs can differentiate into dopaminergic cells when injected in rat models of Parkinson's disease, and they are able to form cochlear hair cell-like cells[121,122]. These SC population do not shown any common markers neither with HSCs nor with NSCs, but they have similarity with MSCs in term of capability to generate mesodermal cell types, including osteoblasts and adipocytes, and expression of typical MSC markers, such as CD73, CD90 and CD105[117]. Moreover, since lamina propria is a connection tissue derived from mesenchyme, these SCs were defined as part of MSC family[117].

Conversely to nervous tissue samples, which are normally used to study molecular abnormality in brain diseases, the isolation of OE-MSCs does not have limitations. The olfactory mucosa is easily accessible in human and can be biopsied under local anaesthesia without any complications, for this reason OE-MSCs could be used for autologous transplantation.

The role of OE-MSCs has been reported in different disease models. For instance, the beneficial effect of OE-MSCs has been shown in a mouse model in which ischemic/hypoxic injury was induced by exicitotoxically. In this brain-injured mouse model, transplanted OE-MSCs were able to migrate toward inflammation area, engraft into lesioned hippocampus, where they exhibited neural differentiation, stimulation of endogenous neurogenesis, and improved the physiological function of tissue[123]. In addition, the regenerative potential of OE-MSCs has been shown in a rodent spinal

cord injury model, where the transplantation of sphere-forming cells derived from OE-MSCs supported axonal regeneration and induced astrocytic hypertrophy in the site of injury[124]. Moreover, it has been demonstrated the ability of OE-MSCs to differentiate into cardiomyocytes. When neurosphere-derived OE-MSCs were injected into infarcted rat heart restored the correct heart and ventricular function.

All these data display the wide differentiation potential of this SC population, supporting them as promising candidates for stem cell-based therapies.

1.4 Leptomeningeal Stem Cells (LeSCs)

LeSCs are a population of nestin positive SCs recently identified in rat leptomeninges by our group[125,126]. The isolation of this SC population has added new insights into the field of neural stem cells (NSCs), which are the major candidates for regenerative therapy in different neurodegenerative disease. Although, NSCs have shown self-renewal properties and neuroglial differentiation, there are some limitations in their use in regenerative medicine, mainly related to the complex localization of these SCs and to the hardly accessible areas of central nervous system (CNV)[127].

It has been demonstrated that neurogenesis from endogenous neural stem/precursor cells is localized in discrete areas of brain, that is the NSC niches, where NSCs are close contact with astrocytes, neuroblast, ependymal cells, endothelial cells. The synergic effect between the surrounding cells and microenvironment maintains NSC homeostasis, by regulating balance between proliferation and self-renewal[128]. NSC niche have been identified in different regions, including hippocampus, subventricular zone (SVZ), olfactory bulb, but also in non-neurogenic areas, such as spinal cord[129].

Meninges are structured in three tissue membranes: the dura mater, which is the outer membrane that envelops the other meningeal layers, and the inner membranes, that are arachnoid and pia mater. The latter constitute the leptomeninges and are linked by arachnoid trabecular that span the subarachnoid space filled with cerebrospinal fluid[130]. Furthermore, leptomeninges are in close proximity of vessels, and penetrate deeply into the CNS parenchyma. In this way, the organization and localization of these membranes help the diffusion and distribution of activated precursor cells at specific sites.

The primary function of meninges and cerebrospinal fluid is to protect the central nervous system (CNS). The leptomeninges constitute a complex microenvironment where different molecules (e.g. SDF-1/CXCR4), various cell types (e.g. pia mater cells, radial glia, neural precursor cells, Cajal Retzius cells, glia limitans cells) and extracellular matrix (e.g. laminin, collagen IV, fibronectin), support the development of cortex[131-137].

In 2009 our group isolated a nestin-positive cell population in brain meninges of embryonic and adult rodents, identified the leptomeningeal compartment as a new NSC niche. Leptomenigeal Stem Cells (LeSCs) were expanded *in vitro* both as

neurospheres, similarly to SVZ-derived NSCs, and as homogeneous cell population with SC features. LeSCs isolated by meningeal biopsies were able differentiate into excitable cells with neural morphology and phenotype, or into oligodendrocytes (**Figure 6A**), and showed the same capabilities also *in vivo*[125,126]. LeSCs showed self-renewal proliferative ability, and expressed doublecortin (DCX) in adult spinal cord meninges[125]. Moreover, nestin-positive cells were found also in human encephalic and spinal cord meninges[125,138]. Other research groups have been shown that human meninges cells expressed neural markers, including neurofilament protein and neuron-specific enolase when cultured *in vitro*[139-141].

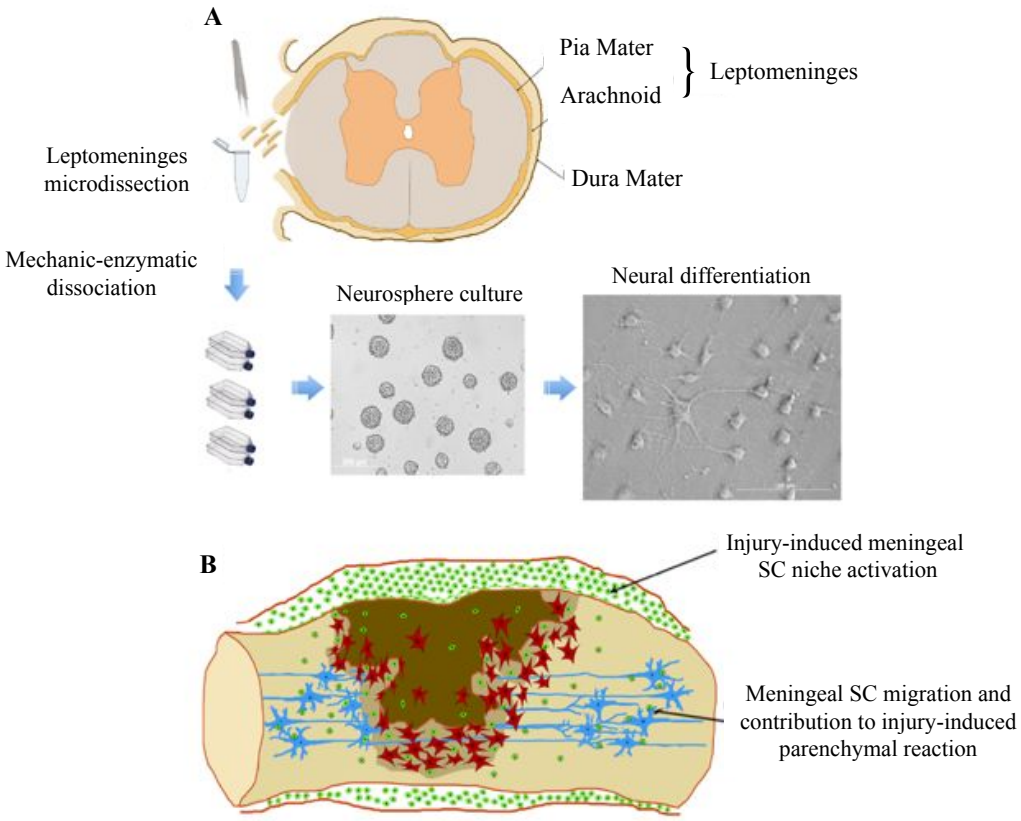

Figure 6. Isolation and function of Leptomeningeal Stem Cells (LeSCs). In (A) it show how LeSCs derived from the inner membrane of leptomeninges (adult spinal cord) can be microdissected, expanded *in vitro* and induced to differentiate into neural cells. (B) A schematic representation of the activation of the SC niche in meninges following spinal cord injury is shown. LeSCs proliferate, increase in number and migrate inside the parenchyma contributing to the parenchymal reaction. (Modified from[130]).

The beneficial effects of LeSCs have been displayed in rat models of spinal cord injury (SCI)[125]. Contusive SCI induced the activation of meningeal niche, which was determined by the increase of thickness, SC proliferation and follow migration of LeSCs toward the site of injury (**Figure 6B**). The trauma induced the overexpression of genes in meningeal spinal cord cells, including stemness-related genes (such as Pou5f1/Oct4 and Nanog), and neural precursor markers, (such as Nestin, Dcx, Pax6 and Klhl1)[142]. *In vivo* tracking analysis by use of specific marker have shown the presence of LeSC in different site of injury. Nestin-negative/DCX-positive cells were located into fibrotic scare, glial scar and also in perilesion parenchyma, demonstrating a differentiation toward neural cell type.

In addition, other groups have been shown the regenerative capabilities of leptomeninges neural stem/progenitor cells in response to ischemia and post-stroke brain, showed their differentiation into neurons, astrocytes and oligodendrocytes[130,143].

Overall, these finding highlights the existence of new neural stem cell niche and highlight the effects of leptomeninges and their stem/precursor cells in the field of neurodegenerative diseases. However, further studies to understand the global mechanism of these SCs need to be clarified.

1.5 Amniotic Fluid Stem Cells (AFSCs)

Amniotic fluid is a rich SC source easily achievable through amniocentesis, during standard diagnostic procedure of prenatal care.

Several authors have shown the presence of different SC subpopulations inside the amniotic fluid. In 2003, Prusa et al. have been isolated for the first time a population of pluripotent SCs derived from amniotic fluids expressing Oct4 (octamer binding transcription factor 4), which is a pluripotent cell marker involved in maintaining of embryonic SCs differentiation and in self-renewal[144,145]. Subsequently, it has been demonstrated that AFSCs transfected with green fluorescent protein gene under both Oct4 and its transcriptional target Rex-1 promoter, were able to activate these promoters, confirming the presence of these transcription factors inside SCs derived from amniotic fluid[146,147].

Successively, other groups have reported the possibility of harvesting amniotic fluid cells displaying features of pluripotent stem cells[148,149], however, the existence of a subpopulation of c-Kit-posivitive SCs into amniotic fluid dates in 2005, when De Coppi et al have been isolated and characterized for the first time a cell population able to generate clonal cell lines, and to differentiate into lineages representative of three embryonic germ layers[150].

The isolation of c-Kit (CD117)-positive AFSCs includes two steps, that is the immune selection of CD117-positive cells derived from AF, following by expansion of these cells in culture (**Figure 7**)[151].

Once AFSCs adhere to plastic, they grow in a feeder layer and adopt a morphology ranging fibroblast-like to an oval-round shape. Moreover, they express MSC markers, such as CD73, CD90 and CD105, adhesion molecules, including CD29 and CD44, they are positive for MHC class I, but do not show positivity for hematopoietic and endothelian markers, including CD14, CD31 CD34, CD45, CD133, and for the MHC class II. Interestingly, AFSCs express also pluripotency-associated markers, such as Oct4 and NANOG and stage-specific embryonic antigen (SSEA-4)[150].

It has been demonstrated that AFSCs are capable to differentiate *in vitro* towards cell lineages deriving from the three germ layers, including adipose, osteoblastic, myogenic, endothelial, neuronal, and hepatic cells, but these properties were not unequivocally confirmed *in vivo*.

Moreover, AFSCs can form embryoid bodies (EBs), a mechanism regulated by mTor pathway, which leads to a decreased expression of SC markers (e.g., nodal and Oct4) and an increased expression of differentiation markers (e.g., nestin-ectodermal, GATA4-endodermal and Brachyury-mesodermal)[152-154].

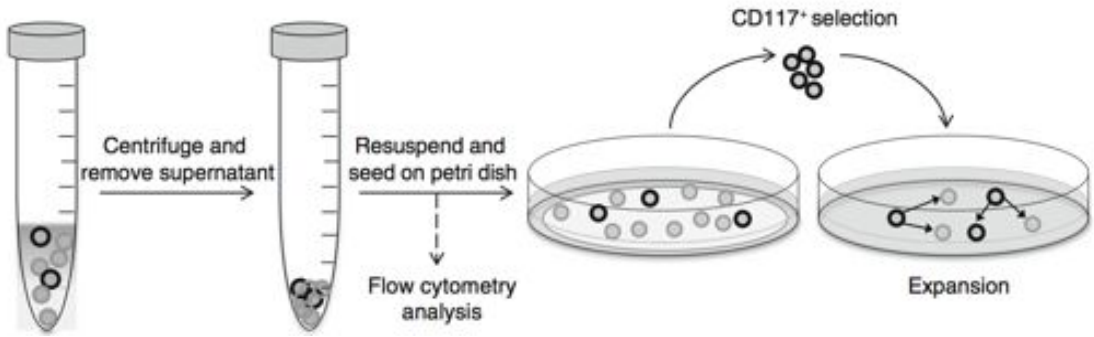

Figure 7. Isolation of c-Kit-positive Amniotic Fluid Stem Cells (AFSCs). Representation of isolation and expansion of CD117 positive cells from fresh amniotic fluid samples. After the first centrifugation, a fraction of the cell pellet can be used to analyze the level of markers present in the total population before culture and selection by flow cytometry. (modified[151]).

Although AFSCs have a high proliferation rate and express pluripontency markers, they did not generate tumors when injected in immunodeficient mice[150,155].

In addition, AFSCs can revert to a functional pluripotent state when cultured in small molecule cocktail, which are chemically induced pluripotent stem cells (iPS). More specifically, when AFSCs were cultured on Matrigel in human ESCs medium in presence of the histrone deacetylase inhibitor (HDACi) valproic acid (VPA), they acquired pluripotency, sharing similarities with human ESC transcriptome and with their capacity to form embryoid bodies (EBs) *in vitro* and teratomas *in vivo*[155,156]. These finding suggest that these iPS derived AFSCs could be utilized as pluripotent stem cells (iPS) for clinical use, but unlike iPS, they can be isolated early in pregnancy during termination procedures and are not at potential risk of virally induced tumorigenicity as they are reprogrammed with VPA.

The therapeutic efficacy of AFSCs has been verified preclinical studies showing their capabilities to regenerate and improve the functionality of injured tissues and to restore cell niche homeostasis in muscle, bone, lung, and kidney[157-161]. The success of AFSCs *in vivo* is also correlated to their ability to cross the endothelial

barrier after systemic injection, thus engrafting into injured tissues[157,162,163].

Recently, it has been displayed that AFSCs systemically injected into rats with cardiac damage did not rejected and were able to restore the functionality of damaged muscle, reducing the apoptosis, muscle atrophy and the level of proinflammatory cytokines[164]. Moreover, when injected into rat models of acute myocardial infarction, AFSCs released different molecules, such as carioprotective and pro-angiogenic factors, which improved the damage and reduced the infarct area[165].

Piccoli et al have shown that AFSCs re-established the muscle cell niche in an *HSA-Cre/Smn*$^{f7/f7}$ mouse model of spinal muscular atrophy, demonstrating the functional and stable long-term integration of these SCs into the skeletal muscle. AFSCs improved the survival rate of these animals, increased the muscle strength, showing their potentiality for the treatment of muscle diseases[158].

The regenerative potential of AFSCs has been also investigated in bone formation and lung injuries. AFSCs cultured 3D scaffold and stimulated with BMP-7 were able to produce mineralized matrix[166]. In similar manner, AFSCs pre-treated with cytokines and cultured for three weeks in a 3D scaffold induced bone repair when injected in a critical sized femoral defect of rats[160].

Also for the therapy of kidney disorders AFSCs have shown beneficial roles. In fact, AFSCs contributed in the nephrogenesis when injected in embryonic mouse models. Moreover, when injected in a model of acute kidney injury, AFSCs accumulated in peritubular capillaries and interstitium where reduced cell apoptosis and increased tubular proliferation[167-169].

When AFSCs integrate in damaged tissues they can carry out their beneficial effects through paracrine mechanisms. For example, AFSCs improved the kidney cell proliferation by release of different molecules, including glial cell line derived neurotrophic factor (GDNF)[161].

Finally, it has been demonstrated that AFSCs can integrate and differentiate into epithelial lung lineage, where they expressed alveolar and bronchiolar epithelial markers, including TFF1, SCP and CC10[157] in a hyperoxia lung damage. Here again, the release of different molecules by AFSCs allowing the recovery of lung structures in a rat model of bronchopulmonary dysplasia induced by hyperoxia[170].

1.6 Cardiac Stem Cells (CSCs)

For a long time the heart was considered as a postmitotic organ with a predetermined number of myocytes. According to this concept, the number of myocytes were established at birth and preserved until the death of individual, and the organ hypertrophy was restricted to myocyte enlargement[171-174].

Today, this old paradigm has been changed thanks to the discovery of SCs localized inside the heart.

CSCs are a population of c-Kit positive SCs that have the capacity to differentiate in caridiomyocytes and coronary vessels. CSCs are localized in cardiac myocardium, inside interstitial microdomains where they support other neighbor cells, including cardiomyocytes and fibroblasts[175]. Inside the heart, CSCs maintain the SC pool through asymmetric divisions, and allow the differentiation of progeny[176].

In adult hearth, there is a balance between functionally-competent CSCs and senescent CSCs; however, in pathological condition this balance can shift toward the senescence, increasing the pool of apoptotic cells and resulting in a deregulation of cardiac niche and myopathy[177,178].

In cardiac niche, CSCs are normally situated in atrial and apical myocardium, close to precursor cells, which are also positive for c-Kit receptor as CSCs, but express some myocyte markers, such as the contractile protein α-sarcomeric actin and the myocyte transcription factor Nkx2.5 (**Figure 8**)[179].

Similarly to BM niche, inside myocardium the oxygen tension regulates the homeostasis of CSCs. For instance, a low concentration of O_2 maintains CSCs in a quiescent and undifferentiated state; on the contrary normoxia could be necessary to regulate the CSC differentiation toward cardiomyocytes[13,180,181]. However, with aging the balance between hypoxia and normoxia is destabilized and produce an accumulation of hypoxic foci. This phenomenon leads to an increase of proliferating CSCs, toward a progressive reduction of CSC telomeres and formation of senescent dysfunctional cardiomyocytes[182].

Several Authors have been shown the existence and the role that CSCs conducts inside myocardium. Resident SCs were labeled with nucleotide analogs or lentiviral fluorescent tags[175-177,181]. The periodic administration of BrdU or ^{3}H-thymidine resulted in labeling of nuclei of cycling cells inside myocardium[183]. The cells with

the higher fluorescence corresponded to the more undifferentiated cells having the lower proliferation rate, while a dilution of the label occurred in cells with a higher proliferation rate, corresponding to cells more differentiated. These experiments allowed the localization of CSCs inside myocardium, which corresponded to the brighter cells in the atria[183]. Moreover, this pool of slow-cycling SCs remained constant during the chasing period, demonstrating that CSCs tend to preserve the pool of undifferentiated SCs in the cardiac niche.

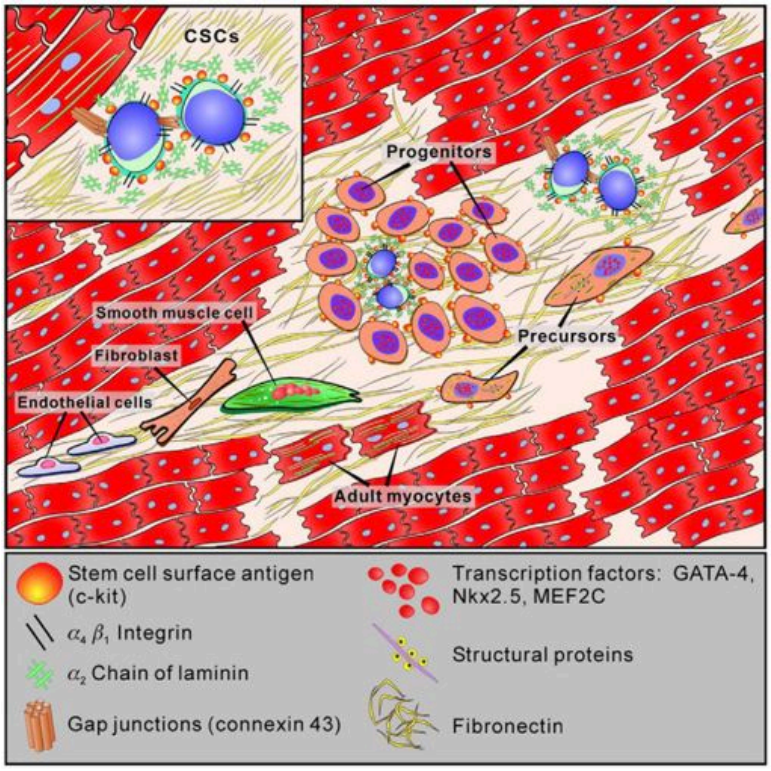

Figure 8. Schematic representation of the cellular and extracellular components of a CSC niche. (**modified from[179]**).

The differentiation potential of CSCs has been demonstrated both *in vitro* and *in vivo.* Transplanted CSCs are able to engraft within infarcted myocardium, differentiating in cardiomyocytes and coronary vessels[184-190].

Thus, CSCs are the more undifferentiated SCs inside the heart, and persist at all ages and with chronic heart failure. These finding have opened the way to a clinical application of these SCs in patients with severe ventricular dysfunction. It has been developed a method to isolate human functionally-competent CSCs from

endomyocardial biopsies from patients with advanced heart failure undergoing cardiac transplantation or implantation of ventricular assist device. When these SCs were cultured *in vitro* showed high myogenic and vasculogenic potential, and a high telomerase activity[191].

In a phase 1 trial SCIPIO (Stem Cell Infusion in Patients with Ischemic cardiomyopathy) c-Kit positive/lineage negative human CSCs are used for the treatment of chronic heart failure of ischemic origin[192,193]. After intracoronary injection, CSCs resulted efficacious in 7 out of 14 patients, while the others did not show adverse effects.

1.7 Lung Stem Cells (LSCs)

LSCs are c-Kit positive SC type isolated and characterized for the first time in 2011. These cells are self-renewing, clonogenic, and multipotent *in vitro*. When human LSCs were injected into mouse lung *in vivo*, they were able to form human bronchioles, alveoli, functionally vessel inside the damaged lung of mouse[194].

Human LSCs are negative for pulmonary lineage markers and do not express epitopes of HSCs or MSCs, excluding their potential derivation from the BM.

These SCs were found localized in bronchioles and in alveolar wall were they interacted with neighbor cells, such as epithelial cells, smooth-muscle cells and fibroblasts by adhesion molecules, which constituted the supporting cells in the LSC niches.

For restoration of damaged lung to occur, the cells inside the organ have to be form both distal airways that distal pulmonary vasculature. The lung is constituted by different cells, which have regenerative potential but with the limitation to form a single cell type. For example, bronchoalveolar stem cells, Clara cells, side population cells, and type II alveolar epithelial cells are able to generate only type I and type II pneumocytes or stromal cells[195-198]. On the contrary LSCs can differentiate in different populations of epithelial cells and into pulmonary vessels, which have endodermal and mesodermal origin respectively[199].

Moreover, the LSCs expressed the genes of pluripotency, that is homeobox transcription factor Nanog (NANOG), octamer-binding transcription factor 3/4 (OCT3/4), sex-determining-region Y–box 2 (SOX2), and Kruppel-like factor 4 (KLF4)[194].

These observations challenge the generally accepted belief that the lung is an organ lacking a hierarchical organization regulated by a compartment of resident SCs nested in the niche.

Aim of the work

Adult SC-based protocols are a promising approach for the treatment of human degenerative diseases[101,106,115,116,200-202]; however, some immunological concerns for their clinical application derive from the possibility that either allogeneic adult SCs may be rejected by the host immune system due to histoincompatibility[203], or SCs themselves may interfere with the physiological functions of host immune system. As previously described, some kinds of SCs, such as MSCs, not only possess regenerative potential but also may interact with and profoundly influence the IECs[25,50]. On the other hand, immune system plays a pivotal role in the pathogenesis and progression of many degenerative diseases; consequently, the application of SC-based approaches could be effective in both regenerating damaged tissues and modulating the pathological immune reactions[100-105,204,205]. For all these reasons, the immunogenicity and immunomodulatory properties of SCs must be carefully addressed before their clinical application.

An important question that rises spontaneously is whether immune modulation is a common property that is shared by all SC compartments or it is a specific feature of some kinds of SCs that are physiologically in contact with the immune system. The answer to this question is still lacking, because a comparative, extensive and standardized characterization *in vitro* and *in vivo* of SC immunological properties has not been carried out so far. In fact, different stimuli, IECs, functional assays, culture conditions and animal models have been employed by many Authors to assess the immunological properties of SCs.

Our study shows for the first time a comparison of different immunological features of various SC types derived from different tissues, in term of immunophenotype, immunomodulation, immunogenicity and anti-apoptotic capabilities. In particular, MSCs of different origin (bone marrow and olfactory mucosa), adult SCs from leptomeninges, amniotic fluid, and SCs isolated from myocardial and lung tissues, were examined by using of standardized assays, previously carried out in our lab[206].

The aim of our study was to assess the role of the immunological licensing of SCs of different origin. In fact, for MSCs it is now clear that the redundant panel of immunoregulatory mechanisms is not constitutively expressed, but its induction requires a process of “licensing” that allows MSCs to exert their immune regulatory

function. As previously described, this process implies: i) the "activation" of MSCs by means of inflammatory cytokines that are early produced by different cells as a consequence of antigen processing and IEC activation, such as IFN-γ or TNF-α; ii) the prevalence of priming stimuli on MSCs over signals that may hamper MSC inhibitory mechanisms, such as the triggering of TLRs by infectious agents or endogenous danger signals; iii) the timing of MSC engagement in the activation process of IECs. Many *in vitro* and *in vivo* data are available supporting the role of MSC licensing in the induction of a measurable and effective immune regulation[50-52]. The failure of some MSC-based protocols for immune modulation in animal models and in human clinical trials may be explained by either the lack of a proper licensing by inflammatory microenvironment or the wrong timing in MSC administration[50]. The optimization of SCs use for immune regulating purposes is required to maximize their beneficial effects. Thus, we compared the effect of licensing on the SCs of different origin above mentioned, to assess whether the immunological properties are equally inducible or there are some peculiarities.

Chapter 2

Materials and Methods

2.1 Isolation and culture of human SCs

BM-MSCs (five samples) were isolated from BM aspirates of healthy donors (informed consent, approved by Ethical Committee of Azienda Ospedaliera Universitaria Integrata Verona; N. 1828, May 12, 2010 ‘‘Institution of cell and tissue collection for biomedical research in Onco-Hematology’’). BM aspirates were cultured in 225-cm^2 flasks at $5x10^5$ nucleated cells/cm^2 concentration in alpha-minimal essential medium (α-MEM), 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin (all from Gibco). After 72 h, non-adherent cells were removed and the medium was replaced twice a week. Full characterization of BM-MSCs has been already described by our group elsewhere[59,60,206].

OE-MSCs (five samples) were obtained under a protocol approved by the local ethical committee (Comité de Protection des Personnes) of Marseille. Informed consent was given by each individual participating in the study, in accordance with the Helsinki convention (1964) and French law relating to biomedical research. OE-MSCs were purified as previously described[117]. Briefly, biopsies from the root of the medial aspect of middle turbinate or the septum in dorsomedial area were washed with Dulbecco modified Eagle medium (DMEM)/HAM F12 and digested for 1 h with dispase II solution (Boehringer). Then, the olfactory epithelium was removed from underlying lamina propria, which was cut into small pieces with 25 gauge needle. Tissue fragments were incubated with collagenase 1A to complete the tissue dissociation and, after centrifugation, the cell pellet was resuspended in α-MEM culture medium supplemented with 10% heat-inactivated adult bovine serum, 100U/mL penicillin, and 100mg/mL streptomycin (all from Gibco). Full characterization of OE-MSCs has been already described by our group elsewhere[117].

LeSCs (five samples) were obtained as previously described[126]from human brain/spinal cord samples including leptomeninges collected during neurosurgical procedures (informed consent, approved by Ethical Committee of Azienda Ospedaliera Universitaria Integrata Verona; N. 1974, June 1, 201i ‘‘Institution of meningeal cell and tissue collection for biomedical research in regenerative medicine for neurodegenerative and neurovascular diseases, and spinal cord injury’’). Leptomeninges were detached from neural parenchyma, dissociated, and washed with α-MEM; adherent cells were cultured in α-MEM, 10% heat-inactivated adult bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin (all from Gibco). Full characterization of LeSCs has been already described by our group elsewhere[125,126]and are part of another specific article.

AFSCs (six samples) were collected from confluent cultures of adherent cells derived from human amniocentesis carried out for diagnostic purposes (cytogenetic analysis), after mother’s informed consent; cells were harvested and immediately subjected to immunoselection. Sorted c-Kit positive AFSCs were grown in α-MEM medium (Gibco) containing heat-inactivated adult bovine serum, 100 U/mL penicillin, and 100mg/mL streptomycin (all from Gibco), supplemented with 18% Chang B and 2% Chang C (Irvine Scientific). Full characterization of AFSCs has been already described by our group elsewhere[150,151].

CSCs and LSCs (three samples each) were obtained from enzymatic dissociation of myocardial and lung tissues, respectively, and cultured as adherent cells as previously described. Full characterization of CSCs and LSCs has been already described by our group elsewhere[184,194].

All SC types were detached (0.05% Tripsin-EDTA; Gibco) and harvested when 80% confluent, and then either reseeded at $10^3/cm^2$ concentration or frozen until use. All experiments were performed between passages 2 and 6.

Each SC type was characterized in terms of phenotype and differentiation potential following ISCT-minimal criteria[32].

2.2 Immunofluorescence

SCs were harvested at 80% confluence and $2x10^4$ cells were seeded on glass dishes pretreated with gelatin or poly-d-lysine (Sigma Aldrich) in 24-well plates. After 24h, cultures were fixed with 4% paraformaldehyde and stained with appropriate antibody: mouse-anti-human CD73-PE (BD Biosciences) for BM-MSCs, rabbit-anti-human nestin (Abcys) for OE-MSCs, mouse-anti-human nestin (BD Biosciences) for LeSCs, rabbit-anti-human c-Kit (DAKO) for AFSCs, CSCs, and LSC; as secondary antibodies, goat-anti-rabbit- AlexaFluor-549, goat-anti-mouse-AlexaFluor-488, and donkey-anti-rabbit-FITC (all from Invitrogen) were used, respectively. Hoechst-33342 or TOPRO-3 (Invitrogen) were added to reveal nuclei. Images were obtained by fluorescence or confocal microscopy at 20x or 40x magnification.

2.3 Immunophenotyping

SCs at 80% confluence were stimulated or not for 48 h with 10 ng/mL IFN-γ and 15 ng/mL TNF-α (R&D Systems). The lack of cytotoxic effects of this inflammatory cytokines was previously confirmed[207]. Rested and primed SCs were stained with the following monoclonal antibodies against human markers: IgG1k-PE, CD54-PE, CD86-PE, CD106-PE, CD200-PE, and HLA-ABC-PE all from BD Biosciences; IgG1k-PE, CD80-PE, IgG1k-FITC, and HLA-DR-FITC all from Beckman Coulter; IgG1k-PE, CD112-PE, CD155-PE, IgG2b-PE, and CD274-PE all from Biolegend; IgG2a-PE, unconjugated IgG2a, MICA/B-PE, ULBP-1-PE, ULBP-2-PE, and unconjugated ULBP-3 all from R&D Systems, and goat-anti-mouse-PE from DAKO.
For staining, 10^5 SCs were incubated with the selected monoclonal antibody or appropriate isotype control in PBS for 15 min at room temperature. For ULBP-3 expression, PE-conjugated goat-anti-mouse IgG $F(ab')^2$ was added, after staining the cells with specific primary unconjugated antibody. According to manufacturer's instruction, ULBP-1 expression was validated by intracellular staining using the Cytofix/Cytoperm kit (BD Biosciences). Data were analyzed by FACSCalibur and FACSCanto (BD Biosciences) and expressed as the ratio of geometric mean

fluorescence intensity (rMFI) obtained for each marker and its isotype-matched negative control.

2.4 Immunomodulatory and survival assays

IECs ($CD3^{pos}$ T cells, $CD19^{pos}$ B cells, and $CD56^{pos}$ NK cells) were purified from peripheral blood using appropriate negative selection kits (Miltenyi Biotec) with at least 95% cell purity, as evaluated by flow cytometry. In each well, rested and primed SCs were seeded with IECs at either $2x10^4$ cell concentration (high ratio, corresponding to a confluent monolayer), or $2x10^3$ cell concentration (low ratio). After SC adhesion, $2x10^5$ T cells, $2x10^4$ B cells, or $2x10^4$ NK cells previously stained with 5 mM carboxyfluorescein succinimidyl ester (CFSE) from Life Technologies, were added. T cells were activated with 0.5 mg/mL cross-linking anti-CD3 and anti-CD28 antibodies (Sanquin) for 6 days in Roswell Park Memorial Istitute (RPMI) supplemented with 10% human AB serum. B cells were activated with 2 mg/mL $F(ab')^2$ anti-human IgM/IgA/IgG (Jackson Immunoresearch), 20IU/mL rhIL-2 (Proleukin; Novartis), 50 ng/mL polyhistidine-tagged CD40 ligand, 5 mg/mL anti-polyhistidine antibody (R&D Systems), and 2.5mg/mL CpG B (Invivogen) for 4 days in RPMI supplemented with 10% FBS (Invitrogen Life Technologies). NK cells were activated by 100 IU/mL rhIL-2 for 6 days in Iscove modified Dulbecco medium (IMDM) supplemented with 10% human AB serum. At the end of coculture, cells were detached by trypsin and stained with PerCP mouse anti-human CD45 monoclonal antibody from BD Biosciences and TOPRO-3 Iodide (Invitrogen Life Technologies); the proliferation was assessed on viable TOPRO-3^{neg} $CD45^{pos}$ cells by FACS analysis as the percentage of cells undergoing at least one cell division. The proliferation rate was obtained according to the following formula: (percentage of $CD45^{pos}$ cell proliferation with SCs)/(percentage of $CD45^{pos}$ cell proliferation without SCs)x100.

To evaluate the molecular pathways involved in immune regulation, the following specific inhibitors were added to SC/T cell cocultures: 1 mM L-N-monomethylarginin (L-NMMA), the inhibitor of inducible NO synthase (iNOS); 5 mM NS-398 (Cayman Chemicals), the inhibitor of cyclooxygenase-2 (COX-2) that is necessary for PGE2 synthesis; 1mM L-1- methyltryptophan (L-1MT), the IDO inhibitor (Sigma-Aldrich); 2

mM tin-protoporphyrin (SnPP), the inhibitor of HO-1 (Frontier Scientific); and 10 mg/mL purified anti-human IFN-γ NA/LE mouse IgG1 (BD Biosciences).

To determine the IEC survival, primed and rested SCs were seeded with IECs at the same ratios used for the immunosuppression assays. Cells were analyzed after either 4 days (B and NK cells) or 6 days of coculture (T cells). Cells were then harvested and stained with Allophycocyanin (APC) mouse anti-human CD45 BD Biosciences and lymphocyte survival was assessed following manufacturer's instructions (PE active caspase-3 apoptosis kit; BD Biosciences). Briefly, fixed and permeabilized cells were stained with PE-anti-caspase-3 antibodies and cell survival was assessed as percentage of active-caspase-3^{neg} CD45pos viable cells by FACS analysis.

2.5 Immunogenicity assay

Resting and primed SC immunogenicity was evaluated in a non-radioactive cytotoxicity assay using rh-IL-2 activated-NK cells as effector cells, following manufacturer's instructions (Delfia Cytotoxicity kit; Perkin Elmer). Briefly, SCs were loaded with bis-acetoxymethyl terpyridine dicarboxylate (BATDA) fluorescent dye and then incubated for 3h with various ratios of allogeneic NK cells preactivated for 48 h with 100 IU/mL rhIL-2. Cytotoxicity was quantified by assessing fluorescence release in coculture supernatants by a time-resolved fluorimeter (Victor^{TM}X4, Perkin Elmer).

2.6 Bioinformatics

Hierarchical cluster analysis of protein expression was used to group SCs with similar expression pattern. Molecular markers with differential expression pattern among various SC types were selected from the rMFI derived from immunophenotyping. The expression data were logarithm transformed and grouped using hierarchical clustering algorithm in Gene Cluster 3.0 program[208]. Heat-map was performed using Java Treeview program[209].

2.7 Statistical analysis

Data were expressed as mean standard deviation, except for immunophenotype data that were expressed as mean ± standard error of the mean. Statistical analysis was performed by Prism software (GraphPad) using the Wilcoxon test to compare the effect of priming on the same SCs, while one-way ANOVA test was used to assess the differences among SCs types. $P < 0.05$ was considered statistically significant.

Chapter 3

Results

3.1 IFN-γ and TNF-α strongly regulate SC phenotype

Previous reports have highlighted the differences among the SC types employed in this study in terms of marker expression, morphology (**Figure 8**.), differentiation potential, *in vivo* engraftment, and regenerative capabilities. The immunophenotype of resting and IFN-γ and TNF-α-primed BM-MSCs, OE-MSCs, LeSCs, AFSCs, CSCs, and LSCs was studied in parallel (**Table 2, Table 3 and Figure 10**), also by using a hierarchical cluster analysis (**Figure 9**). These SC types expressed HLA-ABC molecules at different levels. At baseline, HLA-ABC molecules were more abundant in OE-MSCs and LeSCs than in BM-MSCs, AFSCs, CSCs, and LSCs; however, following inflammatory priming, HLA-ABC molecules increased in all SC categories, and significant changes occurred in LeSCs (2.6-fold), AFSCs (2.2-fold), CSCs (2.0-fold), and LSCs (3.2-fold). In control conditions, HLA-DR was scarcely or partially expressed by BM-MSCs, but after inflammatory priming, it dramatically increased in BM-MSCs, OE- MSCs, and LSCs; low levels of HLA-DR were detected in primed AFSCs, LeSCs, and CSCs. The costimulatory molecules CD80 and CD86 were undetectable in all SC types with the exception of resting CSCs; however, CD86 was no longer detectable in CSCs after inflammatory priming. CD40 was weakly expressed in resting OE-MSCs and AFSCs, but a significant increase in CD40 was observed after inflammatory priming in BM-MSCs (3.2-fold), OE-MSCs (2.0-fold), and LeSCs (3.8-fold). Primed AFSCs did not upregulate CD40, and CSCs and LSCs never expressed this marker.

Two adhesion molecules CD54 (ICAM-1) and CD106 (VCAM-1), which are involved in cell migration and interaction between MSCs and inflammatory cells[85,210,211], were tested. CD54 was highly expressed in OE-MSCs, and low levels of this molecule were detected in the other SCs. CD54 was markedly upregulated in all primed SC types (BM-MSCs, 174.6-fold, OE-MSCs, 39.6-fold, LeSCs, 112.0-fold, AFSCs, 44.7-

fold, CSCs, 47.0-fold, and LSCs, 129.0-fold). CSCs and LSCs did not express CD106 at baseline, and only primed CSCs showed CD106 weakly.

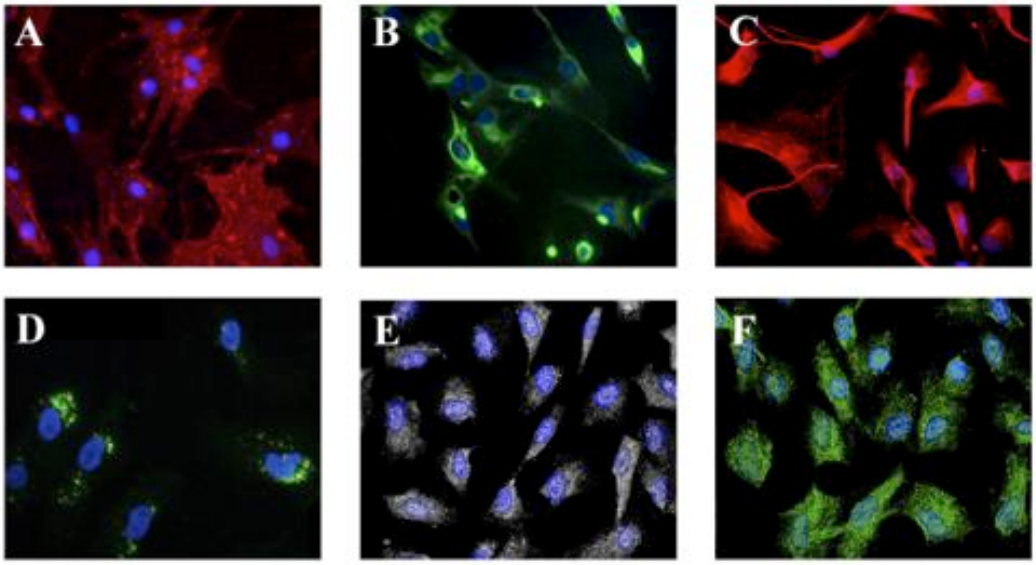

Figure 9. Representative immunofluorescence staining of various human stem cell (SC) types. (**A**) Bone marrow (BM)-mesenchymal stromal cells (MSCs) were stained with anti-CD73-PE (red) and TOPRO-3 (blue); (**B**) olfactory ectomesenchymal SCs (OE-MSCs) were stained with anti-Nestin (green) and Hoechst-33342 (blue); (**C**) leptomeningeal SCs (LeSCs) were stained with anti-Nestin (red) and TOPRO-3 (blue); (**D**) amniotic fluid SCs (AFSCs) were stained with anti-c-Kit (green) and TOPRO-3 (blue); (**E**) cardiac SC (CSCs) were stained with anti-c-Kit (white) and TOPRO-3; (**F**) lung SCs (LSCs) were stained with anti-c- Kit (green) and TOPRO-3 (blue). Scale bars: 50 mm (**A–C**); 20 mm (**D–F**).

Conversely, BM-MSCs, OE-MSCs, LeSCs, and AFSCs constitutively expressed CD106, which was significantly upregulated after priming (BM-MSCs, 4.3-fold, OE-MSCs, 2.0-fold, LeSCs, 12.0-fold, and AFSCs, 14.0-fold).

CD200 and CD274 (PD-L1) modulate the immune response via a cell-to-cell contact-dependent mechanism[212-214]. CD200 was highly expressed in CSCs, modestly present in BM-MSCs and OE-MSCs, and absent in AFSCs, LeSCs, and LSCs. After inflammatory priming, a 2.7-fold downregulation of CD200 occurred in CSCs while no changes were observed in the other SCs. At baseline, CD274 was constitutively expressed in all SCs but mostly in OE-MSCs and AFSCs. Inflammatory priming resulted in a proportional upregulation of CD274 in the various SC categories.

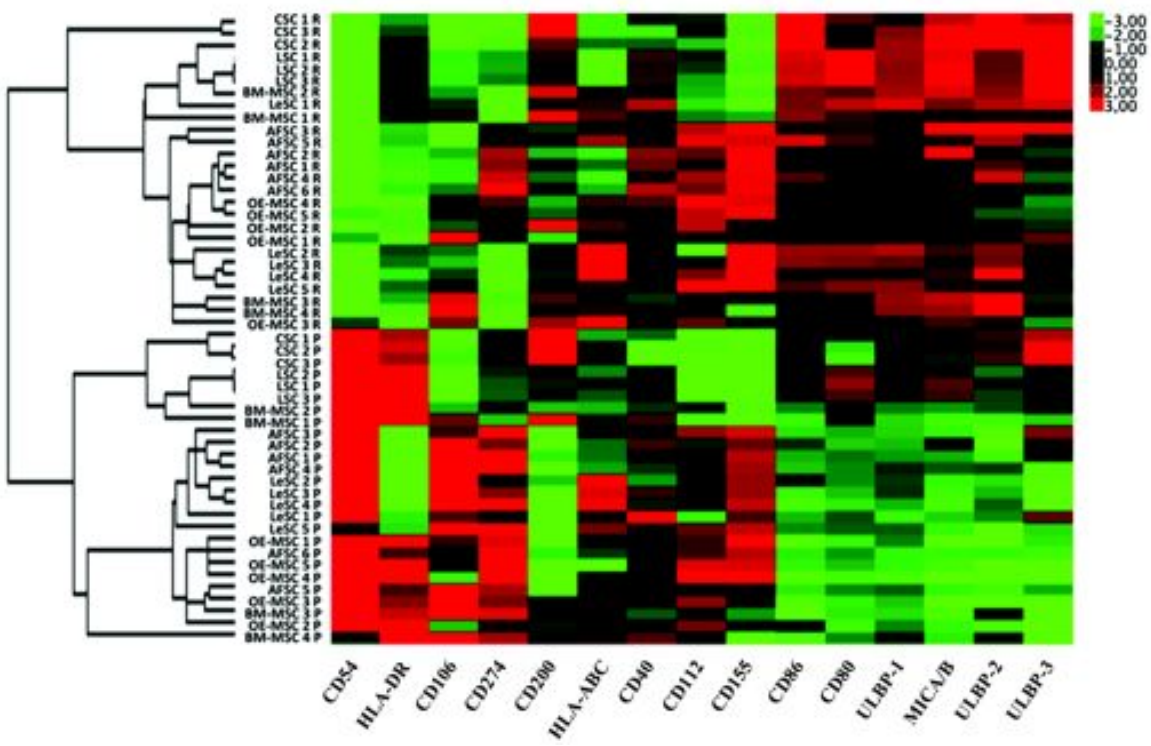

Figure 10. **Hierarchical cluster analysis of protein expression of various human SCs in resting and primed conditions**. Modulation of expression of fifteen proteins in resting and inflammatory-primed conditions. Heat map showed the down (green) or upregulation (red) of protein expression. Different samples were grouped using hierarchical clustering algorithm.

The NK cell-activating ligands were evaluated[96,215]. CD112 (nectin-2) and CD155 Poliovirus receptor (PVR), which stimulate the DNAX Accessory Molecule-1 (DNAM-1) receptor, were expressed at high levels in all SCs at baseline. Inflammatory priming increased by ~ 2-fold the expression of CD112 and CD155 in all SCs but CSCs and LSCs.

In the latter two cases, priming did not alter the levels of CD112 and CD155. The ligands of the NKG2D receptor, MICA/B and ULBPs, were then examined. ULBP-1, ULBP-2, ULBP-3, and MICA/B were not particularly abundant at resting conditions and after inflammatory priming in all SC populations.

Overall, the immunophenotype observed at resting and inflammatory conditions show many similarities among different SC types; moreover, the clustering analysis highlighted a clear distinction between resting and primed SCs, which suggests a common pattern of protein modulation induced by inflammatory mileu (**Figure 9**). These data are in agreement with defective antigen-presenting cell functions

(expression of HLA class I and class II molecules without coexpression of costimulatory molecules) and activating capability toward NK cells due to the partial expression of NK cell-activating receptors possibly leading to SC rejection; nevertheless, the latter activity may be counterbalanced following inflammatory priming by the upregulation of HLA class I molecules triggering inhibitory NK receptors.

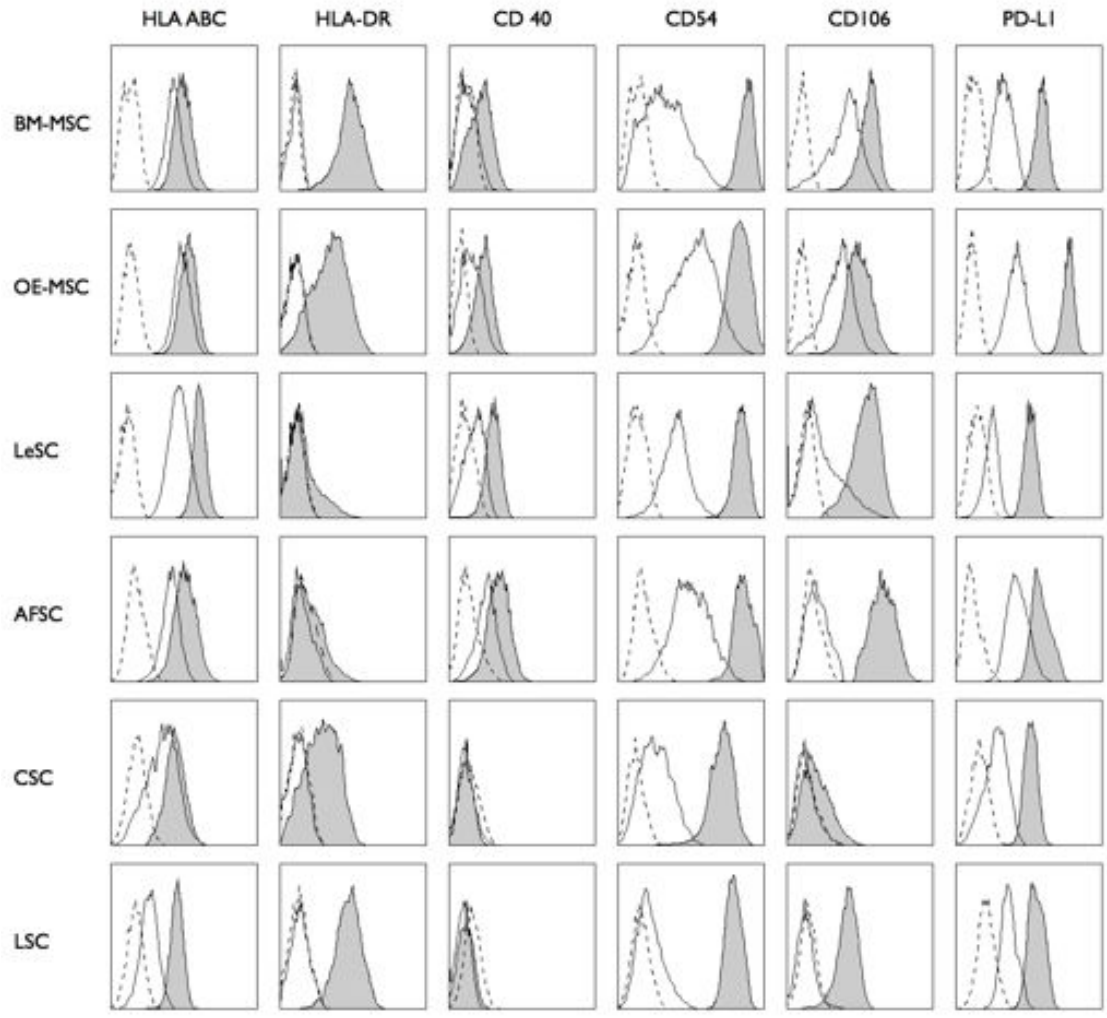

Figure 11. Expression of different markers on various human stem cells (SCs) in resting and primed conditions. Representative FACS analysis of different SC types showing the expression of HLA-ABC, HLA-DR, CD40, CD54 (ICAM-1), CD106 (VCAM-1), and CD274 (PD-L1) at resting and inflammatory conditions. Dashed curve indicates the isotype controls, while open and filled curves indicate resting and primed SCs, respectively.

SC Types Marker	**BM-MSCs**	**OE-MSCs**	**LeSCs**	**AFSCs**	**CSCs**	**LSCs**
MHC and Costimulatory Molecules						
HLA-ABC	16.97±2.48	29.42±5.46	22.83±4.32	11.29±2.98	5.15±0.17	2.74±0.1
	27.37±6.1	***35.4± 5.47***	***59.34±10.2****	***25.3±3.82****	***10.37±0.66****	***8.7±0.6****
HLA-DR	1.42±0.21	1±0	1.04±0.04	1.02±0.02	1±0	1.1±0.04
	21.91±3.61*	***15.04±2.56****	***1.58±0.17***	***2.66±0.91***	***3.53±0.1****	***12.06±0.3****
CD40	1.29±0.14	1.93±0.17	1.19±0.12	2.1±0.26	1±0	1±0
	4.14±0.74*	***3.8±0.27****	***4.55±0.66****	***2.4±0.07***	***1±0***	***1±0***
CD80	1±0	1±0	1±0	1±0	1±0	1±0
	1.36±0.04	***1±0***	***1±0***	***1±0***	***1±0***	***1.07±0.06***
CD86	1.24±0.07	1.09±0.09	1.11±0.05	1.26±0.16	2.2±0.15	1±0
	1.02±0.02	***1.34±0.31***	***1.18±0.18***	***1.17±0.09***	***1.06±0.05****	***1±0***
Adhesion Molecules						
CD54	6.83±2.68	35.28±6.51	9.67±1.2	14.04±4.78	4.43±0.9	2.1±0.14
	1193±98**	***1400±156*****	***1082±101*****	***626±60.9*****	***208±14.54*****	***271±13.5*****
CD106	5.8±2.08	6.11±1.54	2.06±0.19	1.89±0.27	1±0	1±0
	25.3±10.62	***12.47±4.62***	***25±5.12****	***26.58±7.3****	***1.57±0.07****	***5.16±0.4****
Immunomodulatory						
CD200	3.47±1.56	2.82±0.76	1.06±0.02	1.17±0.06	17.24±6.64	1±0
	3.73±1.06	***1.86±0.45***	***1.15±0.12***	***1.18±0.1***	***6.03±0.42***	***1±0***
PD-L1	5.28±0.55	15.68±2.68	2.17±0.14	23.88±4.2	3.72±0.21	4.37±0.1
	76.14±6.65**	***96.42±26.51****	***60.45±17.3****	***80.2±12.7*****	***12.63±0.38*****	***8.4±1.17*****

Table 2. Different expression of various markers involved in immunological effects of SCs. Cells were cultured for 48 h under normal or inflammatory conditions, stained with different antibodies and analyzed by flow cytometry. Data are presented as mean ± SEM of relative Mean Fluorescence Intensity (rMFI) of five (BM-MSCs, OE-MSCs, AFSCs, and LeSCs) or three (CSCs, LSCs) different experiments derived from resting (upper) and primed (below) SCs for each marker.*P < 0.05, **P < 0.01, ***P < 0.001. SC, stem cell; BM-MSC, bone marrow-mesenchymal stromal cells; OE-MSC, olfactory ectomesenchymal SCs; LeSC, leptomeningeal SCs; AFS, amniotic fluid SCs; CSC, cardiac SCs; LSC, lung SCs.

SC Types Marker	**BM-MSCs**	**OE-MSCs**	**LeSCs**	**AFSCs**	**CSCs**	**LSCs**
Activating NK ligands						
CD112	11.87±2	30.86±4.75	12.5±4.33	23.7±1.37	7.18±1.21	4.95±0.26
	26.45±5.67*	***62.38±8.78*****	***27.87±5.07*****	***33.8±4.2***	***5.23±0.02***	***3.54±0.16***
CD155	17.9±3.78	57.86±12.8	57.82±20.35	78.48±17.9	7.9±1.47	6.23±0.44
	29.48±7.84	***128.5±31***	***103.8±12****	***102.7±13.04***	***6.88±0.04***	***8.2±0.3***
MICA/B	1.7±0.24	1.19±0.17	1.07±0.08	1.75±0.4	1.57±0.06	1.15±0.02
	1.16±0.1	***1.06±0.06***	***1±0***	***1.43±0.36***	***1.03±0.03***	***1.18±0.08***
ULBP-1	1.79±0.21	1.43±0.08	1.43±0.04	1.23±0.02	1.14±0.06	1±0
	1.67±0.27	***1.58±0.13***	***1.47±0.16***	***1.46±0.1***	***1.31±0.06***	***1±0***
ULBP-2	3.73±1.8	2.47±0.28	2.31±0.45	3.12±0.55	3.84±0.02	1.4±0.01
	3.04±0.66	***1.54±0.23****	***2±0.3***	***1.88±0.23****	***2.38±0.08***	***1.12±0.06***
ULBP-3	2.3±1.04	1.3±0.27	1.16±0.12	1.64±0.59	2.2±0.07	3.24±0
	1.35±0.2	***1.15±0.1***	***1.54±0.46***	***2.38±0.68***	***2.6±0.11****	***1.04±0.03*****

Table 3. **Different expression of NK activating ligands on SCs.** Cells were cultured for 48 h under normal or inflammatory conditions, stained with different antibodies and analyzed by flow cytometry. Data are presented as mean ± SEM of relative Mean Fluorescence Intensity (rMFI) of five (BM-MSCs, OE-MSCs, AFSCs, and LeSCs) or three (CSCs, LSCs) different experiments derived from resting (upper) and primed (below) SCs for each marker.*P < 0.05, **P < 0.01, ***P < 0.001. SC, stem cell; BM-MSC, bone marrow-mesenchymal stromal cells; OE-MSC, olfactory ectomesenchymal SCs; LeSC, leptomeningeal SCs; AFS, amniotic fluid SCs; CSC, cardiac SCs; LSC, lung SCs.

3.2 SCs share immunomodulatory properties

MSCs from several organs exert comparable regulatory effects on cells belonging to the innate and acquired immunity. Thus, we determined whether the variety of SC types included in the current study possessed similar immunological properties. Resting or primed SCs were cocultured with purified T-, NK-, and B cells to evaluate their ability to induce proliferation of unstimulated IECs. All SC types neither activated nor promoted IEC growth (**data not shown**). Then, the role of SCs in IEC replication was assessed by using different IEC:SC ratios, which ranged from 10:1 (high ratio) to 100:1 (low ratio) for T cells, and from 1:1 (high ratio) to 10:1 (low ratio) for NK and B cells, according to the standardized approach previously used with BM-MSCs[206]. At resting and primed conditions, none of the SC types displayed a modulatory function on IECs at low ratios (**data not shown**). Conversely, at high ratios, all SCs inhibited T cell division at resting conditions, and significantly more following pretreatment with inflammatory cytokines (**Figure 11**). OE-MSCs, LeSCs, CSCs, and LSCs showed a greater immunomodulatory effect than AFSCs and BM-MSCs.

All SCs with the exception of LeSCs displayed inhibitory properties on NK cells in absence of pretreatment with IFN-γ and TNF-α (**Figure 12**). Resting and primed OE-MSCs inhibited proliferation of NK cells by more than 90%. Resting LSCs and CSCs had an intermediate effect on NK cell proliferation, and BM-MSCs and AFSCs had the lowest. Primed BM-MSCs had a partial enhanced immunosuppressive activity, a response that was not detected in AFSCs, LSCs, and CSCs. LeSCs had no effect at baseline but, following priming, manifested a strong immunodulatory function comparable to OE-MSCs, and higher than BM-MSCs.

In the absence of inflammatory stimuli, MSCs cannot inhibit B cell growth due to the lack of IFN-γ release[59]. Although resting LSCs enhanced B cell proliferation, the other resting SC types failed to demonstrate any immunomodulatory function on B cells (**Figure 13**). However, primed SCs inhibited B cell replication. OE-MSCs and LeSCs showed an effect that was significantly greater than in the other SCs including BM-MSCs. Collectively, distinct SC categories possess common immunomodulatory properties, which at times differ qualitative and quantitatively (**Table 4).**

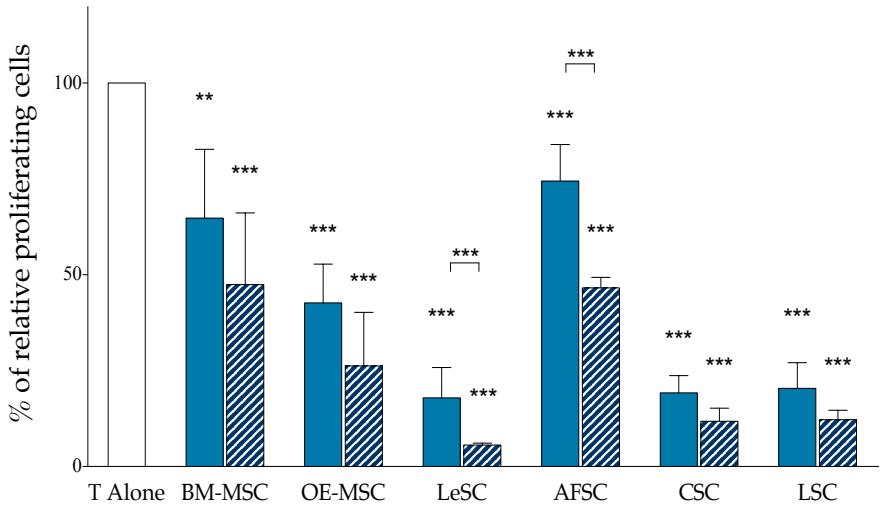

Figure 12. HumanSC inhibitory effect on stimulated T cell proliferation. Human T cells (were stimulated with anti-CD3 and anti-CD28 and cultured alone (□ bar) or in the presence of resting (■ bars) or primed (▨ bars) allogeneic human SCs. At the end of coculture, lymphocyte proliferation was assessed using carboxyfluorescein succinimidyl ester (CFSE) dilution method, as described in Materials and Methods section. CFSE fluorescence was analyzed after 6 days at 10:1 T/SC ratio. The results are expressed as relative proliferation percentage of IEC, normalized to IEC cultured alone (100%). Error bars represented mean ± SD of five independent experiments for BM-MSCs, OE-MSCs, LeSCs, and AFSCs and three independent experiments for CSCs and LSCs. **P < 0.01, ***P < 0.001.

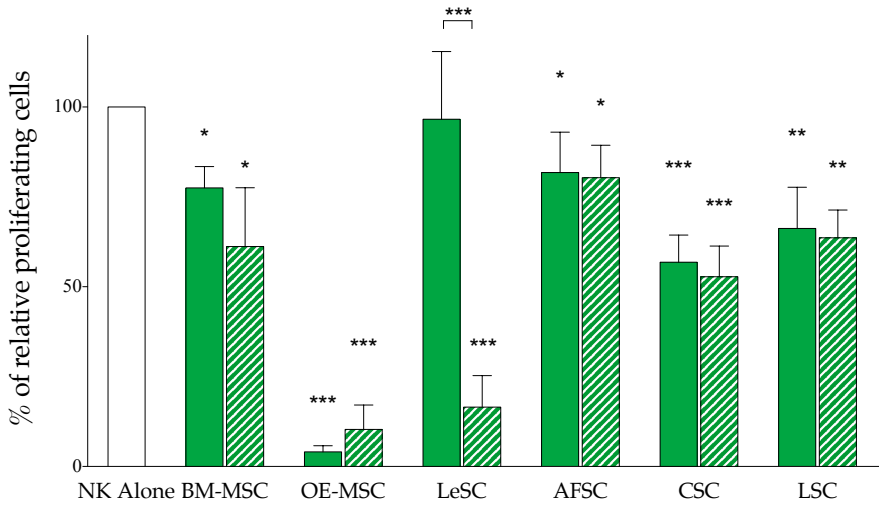

Figure 13. Human SC inhibitory effect on stimulated NK cell proliferation. Human NK cells were stimulated with IL-2 and cultured alone (□ bar) or in the presence of resting (■ bars) or primed (▨ bars) allogeneic human SCs. CFSE fluorescence was analyzed after 6 days at 1:1 NK/SC ratio. The results are expressed as relative proliferation percentage of IEC, normalized to IEC cultured alone (100%). Error bars represented mean ± SD of five independent experiments for BM-MSCs, OE-MSCs, LeSCs, and AFSCs and three inde- pendent experiments for CSCs and LSCs. *P < 0.05, **P < 0.01, ***P < 0.001.

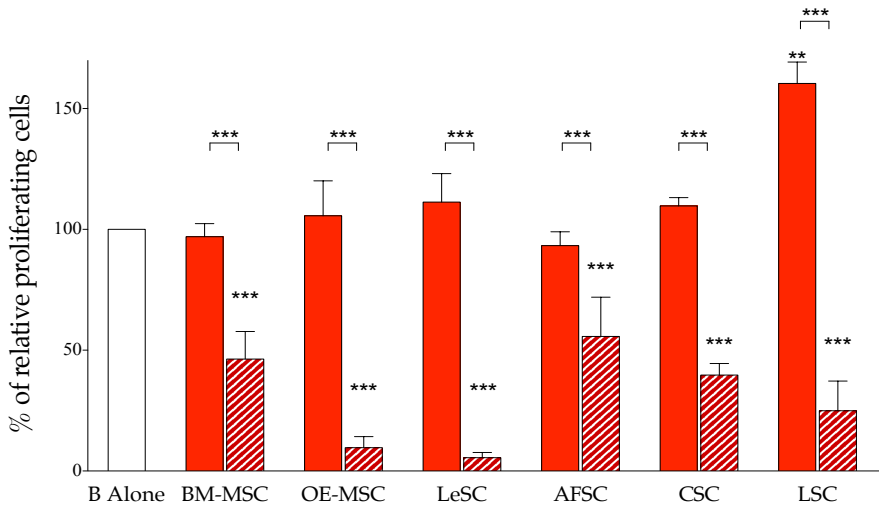

Figure 14. HumanSC inhibitory effect on stimulated B cell proliferation. Human B cells were stimulated with specific F(ab')^2 anti-human IgM/IgA/IgG, rhIL-2, CD40 ligand, anti-polyhistidine antibody, and CpG, and then were cultured alone (□ bar) or in the presence of resting (■ bars) or primed (▨ bars) allogeneic human SCs. CFSE fluorescence was analyzed after 4 days at 1:1 B/SC. The results are expressed as relative proliferation percentage of IEC, normalized to IEC cultured alone (100%). Error bars represented mean ± SD of five independent experiments for BM-MSCs, OE-MSCs, LeSCs, and AFSCs and three inde- pendent experiments for CSCs and LSCs. **P < 0.01, ***P < 0.001.

3.3 Molecular pathway in SC-mediated immunomodulation

To dissect the mechanisms responsible for the impact of SCs on T cell division, specific inhibitors (L-1MT, LNMMA, NS-398, and snPP) were added to SC-T cell cultures at doses that did not alter T cell viability and growth. The neutralizing anti-IFN-γ monoclonal antibody was also used to define the role of this cytokine in immune modulation. With the exception of resting OE-MSCs, L-1MT completely rescued T cell proliferation in the presence of resting or primed SCs; this result indicates that IDO is a major determinant of the anti-proliferative effects that SCs have on T cells (**Figure 14**). Conversely, inhibitors COX-2 (NS-398), HO-1 (snPP), and IFN-γ blocking antibody only partly restored T cell replication. The iNOS inhibitor LNMMA, however, failed to restore T cell division in the presence of either resting or primed human SCs (**data not shown**). NS-398 interfered with PGE2 synthesis attenuating the effects of resting LeSCs and primed BM-MSCs. Interestingly, snPP-mediated HO-1 inhibition modestly reactivated CSC-induced T cell suppression.
Collectively, our observations suggest that the anti-proliferative effects of SC types on T cells is predominantly driven by the activation of IDO and the degradation of tryptophan (**Figure 14**), an essential factor for T cell division and the generation of kynurenine, a critical immunomodulatory molecule.

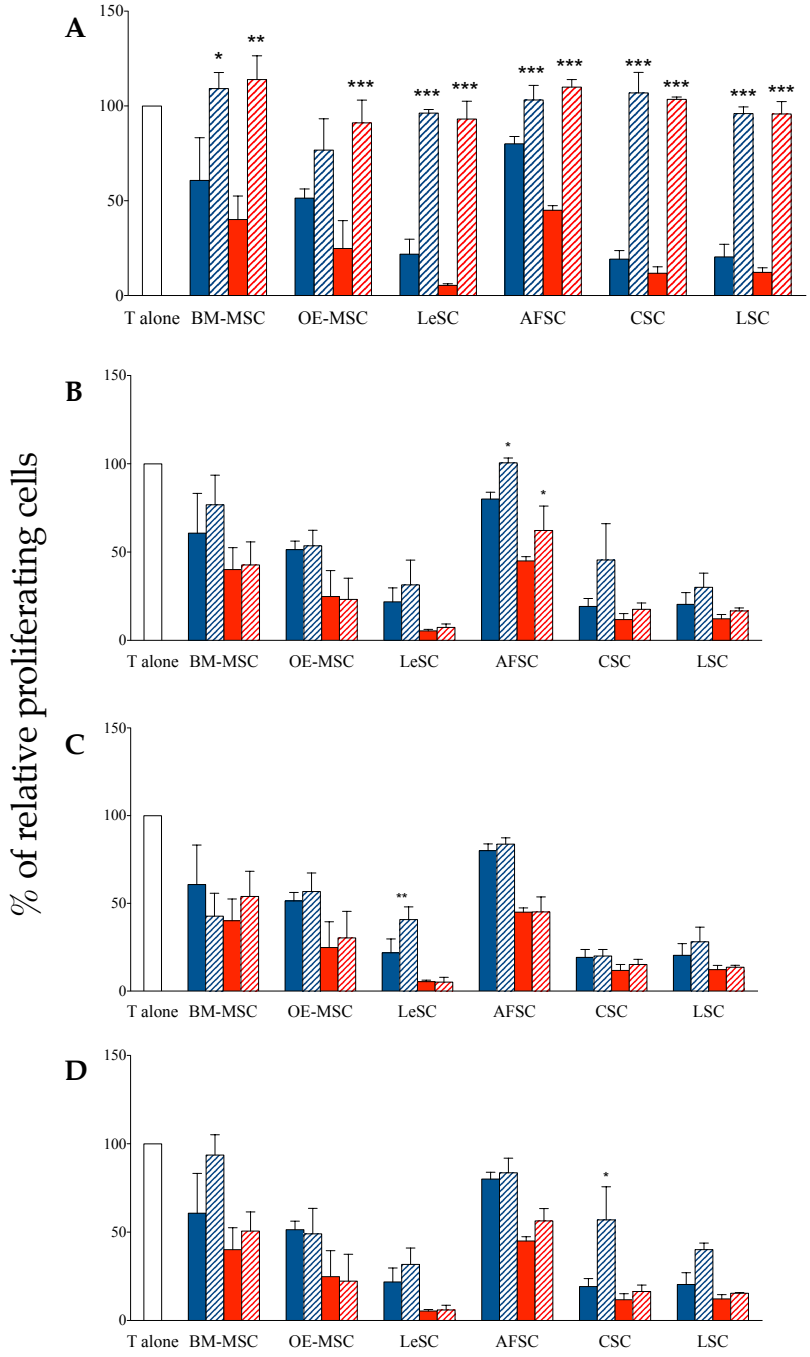

Figure 15. Effect of specific inhibitors on T cell proliferation. Stimulated T cells were cultured alone (□ bars)or in presence of different resting (■ bars) and primed (■ bars) SC types (at 10:1 T/SCs ratio). In each coculture the following inhibitors were added both on resting (▨ bars) and primed (▨) SC/T cell coculture: L-1MT (A), Anti-IFN-g (B), NS-398 (C), and snPP (D). After 6 days, cells were harvested and T cell proliferation was evaluated by FACS analysis. The results are expressed as relative proliferation percentage of T cells, normalized to T cells cultured alone (100%). Error bars represented mean ± SD of five independent experiments for BM-MSCs, OE-MSCs, LeSCs, and AFSCs and three independent experiments for CSCs and LSCs. *P < 0.05, **P < 0.01, ***P < 0.001.

3.4 SC immunogenicity and NK-mediated lysis

Treatment of BM-MSCs with inflammatory cytokines reduces the susceptibility of these cells to NK cell-mediated lysis[96]. This phenomenon is initiated by the expression in BM-MSCs of surface molecules, induced by the inflammatory milieu, which have the ability to activate or inhibit NK receptors[215,216]. These ligands, including CD112, CD155, MICA/B, ULBPs, and HLA class I and II molecules, are present in the SC types studied here (**Table 2 and Table 3**). To assess whether SCs are sensitive to NK cell-mediated cytotoxicity, BATDA stained-SCs were cocultured with different ratios of IL-2-stimulated NK cells. Cytotoxicity activity was measured by the quantity of BATDA released in the medium that is proportional to NK cell-mediated lysis. NK cells were capable of partially lysing resting SCs, and this effect was directly proportional to the increase of NK/SC ratio (**Figure 15**). However, an opposite effect was observed when SCs were pretreated with IFN-γ and TNF-α; SCs were significantly less vulnerable, suggesting that the differential expression of surface molecules attenuates the susceptibility of SCs to NK-mediated lysis.

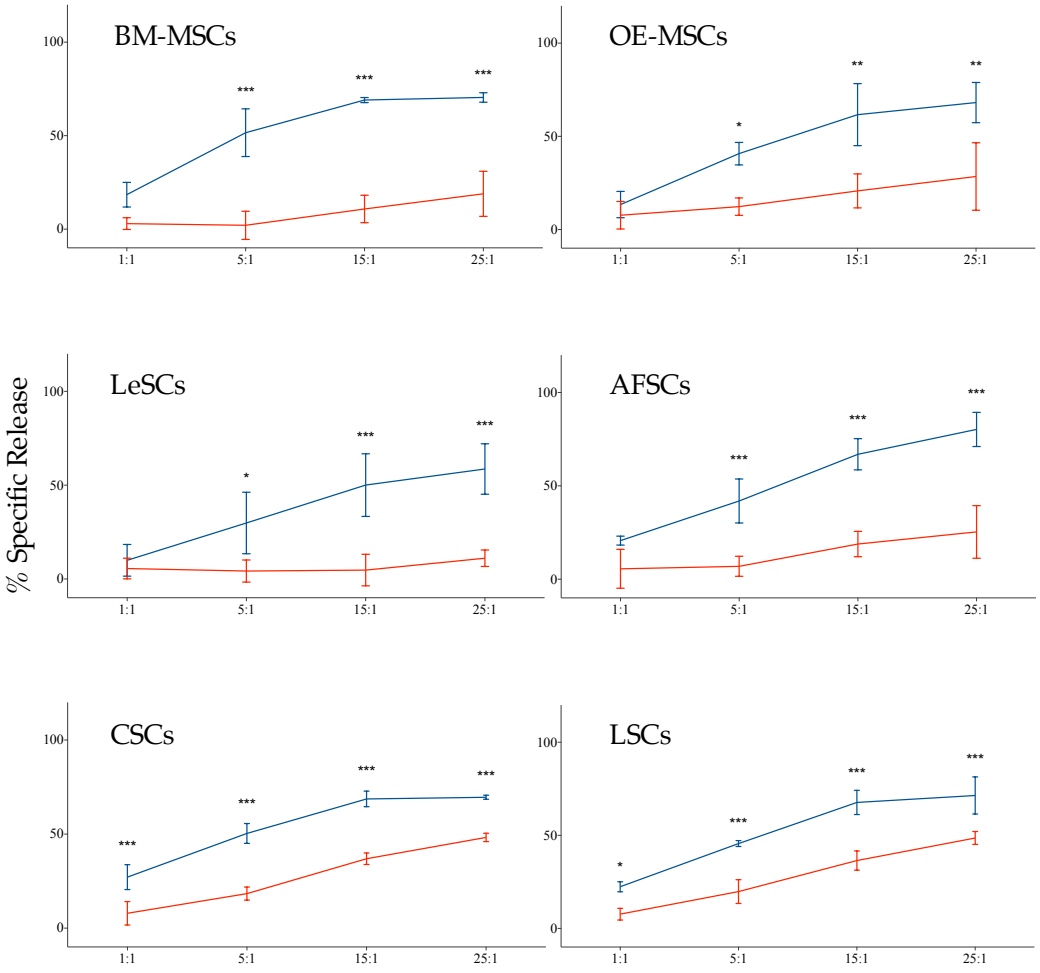

Figure 16. Immunogenicity of resting and primed human SCs. To evaluate NK cytotoxicity, SCs were labeled with BATDA (as reported in Materials and Methods section) and cocultured with IL-2-stimulated NK cells at different NK/SC ratios. The amount of cytotoxicity was calculated as release of fluorescence by lysed SCs, and detected by time-resolved fluorimeter (Victor X4 Multilabel Plate Reader, PerkinElmer). Each graphic shows the results obtained from five independent experiments, in which resting (blu line) and primed (red line) SCs were used as target cells. Data are expressed as percentage of fluorescence release. Error bars represented mean ± SD of five independent experiments for BM- MSCs, OE-MSCs, LeSCs, and AFSCs and three independent experiments for CSCs and LSCs. *P < 0.05, **P < 0.01, ***P < 0.001. BATDA, bis-acetoxymethyl terpyridine dicarboxylate.

3.5 SC and lymphocyte survival

Resting MSCs prevent T cell apoptosis promoted by the engagement of T cell receptors[217]. On the basis of these observations, we tested whether different SC types were capable of protecting IEC survival and whether this effect was modulated by inflammatory priming. Unstimulated T, NK, and B cells were cultured alone or in presence of either resting or primed SCs at different ratios. After 4 days (B and NK cells) and 6 days (T cells), cocultured cells were harvested and stained with anti-active-caspase 3 antibody, and analyzed by flow cytometry. Resting and primed SCs significantly reduced IEC apoptosis (**Figure 16**). This phenomenon was less apparent in T cells, possibly due to the higher survival of this cell type with respect to B and NK cells. We also found a dose-dependent anti-apoptotic effect of BM-MSCs, OE-MSCs, and LeSCs on B cells.

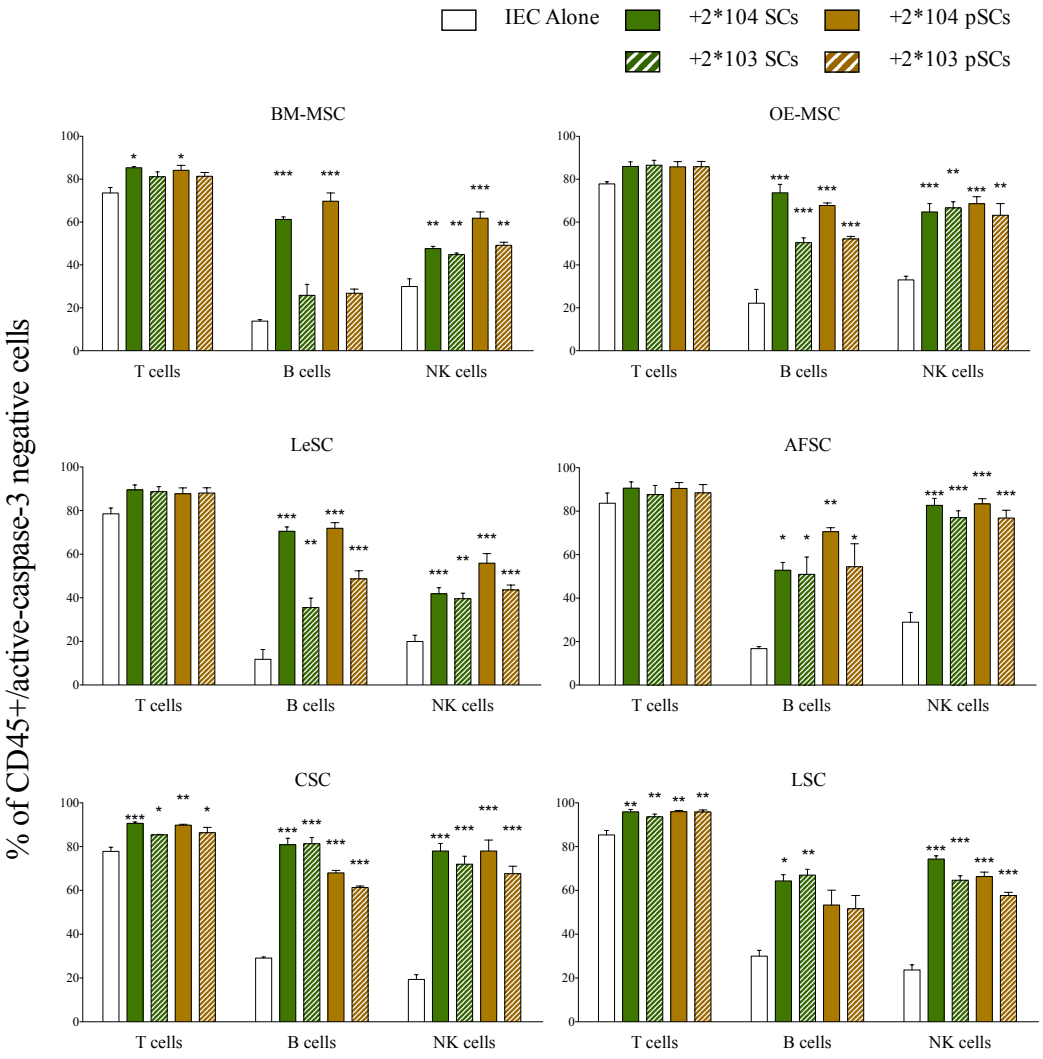

Figure 17. Trophic support of resting and primed human SCs on different immune effectors. Resting and primed SCs were cocultured with unstimulated human IECs (ie, T, B, and NK cells). At the end of coculture, immune cell survival was detected by measuring cytosolic active-caspase-3 (as reported in Materials and Methods section). The results are expressed as percentage of caspase-$3^{neg}CD45^{pos}$ cells, and the analysis was performed by flow cytometry. Error bars represented mean ± SD of five independent experiments for BM-MSCs, OE-MSCs, LeSCs, and AFSCs and three independent experiments for CSCs and LSCs. *P < 0.05, **P < 0.01, ***P < 0.001.

SC Types/IECs	Immune Modulatory Effect			Molecular Mechanism	Immunogenicity
	T	**NK**	**B**	**T**	**NK**
BM-MSCs	+	+	≅	IDO	***
pBM-MSCs	++	+	++	***IDO***	*
OE-MSCs	++	+++	-	//	***
pOE-MSCs	++	+++	+++	***IDO***	*
LeSCs	+++	≅	-	IDO/COX-2	***
pLeSCs	+++	+++	+++	***IDO***	*
AFSCs	+	≅	≅	IDO/IFN-γ	***
pAFSCs	++	?	+	***IDO/IFN-γ***	*
CSCs	+++	+	-	IDO/HO-1	***
pCSCs	+++	+	++	***IDO***	**
LSCs	+++	+	-	IDO	***
pLSCs	+++	+	+++	***IDO***	**

Inhibition Range (%)	**Symbol**
Supportive effect	-
0-20	≅
20-50	+
50-75	++
>75	+++
Susceptibility to NK-mediated lysis (%)	**Symbol**
0-30	*
30-50	**
>50	**

Table 4. Results derived from immunomodulatory and immunogenicity assays are summarized to compare different SC type behavior; p:inflammatory primed. For immunogenicity assay, NK:SC ratio is 25:1

Chapter 4

Conclusions

Cell loss, inflammation, immune system activation, and cell renewal occur following tissue injury. Alterations in this sequence of events result in inadequate organ repair giving rise to pathologic states[25,205,218,219]. Inflammatory priming has been viewed as a requirement for MSCs to have immune modulatory properties *in vitro* and *in vivo*[25,50,101,106,115,204,220]. The origin of MSCs, the secretion of soluble factors conferring proper SC licensing, and the level of inflammation at the site of cell delivery may account for some of the differences reported in the literature[50]. However, the immune modulatory function of BM-MSCs has been documented in graft-versus-host disease[101,106,115], autoimmune encephalomyelitis[102], sepsis[201], collagen-induced arthritis, and bowel inflammation[103-105]. But whether other SC categories have the ability to exert comparable effects was unknown and our results provide novel information supporting the view that a variety of SCs share the critical aspect of being immune privileged.

An important distinction has to be emphasized among the SC types analyzed here. It is generally considered that the regenerative potential of transplanted SCs may be exerted either directly, through the engraftment of SCs inside the damaged tissue followed by proliferation and differentiation into novel terminally differentiated cell progeny, or indirectly, through the release of soluble factors favoring the healing processes mediated by resident SCs and contrasting the negative effect of inflammatory phenomena. The predominant therapeutic efficacy of MSCs and MSC-like SCs is mediated via a paracrine mechanism associated with the release of several cytokines that profoundly influence the response of IECs[59,65,83,95,221-224]. This principle applies to all SCs that modulate inflammation and have a limited capacity to generate a specialized progeny. However, they indirectly activate resident SCs, enhancing the repair of the organ[225-227]. Because of these characteristics, therapeutically, MSCs and MSC-like SCs may have to be repeatedly employed to exert their role long-term, in absence of clear evidence of their homing and persistence inside the

tissues[100,102-105,204,205]. Conversely, the delivery of tissue-specific multipotent adult SCs, such as CSCs and LSCs, to the corresponding damaged organ fails to stimulate the resident SCs nested in proximity to or distant from the injured parenchyma[196,228]. Their exclusive beneficial effect is linked to their engraftment, expansion, and ultimately the regeneration of functionally-competent cells and vessels[184,194]. Thus, two important aspects have to be discussed. First, the recognition obtained in the current study *in vitro* that the analyzed SCs interfere with the inflammatory microenvironment is strongly consistent with their inherent ability to home to and divide within the damaged tissue, which has been previously demonstrated experimentally[123-126,157,158,194,196,228], in absence of inflammation in the parenchyma surrounding the integrated SCs shortly after their administration, or chronically in the regions adjacent to the regenerated tissues. In other words, our data suggest that the immune modulatory properties of SCs here described *in vitro* for the first time may have a role *in vivo* in favoring their regenerative potential. Secondly, all SCs become structurally and functionally coupled with the cells of the recipient organ, creating new niches in which the SCs can divide asymmetrically[123-126,157,158,194,196,228], thus ensuring the preservation of the SC pool and the formation of parenchymal and vascular cells[176,184,194]. It is likely that the potential clinical implementation of these SC populations is significantly strengthened by their immunomodulatory function. *In vivo* studies with each SC type are mandatory to assess the role of these immunological features in SC engraftment and regenerative potential. Further *in vivo* studies in animal models will clarify the immunological results obtained here.

As far as the general features related to the acquisition of the immune regulatory functions are concerned, the immunophenotype of the different SCs revealed a common switch, in response to inflammatory priming, from a resting to an activated immunosuppressive pattern. This change included the upregulation of HLA class I, the adhesion molecules ICAM-1 and VCAM-1, and the immunosuppressive molecule PD-L1 (CD274). Importantly, ICAM-1, VCAM-1, and PD-L1 strongly bind to IECs promoting cell-to-cell contact and exposing immune cells to immunosuppressive molecules[85,212,213]. A *de novo* expression of MHC class II was detected and, although this adaptation may theoretically favor an immune cell response, lymphocyte activation was never detected when cocultured with the variety of SC types investigated here. This result may reflect the absent or weak expression of the co-

stimulatory molecules CD80 and CD86.

Our observations indicate that resting and primed SCs enhance the viability of unstimulated IECs rather than inhibiting their function. All SC types show an anti-apoptotic effect on T, B, and NK cells, suggesting that their potential inhibitory role[50] is not a constitutive property of SCs, but is acquired through IEC activation. If SCs are not inflammatory-primed and are challenged with activated NK cells, the resulting interaction is SC lysis. However, IFN-γ and TNF-α licensing makes SCs resistant to NK cells, a characteristic particularly apparent for B cell proliferation. A similar requirement has previously been shown for MSCs[96].

Here, we found that the exposure to inflammatory milieu leads to IDO activation that becomes the central immunosuppressive enzyme affecting T cell proliferation in all SC types, as shown previously also by other authors[65,223], even if some other molecules may be involved. The discrepancies among different published data could be related to different experimental approaches; thus, method and assay standardization is required to obtain comparable results, as suggested by ISCT MSC Committee[229].

In summary, our findings have provided new information concerning the immune modulatory properties of several SC types, an attribute that was previously unknown. It is now clear that immunomodulation is not a peculiar feature of MSC-like cells, but actually a general property of SCs that may be induced or enhanced by inflammatory stimuli. SC niches may be viewed as immunological structures playing a fundamental role in tissue homeostasis, by regulating the interplay of SCs and IECs, which promotes cell survival. With persistent antigen activation and immune responses, the immunological SC niche may become an important variable of the pathogenesis and progression of degenerative diseases. Understanding these mechanisms may help identifying novel therapeutic strategies or recognizing the most effective SC class able to interfere with damage-mediated inflammation, and to induce tissue regeneration and organ repair.

References

1. Siminovitch L, EA McCulloch and JE Till. (1963). The Distribution of Colony-Forming Cells among Spleen Colonies. J Cell Physiol 62:327-36.
2. Evans MJ and MH Kaufman. (1981). Establishment in culture of pluripotential cells from mouse embryos. Nature 292:154-6.
3. Martin GR. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A 78:7634-8.
4. Betschinger J, F Eisenhaber and JA Knoblich. (2005). Phosphorylation-induced autoinhibition regulates the cytoskeletal protein Lethal (2) giant larvae. Curr Biol 15:276-82.
5. Knoblich JA. (2008). Mechanisms of asymmetric stem cell division. Cell 132:583-97.
6. Li L and T Xie. (2005). Stem cell niche: structure and function. Annu Rev Cell Dev Biol 21:605-31.
7. Morrison SJ and DT Scadden. (2014). The bone marrow niche for haematopoietic stem cells. Nature 505:327-34.
8. Spangrude GJ, S Heimfeld and IL Weissman. (1988). Purification and characterization of mouse hematopoietic stem cells. Science 241:58-62.
9. Levesque JP, IG Winkler, J Hendy, B Williams, F Helwani, V Barbier, B Nowlan and SK Nilsson. (2007). Hematopoietic progenitor cell mobilization results in hypoxia with increased hypoxia-inducible transcription factor-1 alpha and vascular endothelial growth factor A in bone marrow. Stem Cells 25:1954-65.
10. Winkler IG, V Barbier, R Wadley, AC Zannettino, S Williams and JP Levesque. (2010). Positioning of bone marrow hematopoietic and stromal cells relative to blood flow in vivo: serially reconstituting hematopoietic stem cells reside in distinct nonperfused niches. Blood 116:375-85.
11. Parmar K, P Mauch, JA Vergilio, R Sackstein and JD Down. (2007). Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. Proc Natl Acad Sci U S A 104:5431-6.
12. Jang YY and SJ Sharkis. (2007). A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. Blood 110:3056-63.
13. Takubo K, N Goda, W Yamada, H Iriuchishima, E Ikeda, Y Kubota, H Shima, RS Johnson, A Hirao, M Suematsu and T Suda. (2010). Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. Cell Stem Cell 7:391-402.
14. Forristal CE, IG Winkler, B Nowlan, V Barbier, G Walkinshaw and JP Levesque. (2013). Pharmacologic stabilization of HIF-1alpha increases hematopoietic stem cell quiescence in vivo and accelerates blood recovery after severe irradiation. Blood 121:759-69.
15. Taichman RS and SG Emerson. (1994). Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. J Exp Med 179:1677-82.

16. Yoshihara H, F Arai, K Hosokawa, T Hagiwara, K Takubo, Y Nakamura, Y Gomei, H Iwasaki, S Matsuoka, K Miyamoto, H Miyazaki, T Takahashi and T Suda. (2007). Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. Cell Stem Cell 1:685-97.
17. Jung Y, J Wang, A Schneider, YX Sun, AJ Koh-Paige, NI Osman, LK McCauley and RS Taichman. (2006). Regulation of SDF-1 (CXCL12) production by osteoblasts; a possible mechanism for stem cell homing. Bone 38:497-508.
18. Sugiyama T, H Kohara, M Noda and T Nagasawa. (2006). Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity 25:977-88.
19. Omatsu Y, T Sugiyama, H Kohara, G Kondoh, N Fujii, K Kohno and T Nagasawa. (2010). The essential functions of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. Immunity 33:387-99.
20. Tokoyoda K, T Egawa, T Sugiyama, BI Choi and T Nagasawa. (2004). Cellular niches controlling B lymphocyte behavior within bone marrow during development. Immunity 20:707-18.
21. Friedenstein AJ, KV Petrakova, AI Kurolesova and GP Frolova. (1968). Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 6:230-47.
22. Friedenstein AJ, JF Gorskaja and NN Kulagina. (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 4:267-74.
23. Dexter TM, TD Allen and LG Lajtha. (1977). Conditions controlling the proliferation of haemopoietic stem cells in vitro. J Cell Physiol 91:335-44.
24. Caplan AI. (1991). Mesenchymal stem cells. J Orthop Res 9:641-50.
25. Pittenger MF, AM Mackay, SC Beck, RK Jaiswal, R Douglas, JD Mosca, MA Moorman, DW Simonetti, S Craig and DR Marshak. (1999). Multilineage potential of adult human mesenchymal stem cells. Science 284:143-7.
26. Zuk PA, M Zhu, P Ashjian, DA De Ugarte, JI Huang, H Mizuno, ZC Alfonso, JK Fraser, P Benhaim and MH Hedrick. (2002). Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13:4279-95.
27. In 't Anker PS, SA Scherjon, C Kleijburg-van der Keur, GM de Groot-Swings, FH Claas, WE Fibbe and HH Kanhai. (2004). Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells 22:1338-45.
28. Nadri S and M Soleimani. (2007). Comparative analysis of mesenchymal stromal cells from murine bone marrow and amniotic fluid. Cytotherapy 9:729-37.
29. Wang HS, SC Hung, ST Peng, CC Huang, HM Wei, YJ Guo, YS Fu, MC Lai and CC Chen. (2004). Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells 22:1330-7.
30. Kawashima N. (2012). Characterisation of dental pulp stem cells: a new horizon for tissue regeneration? Arch Oral Biol 57:1439-58.
31. Horwitz EM, K Le Blanc, M Dominici, I Mueller, I Slaper-Cortenbach, FC Marini, RJ Deans, DS Krause, A Keating and T International Society for Cellular. (2005). Clarification of the nomenclature for MSC: The

International Society for Cellular Therapy position statement. Cytotherapy 7:393-5.

32. Dominici M, K Le Blanc, I Mueller, I Slaper-Cortenbach, F Marini, D Krause, R Deans, A Keating, D Prockop and E Horwitz. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315-7.
33. Oswald J, S Boxberger, B Jorgensen, S Feldmann, G Ehninger, M Bornhauser and C Werner. (2004). Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 22:377-84.
34. Makino S, K Fukuda, S Miyoshi, F Konishi, H Kodama, J Pan, M Sano, T Takahashi, S Hori, H Abe, J Hata, A Umezawa and S Ogawa. (1999). Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest 103:697-705.
35. Snykers S, J De Kock, V Rogiers and T Vanhaecke. (2009). In vitro differentiation of embryonic and adult stem cells into hepatocytes: state of the art. Stem Cells 27:577-605.
36. Arthur A, G Rychkov, S Shi, SA Koblar and S Gronthos. (2008). Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells 26:1787-95.
37. Bianco P, X Cao, PS Frenette, JJ Mao, PG Robey, PJ Simmons and CY Wang. (2013). The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. Nat Med 19:35-42.
38. Roberts EW, A Deonarine, JO Jones, AE Denton, C Feig, SK Lyons, M Espeli, M Kraman, B McKenna, RJ Wells, Q Zhao, OL Caballero, R Larder, AP Coll, S O'Rahilly, KM Brindle, SA Teichmann, DA Tuveson and DT Fearon. (2013). Depletion of stromal cells expressing fibroblast activation protein-alpha from skeletal muscle and bone marrow results in cachexia and anemia. J Exp Med 210:1137-51.
39. Zhao H, J Feng, K Seidel, S Shi, O Klein, P Sharpe and Y Chai. (2014). Secretion of shh by a neurovascular bundle niche supports mesenchymal stem cell homeostasis in the adult mouse incisor. Cell Stem Cell 14:160-73.
40. Zhou BO, R Yue, MM Murphy, JG Peyer and SJ Morrison. (2014). Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. Cell Stem Cell 15:154-68.
41. Mendez-Ferrer S, TV Michurina, F Ferraro, AR Mazloom, BD Macarthur, SA Lira, DT Scadden, A Ma'ayan, GN Enikolopov and PS Frenette. (2010). Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 466:829-34.
42. Ren G, X Chen, F Dong, W Li, X Ren, Y Zhang and Y Shi. (2012). Concise review: mesenchymal stem cells and translational medicine: emerging issues. Stem Cells Transl Med 1:51-8.
43. Crisostomo PR, Y Wang, TA Markel, M Wang, T Lahm and DR Meldrum. (2008). Human mesenchymal stem cells stimulated by TNF-alpha, LPS, or hypoxia produce growth factors by an NF kappa B- but not JNK-dependent mechanism. Am J Physiol Cell Physiol 294:C675-82.
44. Aggarwal S and MF Pittenger. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105:1815-22.
45. Togel F, Z Hu, K Weiss, J Isaac, C Lange and C Westenfelder. (2005). Administered mesenchymal stem cells protect against ischemic acute renal

failure through differentiation-independent mechanisms. Am J Physiol Renal Physiol 289:F31-42.
46. Prockop DJ, DJ Kota, N Bazhanov and RL Reger. (2010). Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). J Cell Mol Med 14:2190-9.
47. Bai L, DP Lennon, AI Caplan, A DeChant, J Hecker, J Kranso, A Zaremba and RH Miller. (2012). Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models. Nat Neurosci 15:862-70.
48. Lee RH, AA Pulin, MJ Seo, DJ Kota, J Ylostalo, BL Larson, L Semprun-Prieto, P Delafontaine and DJ Prockop. (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.
49. Wang Y, X Chen, W Cao and Y Shi. (2014). Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 15:1009-16.
50. Krampera M. (2011). Mesenchymal stromal cell 'licensing': a multistep process. Leukemia 25:1408-14.
51. Bernardo ME and WE Fibbe. (2013). Mesenchymal stromal cells: sensors and switchers of inflammation. Cell Stem Cell 13:392-402.
52. Shi Y, J Su, AI Roberts, P Shou, AB Rabson and G Ren. (2012). How mesenchymal stem cells interact with tissue immune responses. Trends Immunol 33:136-43.
53. Gordon S and A Mantovani. (2011). Diversity and plasticity of mononuclear phagocytes. Eur J Immunol 41:2470-2.
54. Waterman RS, SL Tomchuck, SL Henkle and AM Betancourt. (2010). A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. PLoS One 5:e10088.
55. Mantovani A, SK Biswas, MR Galdiero, A Sica and M Locati. (2013). Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol 229:176-85.
56. Raicevic G, R Rouas, M Najar, P Stordeur, HI Boufker, D Bron, P Martiat, M Goldman, MT Nevessignsky and L Lagneaux. (2010). Inflammation modifies the pattern and the function of Toll-like receptors expressed by human mesenchymal stromal cells. Hum Immunol 71:235-44.
57. Brandau S, M Jakob, H Hemeda, K Bruderek, S Janeschik, F Bootz and S Lang. (2010). Tissue-resident mesenchymal stem cells attract peripheral blood neutrophils and enhance their inflammatory activity in response to microbial challenge. J Leukoc Biol 88:1005-15.
58. Cassatella MA, F Mosna, A Micheletti, V Lisi, N Tamassia, C Cont, F Calzetti, M Pelletier, G Pizzolo and M Krampera. (2011). Toll-like receptor-3-activated human mesenchymal stromal cells significantly prolong the survival and function of neutrophils. Stem Cells 29:1001-11.
59. Krampera M, L Cosmi, R Angeli, A Pasini, F Liotta, A Andreini, V Santarlasci, B Mazzinghi, G Pizzolo, F Vinante, P Romagnani, E Maggi, S Romagnani and F Annunziato. (2006). Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells 24:386-98.

60. Krampera M, S Sartoris, F Liotta, A Pasini, R Angeli, L Cosmi, A Andreini, F Mosna, B Bonetti, E Rebellato, MG Testi, F Frosali, G Pizzolo, G Tridente, E Maggi, S Romagnani and F Annunziato. (2007). Immune regulation by mesenchymal stem cells derived from adult spleen and thymus. Stem Cells Dev 16:797-810.
61. Groh ME, B Maitra, E Szekely and ON Koc. (2005). Human mesenchymal stem cells require monocyte-mediated activation to suppress alloreactive T cells. Exp Hematol 33:928-34.
62. Di Nicola M, C Carlo-Stella, M Magni, M Milanesi, PD Longoni, P Matteucci, S Grisanti and AM Gianni. (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 99:3838-43.
63. Liu H, DM Kemeny, BC Heng, HW Ouyang, AJ Melendez and T Cao. (2006). The immunogenicity and immunomodulatory function of osteogenic cells differentiated from mesenchymal stem cells. J Immunol 176:2864-71.
64. Tse WT, JD Pendleton, WM Beyer, MC Egalka and EC Guinan. (2003). Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation 75:389-97.
65. Meisel R, A Zibert, M Laryea, U Gobel, W Daubener and D Dilloo. (2004). Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 103:4619-21.
66. Xu G, Y Zhang, L Zhang, G Ren and Y Shi. (2007). The role of IL-6 in inhibition of lymphocyte apoptosis by mesenchymal stem cells. Biochem Biophys Res Commun 361:745-50.
67. Djouad F, LM Charbonnier, C Bouffi, P Louis-Plence, C Bony, F Apparailly, C Cantos, C Jorgensen and D Noel. (2007). Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. Stem Cells 25:2025-32.
68. Zhang W, W Ge, C Li, S You, L Liao, Q Han, W Deng and RC Zhao. (2004). Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. Stem Cells Dev 13:263-71.
69. Beyth S, Z Borovsky, D Mevorach, M Liebergall, Z Gazit, H Aslan, E Galun and J Rachmilewitz. (2005). Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. Blood 105:2214-9.
70. Klyushnenkova E, JD Mosca, V Zernetkina, MK Majumdar, KJ Beggs, DW Simonetti, RJ Deans and KR McIntosh. (2005). T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. J Biomed Sci 12:47-57.
71. Potian JA, H Aviv, NM Ponzio, JS Harrison and P Rameshwar. (2003). Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. J Immunol 171:3426-34.
72. Sato K, K Ozaki, I Oh, A Meguro, K Hatanaka, T Nagai, K Muroi and K Ozawa. (2007). Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood 109:228-34.

73. Chabannes D, M Hill, E Merieau, J Rossignol, R Brion, JP Soulillou, I Anegon and MC Cuturi. (2007). A role for heme oxygenase-1 in the immunosuppressive effect of adult rat and human mesenchymal stem cells. Blood 110:3691-4.
74. Selmani Z, A Naji, I Zidi, B Favier, E Gaiffe, L Obert, C Borg, P Saas, P Tiberghien, N Rouas-Freiss, ED Carosella and F Deschaseaux. (2008). Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. Stem Cells 26:212-22.
75. Oh I, K Ozaki, K Sato, A Meguro, R Tatara, K Hatanaka, T Nagai, K Muroi and K Ozawa. (2007). Interferon-gamma and NF-kappaB mediate nitric oxide production by mesenchymal stromal cells. Biochem Biophys Res Commun 355:956-62.
76. Su J, X Chen, Y Huang, W Li, J Li, K Cao, G Cao, L Zhang, F Li, AI Roberts, H Kang, P Yu, G Ren, W Ji, Y Wang and Y Shi. (2014). Phylogenetic distinction of iNOS and IDO function in mesenchymal stem cell-mediated immunosuppression in mammalian species. Cell Death Differ 21:388-96.
77. Taher YA, BJ Piavaux, R Gras, BC van Esch, GA Hofman, N Bloksma, PA Henricks and AJ van Oosterhout. (2008). Indoleamine 2,3-dioxygenase-dependent tryptophan metabolites contribute to tolerance induction during allergen immunotherapy in a mouse model. J Allergy Clin Immunol 121:983-91 e2.
78. Swardfager W, N Herrmann, Y Dowlati, PI Oh, A Kiss, SE Walker and KL Lanctot. (2009). Indoleamine 2,3-dioxygenase activation and depressive symptoms in patients with coronary artery disease. Psychoneuroendocrinology 34:1560-6.
79. Ling W, J Zhang, Z Yuan, G Ren, L Zhang, X Chen, AB Rabson, AI Roberts, Y Wang and Y Shi. (2014). Mesenchymal stem cells use IDO to regulate immunity in tumor microenvironment. Cancer Res 74:1576-87.
80. Nguyen NT, A Kimura, T Nakahama, I Chinen, K Masuda, K Nohara, Y Fujii-Kuriyama and T Kishimoto. (2010). Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. Proc Natl Acad Sci U S A 107:19961-6.
81. Mezrich JD, JH Fechner, X Zhang, BP Johnson, WJ Burlingham and CA Bradfield. (2010). An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. J Immunol 185:3190-8.
82. Munn DH and AL Mellor. (2013). Indoleamine 2,3 dioxygenase and metabolic control of immune responses. Trends Immunol 34:137-43.
83. Ren G, L Zhang, X Zhao, G Xu, Y Zhang, AI Roberts, RC Zhao and Y Shi. (2008). Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2:141-50.
84. Porterfield DM, JD Laskin, SK Jung, RP Malchow, B Billack, PJ Smith and DE Heck. (2001). Proteins and lipids define the diffusional field of nitric oxide. Am J Physiol Lung Cell Mol Physiol 281:L904-12.
85. Ren G, X Zhao, L Zhang, J Zhang, A L'Huillier, W Ling, AI Roberts, AD Le, S Shi, C Shao and Y Shi. (2010). Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in

mesenchymal stem cells are critical for immunosuppression. J Immunol 184:2321-8.

86. Rasmusson I, O Ringden, B Sundberg and K Le Blanc. (2005). Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. Exp Cell Res 305:33-41.
87. Burr SP, F Dazzi and OA Garden. (2013). Mesenchymal stromal cells and regulatory T cells: the Yin and Yang of peripheral tolerance? Immunol Cell Biol 91:12-8.
88. English K, JM Ryan, L Tobin, MJ Murphy, FP Barry and BP Mahon. (2009). Cell contact, prostaglandin E(2) and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4+CD25(High) forkhead box P3+ regulatory T cells. Clin Exp Immunol 156:149-60.
89. Maccario R, M Podesta, A Moretta, A Cometa, P Comoli, D Montagna, L Daudt, A Ibatici, G Piaggio, S Pozzi, F Frassoni and F Locatelli. (2005). Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. Haematologica 90:516-25.
90. Akiyama K, C Chen, D Wang, X Xu, C Qu, T Yamaza, T Cai, W Chen, L Sun and S Shi. (2012). Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. Cell Stem Cell 10:544-55.
91. Melief SM, SB Geutskens, WE Fibbe and H Roelofs. (2013). Multipotent stromal cells skew monocytes towards an anti-inflammatory interleukin-10-producing phenotype by production of interleukin-6. Haematologica 98:888-95.
92. Nauta AJ, AB Kruisselbrink, E Lurvink, R Willemze and WE Fibbe. (2006). Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. J Immunol 177:2080-7.
93. Jiang XX, Y Zhang, B Liu, SX Zhang, Y Wu, XD Yu and N Mao. (2005). Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 105:4120-6.
94. Casado JG, R Tarazona and FM Sanchez-Margallo. (2013). NK and MSCs crosstalk: the sense of immunomodulation and their sensitivity. Stem Cell Rev 9:184-9.
95. Spaggiari GM, A Capobianco, H Abdelrazik, F Becchetti, MC Mingari and L Moretta. (2008). Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 111:1327-33.
96. Spaggiari GM, A Capobianco, S Becchetti, MC Mingari and L Moretta. (2006). Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood 107:1484-90.
97. Corcione A, F Benvenuto, E Ferretti, D Giunti, V Cappiello, F Cazzanti, M Risso, F Gualandi, GL Mancardi, V Pistoia and A Uccelli. (2006). Human mesenchymal stem cells modulate B-cell functions. Blood 107:367-72.
98. Van Gool SW, P Vandenberghe, M de Boer and JL Ceuppens. (1996). CD80, CD86 and CD40 provide accessory signals in a multiple-step T-cell activation model. Immunol Rev 153:47-83.

99. Menard C and K Tarte. (2013). Immunoregulatory properties of clinical grade mesenchymal stromal cells: evidence, uncertainties, and clinical application. Stem Cell Res Ther 4:64.
100. Sun L, K Akiyama, H Zhang, T Yamaza, Y Hou, S Zhao, T Xu, A Le and S Shi. (2009). Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. Stem Cells 27:1421-32.
101. Polchert D, J Sobinsky, G Douglas, M Kidd, A Moadsiri, E Reina, K Genrich, S Mehrotra, S Setty, B Smith and A Bartholomew. (2008). IFN-gamma activation of mesenchymal stem cells for treatment and prevention of graft versus host disease. Eur J Immunol 38:1745-55.
102. Rafei M, E Birman, K Forner and J Galipeau. (2009). Allogeneic mesenchymal stem cells for treatment of experimental autoimmune encephalomyelitis. Mol Ther 17:1799-803.
103. Gonzalez MA, E Gonzalez-Rey, L Rico, D Buscher and M Delgado. (2009). Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum 60:1006-19.
104. Fiorina P, M Jurewicz, A Augello, A Vergani, S Dada, S La Rosa, M Selig, J Godwin, K Law, C Placidi, RN Smith, C Capella, S Rodig, CN Adra, M Atkinson, MH Sayegh and R Abdi. (2009). Immunomodulatory function of bone marrow-derived mesenchymal stem cells in experimental autoimmune type 1 diabetes. J Immunol 183:993-1004.
105. Zhang Q, S Shi, Y Liu, J Uyanne, Y Shi and AD Le. (2009). Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 183:7787-98.
106. Le Blanc K, I Rasmusson, B Sundberg, C Gotherstrom, M Hassan, M Uzunel and O Ringden. (2004). Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 363:1439-41.
107. Sun L, D Wang, J Liang, H Zhang, X Feng, H Wang, B Hua, B Liu, S Ye, X Hu, W Xu, X Zeng, Y Hou, GS Gilkeson, RM Silver, L Lu and S Shi. (2010). Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. Arthritis Rheum 62:2467-75.
108. Dalal J, K Gandy and J Domen. (2012). Role of mesenchymal stem cell therapy in Crohn's disease. Pediatr Res 71:445-51.
109. Augello A, R Tasso, SM Negrini, R Cancedda and G Pennesi. (2007). Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. Arthritis Rheum 56:1175-86.
110. Djouad F, V Fritz, F Apparailly, P Louis-Plence, C Bony, J Sany, C Jorgensen and D Noel. (2005). Reversal of the immunosuppressive properties of mesenchymal stem cells by tumor necrosis factor alpha in collagen-induced arthritis. Arthritis Rheum 52:1595-603.
111. Youd M, C Blickarz, L Woodworth, T Touzjian, A Edling, J Tedstone, M Ruzek, R Tubo, J Kaplan and T Lodie. (2010). Allogeneic mesenchymal stem cells do not protect NZBxNZW F1 mice from developing lupus disease. Clin Exp Immunol 161:176-86.

112. Sudres M, F Norol, A Trenado, S Gregoire, F Charlotte, B Levacher, JJ Lataillade, P Bourin, X Holy, JP Vernant, D Klatzmann and JL Cohen. (2006). Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. J Immunol 176:7761-7.
113. Tisato V, K Naresh, J Girdlestone, C Navarrete and F Dazzi. (2007). Mesenchymal stem cells of cord blood origin are effective at preventing but not treating graft-versus-host disease. Leukemia 21:1992-9.
114. Han X, Q Yang, L Lin, C Xu, C Zheng, X Chen, Y Han, M Li, W Cao, K Cao, Q Chen, G Xu, Y Zhang, J Zhang, RJ Schneider, Y Qian, Y Wang, G Brewer and Y Shi. (2014). Interleukin-17 enhances immunosuppression by mesenchymal stem cells. Cell Death Differ 21:1758-68.
115. Le Blanc K, F Frassoni, L Ball, F Locatelli, H Roelofs, I Lewis, E Lanino, B Sundberg, ME Bernardo, M Remberger, G Dini, RM Egeler, A Bacigalupo, W Fibbe, O Ringden, B Developmental Committee of the European Group for and T Marrow. (2008). Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 371:1579-86.
116. Constantin G, S Marconi, B Rossi, S Angiari, L Calderan, E Anghileri, B Gini, SD Bach, M Martinello, F Bifari, M Galie, E Turano, S Budui, A Sbarbati, M Krampera and B Bonetti. (2009). Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoimmune encephalomyelitis. Stem Cells 27:2624-35.
117. Delorme B, E Nivet, J Gaillard, T Häupl, J Ringe, A Devèze, J Magnan, J Sohier, M Khrestchatisky, FS Roman, P Charbord, L Sensebé, P Layrolle and F Féron. (2010). The Human Nose Harbors a Niche of Olfactory Ectomesenchymal Stem Cells Displaying Neurogenic and Osteogenic Properties. Stem Cells and Development 19:853-866.
118. Tome M, SL Lindsay, JS Riddell and SC Barnett. (2009). Identification of nonepithelial multipotent cells in the embryonic olfactory mucosa. Stem Cells 27:2196-208.
119. Leung CT, PA Coulombe and RR Reed. (2007). Contribution of olfactory neural stem cells to tissue maintenance and regeneration. Nat Neurosci 10:720-6.
120. Feron F, A Mackay-Sim, JL Andrieu, KI Matthaei, A Holley and G Sicard. (1999). Stress induces neurogenesis in non-neuronal cell cultures of adult olfactory epithelium. Neuroscience 88:571-83.
121. Murrell W, A Wetzig, M Donnellan, F Feron, T Burne, A Meedeniya, J Kesby, J Bianco, C Perry, P Silburn and A Mackay-Sim. (2008). Olfactory mucosa is a potential source for autologous stem cell therapy for Parkinson's disease. Stem Cells 26:2183-92.
122. Doyle KL, A Kazda, Y Hort, SM McKay and S Oleskevich. (2007). Differentiation of adult mouse olfactory precursor cells into hair cells in vitro. Stem Cells 25:621-7.
123. Nivet E, M Vignes, SD Girard, C Pierrisnard, N Baril, A Devèze, J Magnan, F Lanté, M Khrestchatisky, F Féron and FS Roman. (2011). Engraftment of human nasal olfactory stem cells restores neuroplasticity in mice with hippocampal lesions. Journal of Clinical Investigation 121:2808-2820.

124. Toft A, M Tome, SL Lindsay, SC Barnett and JS Riddell. (2012). Transplant-mediated repair properties of rat olfactory mucosal OM-I and OM-II sphere-forming cells. J Neurosci Res 90:619-31.
125. Decimo I, F Bifari, FJ Rodriguez, G Malpeli, S Dolci, V Lavarini, S Pretto, S Vasquez, M Sciancalepore, A Montalbano, V Berton, M Krampera and G Fumagalli. (2011). Nestin- and doublecortin-positive cells reside in adult spinal cord meninges and participate in injury-induced parenchymal reaction. Stem Cells 29:2062-76.
126. Bifari F, I Decimo, C Chiamulera, E Bersan, G Malpeli, J Johansson, V Lisi, B Bonetti, G Fumagalli, G Pizzolo and M Krampera. (2009). Novel stem/progenitor cells with neuronal differentiation potential reside in the leptomeningeal niche. J Cell Mol Med 13:3195-208.
127. Rosser AE, R Zietlow and SB Dunnett. (2007). Stem cell transplantation for neurodegenerative diseases. Curr Opin Neurol 20:688-92.
128. Scadden DT. (2006). The stem-cell niche as an entity of action. Nature 441:1075-9.
129. Johansson CB, S Momma, DL Clarke, M Risling, U Lendahl and J Frisen. (1999). Identification of a neural stem cell in the adult mammalian central nervous system. Cell 96:25-34.
130. Decimo I, G Fumagalli, V Berton, M Krampera and F Bifari. (2012). Meninges: from protective membrane to stem cell niche. Am J Stem Cells 1:92-105.
131. Borrell V and O Marin. (2006). Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. Nat Neurosci 9:1284-93.
132. Trommsdorff M, M Gotthardt, T Hiesberger, J Shelton, W Stockinger, J Nimpf, RE Hammer, JA Richardson and J Herz. (1999). Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. Cell 97:689-701.
133. Madhavan L, V Ourednik and J Ourednik. (2006). Increased "vigilance" of antioxidant mechanisms in neural stem cells potentiates their capability to resist oxidative stress. Stem Cells 24:2110-2119.
134. Marin-Padilla M. (1998). Cajal-Retzius cells and the development of the neocortex. Trends in Neurosciences 21:64-71.
135. Costa C, B Harding and AJ Copp. (2001). Neuronal migration defects in the Dreher (Lmx1a) mutant mouse: Role of disorders of the glial limiting membrane. Cerebral Cortex 11:498-505.
136. Haubst N, E Georges-Labouesse, A De Arcangelis, U Mayer and M Gotz. (2006). Basement membrane attachment is dispensable for radial glial cell fate and for proliferation, but affects positioning of neuronal subtypes. Development 133:3245-3254.
137. Grimpe B, SC Dong, C Doller, K Temple, AT Malouf and J Silver. (2002). The critical role of basement membrane-independent laminin gamma 1 chain during axon regeneration in the CNS. Journal of Neuroscience 22:3144-3160.
138. Petricevic J, G Forempoher, L Ostojic, S Mardesic-Brakus, S Andjelinovic, K Vukojevic and M Saraga-Babic. (2011). Expression of nestin, mesothelin and epithelial membrane antigen (EMA) in developing and adult human meninges and meningiomas. Acta Histochemica 113:703-711.

139. Degiorgio LA, KFR Sheu and JP Blass. (1994). Culture from Human Leptomeninges of Cells Containing Neurofilament Protein and Neuron-Specific Enolase. Journal of the Neurological Sciences 124:141-148.
140. DeGiorgio LA, JJ Bernstein and JP Blass. (1997). Implantation of cultured human leptomeningeal cells into rat brain. International Journal of Developmental Neuroscience 15:231-238.
141. Bernstein JJ, SM Karp, WJ Goldberg, LA DeGiorgio and JP Blass. (1996). Human leptomeningeal-derived cells express GFAP and HLADR when grafted into rat spinal cord. International Journal of Developmental Neuroscience 14:681-687.
142. Decimo F, C Capristo, R Amelio, N Maiello, AF Capristo and M Miraglia Del Giudice. (2011). Evaluation of bronchial hyperreactivity with mannitol dry powder challenge test in a paediatric population with intermittent allergic asthma or allergic rhinitis. Int J Immunopathol Pharmacol 24:1069-74.
143. Nakagomi T, Z Molnar, A Nakano-Doi, A Taguchi, O Saino, S Kubo, M Clausen, H Yoshikawa, N Nakagomi and T Matsuyama. (2011). Ischemia-Induced Neural Stem/Progenitor Cells in the Pia Mater Following Cortical Infarction. Stem Cells and Development 20:2037-2051.
144. Prusa AR, E Marton, M Rosner, G Bernaschek and M Hengstschlager. (2003). Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research? Hum Reprod 18:1489-93.
145. Niwa H, J Miyazaki and AG Smith. (2000). Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nature Genetics 24:372-376.
146. Stefanidis K, D Loutradis, V Anastasiadou, R Bletsa, E Kiapekou, P Drakakis, P Beretsos, E Elenis, S Mesogitis and A Antsaklis. (2008). Oxytocin receptor- and Oct-4-expressing cells in human amniotic fluid. Gynecological Endocrinology 24:280-284.
147. Karlmark KR, A Freilinger, E Marton, M Rosner, G Lubec and M Hengstschlager. (2005). Activation of ectopic Oct-4 and Rex-1 promoters in human amniotic fluid cells. Int J Mol Med 16:987-92.
148. Tsai MS, SM Hwang, YL Tsai, FC Cheng, JL Lee and YJ Chang. (2006). Clonal amniotic fluid-derived stem cells express characteristics of both mesenchymal and neural stem cells. Biol Reprod 74:545-51.
149. Kim J, Y Lee, H Kim, KJ Hwang, HC Kwon, SK Kim, DJ Cho, SG Kang and J You. (2007). Human amniotic fluid-derived stem cells have characteristics of multipotent stem cells. Cell Proliferation 40:75-90.
150. De Coppi P, G Bartsch, Jr., MM Siddiqui, T Xu, CC Santos, L Perin, G Mostoslavsky, AC Serre, EY Snyder, JJ Yoo, ME Furth, S Soker and A Atala. (2007). Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol 25:100-6.
151. Pozzobon M, M Piccoli, AA Schiavo, A Atala and P De Coppi. (2013). Isolation of c-Kit+ human amniotic fluid stem cells from second trimester. Methods Mol Biol 1035:191-8.
152. Valli A, M Rosner, C Fuchs, N Siegel, CE Bishop, H Dolznig, U Madel, W Feichtinger, A Atala and M Hengstschlager. (2010). Embryoid body formation of human amniotic fluid stem cells depends on mTOR. Oncogene 29:966-77.

153. Fuchs C, M Rosner, H Dolznig, M Mikula, N Kramer and M Hengstschlager. (2012). Tuberin and PRAS40 are anti-apoptotic gatekeepers during early human amniotic fluid stem-cell differentiation. Hum Mol Genet 21:1049-61.
154. Siegel N, A Valli, C Fuchs, M Rosner and M Hengstschlager. (2009). Expression of mTOR pathway proteins in human amniotic fluid stem cells. International Journal of Molecular Medicine 23:779-784.
155. Moschidou D, S Mukherjee, MP Blundell, K Drews, GN Jones, H Abdulrazzak, B Nowakowska, A Phoolchund, K Lay, TS Ramasamy, M Cananzi, D Nettersheim, M Sullivan, J Frost, G Moore, JR Vermeesch, NM Fisk, AJ Thrasher, A Atala, J Adjaye, H Schorle, P De Coppi and PV Guillot. (2012). Valproic acid confers functional pluripotency to human amniotic fluid stem cells in a transgene-free approach. Mol Ther 20:1953-67.
156. Moschidou D, S Mukherjee, MP Blundell, GN Jones, AJ Atala, AJ Thrasher, NM Fisk, P De Coppi and PV Guillot. (2013). Human mid-trimester amniotic fluid stem cells cultured under embryonic stem cell conditions with valproic acid acquire pluripotent characteristics. Stem Cells Dev 22:444-58.
157. Carraro G, L Perin, S Sedrakyan, S Giuliani, C Tiozzo, J Lee, G Turcatel, SP De Langhe, B Driscoll, S Bellusci, P Minoo, A Atala, RE De Filippo and D Warburton. (2008). Human amniotic fluid stem cells can integrate and differentiate into epithelial lung lineages. Stem Cells 26:2902-11.
158. Piccoli M, C Franzin, E Bertin, L Urbani, B Blaauw, A Repele, E Taschin, A Cenedese, GF Zanon, I Andre-Schmutz, A Rosato, J Melki, M Cavazzana-Calvo, M Pozzobon and P De Coppi. (2012). Amniotic fluid stem cells restore the muscle cell niche in a HSA-Cre, Smn(F7/F7) mouse model. Stem Cells 30:1675-84.
159. Riccio M, T Maraldi, A Pisciotta, GB La Sala, A Ferrari, G Bruzzesi, A Motta, C Migliaresi and A De Pol. (2012). Fibroin scaffold repairs critical-size bone defects in vivo supported by human amniotic fluid and dental pulp stem cells. Tissue Eng Part A 18:1006-13.
160. Rodrigues MT, BK Lee, SJ Lee, ME Gomes, RL Reis, A Atala and JJ Yoo. (2012). The effect of differentiation stage of amniotic fluid stem cells on bone regeneration. Biomaterials 33:6069-78.
161. Rota C, B Imberti, M Pozzobon, M Piccoli, P De Coppi, A Atala, E Gagliardini, C Xinaris, V Benedetti, AS Fabricio, E Squarcina, M Abbate, A Benigni, G Remuzzi and M Morigi. (2012). Human amniotic fluid stem cell preconditioning improves their regenerative potential. Stem Cells Dev 21:1911-23.
162. Ghionzoli M, M Cananzi, A Zani, CA Rossi, FF Leon, A Pierro, S Eaton and P De Coppi. (2010). Amniotic fluid stem cell migration after intraperitoneal injection in pup rats: implication for therapy. Pediatr Surg Int 26:79-84.
163. Bollini S, M Pozzobon, M Nobles, J Riegler, X Dong, M Piccoli, A Chiavegato, AN Price, M Ghionzoli, KK Cheung, A Cabrelle, PR O'Mahoney, E Cozzi, S Sartore, A Tinker, MF Lythgoe and P De Coppi. (2011). In vitro and in vivo cardiomyogenic differentiation of amniotic fluid stem cells. Stem Cell Rev 7:364-80.
164. Castellani C, G Vescovo, B Ravara, C Franzin, M Pozzobon, R Tavano, L Gorza, E Papini, R Vettor, P De Coppi, G Thiene and A Angelini. (2013). The

contribution of stem cell therapy to skeletal muscle remodeling in heart failure. International Journal of Cardiology 168:2014-2021.

165. Bollini S, KK Cheung, J Riegler, XB Dong, N Smart, M Ghionzoli, SP Loukogeorgakis, P Maghsoudlou, KN Dube, PR Riley, MF Lythgoe and P De Coppi. (2011). Amniotic Fluid Stem Cells Are Cardioprotective Following Acute Myocardial Infarction. Stem Cells and Development 20:1985-1994.
166. Maraldi T, M Riccio, E Resca, A Pisciotta, GB La Sala, A Ferrari, G Bruzzesi, A Motta, C Migliaresi, L Marzona and A De Pol. (2011). Human amniotic fluid stem cells seeded in fibroin scaffold produce in vivo mineralized matrix. Tissue Eng Part A 17:2833-43.
167. Perin L, S Sedrakyan, S Giuliani, S Da Sacco, G Carraro, L Shiri, KV Lemley, M Rosol, S Wu, A Atala, D Warburton and RE De Filippo. (2010). Protective effect of human amniotic fluid stem cells in an immunodeficient mouse model of acute tubular necrosis. PLoS One 5:e9357.
168. Perin L, S Giuliani, D Jin, S Sedrakyan, G Carraro, R Habibian, D Warburton, A Atala and RE De Filippo. (2007). Renal differentiation of amniotic fluid stem cells. Cell Proliferation 40:936-948.
169. Hauser PV, R De Fazio, S Bruno, S Sdei, C Grange, B Bussolati, C Benedetto and G Camussi. (2010). Stem Cells Derived from Human Amniotic Fluid Contribute to Acute Kidney Injury Recovery. American Journal of Pathology 177:2011-2021.
170. Grisafi D, M Pozzobon, A Dedja, V Vanzo, R Tomanin, A Porzionato, V Macchi, R Salmaso, M Scarpa, E Cozzi, A Fassina, F Navaglia, C Maran, M Onisto, L Caenazzo, P De Coppi, R De Caro, L Chiandetti and P Zaramella. (2013). Human Amniotic Fluid Stem Cells Protect Rat Lungs Exposed to Moderate Hyperoxia. Pediatric Pulmonology 48:1070-1080.
171. Murry CE, LJ Field and P Menasche. (2005). Cell-based cardiac repair - Reflections at the 10-year point. Circulation 112:3174-3183.
172. Laflamme MA and CE Murry. (2005). Regenerating the heart. Nature Biotechnology 23:845-856.
173. Rubart M and LJ Field. (2006). Cardiac regeneration: Repopulating the heart. Annual Review of Physiology 68:29-49.
174. Hansson EM, ME Lindsay and KR Chien. (2009). Regeneration Next: Toward Heart Stem Cell Therapeutics. Cell Stem Cell 5:364-377.
175. Urbanek K, D Cesselli, M Rota, A Nascimbene, A De Angelis, T Hosoda, C Bearzi, A Boni, R Bolli, J Kajstura, P Anversa and A Leri. (2006). Stem cell niches in the adult mouse heart. Proc Natl Acad Sci U S A 103:9226-31.
176. Hosoda T, D D'Amario, MC Cabral-Da-Silva, H Zheng, ME Padin-Iruegas, B Ogorek, J Ferreira-Martins, S Yasuzawa-Amano, K Amano, N Ide-Iwata, W Cheng, M Rota, K Urbanek, J Kajstura, P Anversa and A Leri. (2009). Clonality of mouse and human cardiomyogenesis in vivo. Proc Natl Acad Sci U S A 106:17169-74.
177. Gonzalez A, M Rota, D Nurzynska, Y Misao, J Tillmanns, C Ojaimi, ME Padin-Iruegas, P Mueller, G Esposito, C Bearzi, S Vitale, B Dawn, SK Sanganalmath, M Baker, TH Hintze, R Bolli, K Urbanek, T Hosoda, P Anversa, J Kajstura and A Leri. (2008). Activation of cardiac progenitor cells reverses the failing heart senescent phenotype and prolongs lifespan. Circulation Research 102:597-606.

178. Torella D, M Rota, D Nurzynska, E Musso, A Monsen, I Shiraishi, E Zias, K Walsh, A Rosenzweig, MA Sussman, K Urbanek, B Nadal-Ginard, J Kajstura, P Anversa and A Leri. (2004). Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. Circulation Research 94:514-524.
179. Leri A, M Rota, T Hosoda, P Goichberg and P Anversa. (2014). Cardiac stem cell niches. Stem Cell Research 13:631-646.
180. Miharada K, G Karlsson, M Rehn, E Rorby, K Siva, J Cammenga and S Karlsson. (2011). Cripto Regulates Hematopoietic Stem Cells as a Hypoxic-Niche-Related Factor through Cell Surface Receptor GRP78. Cell Stem Cell 9:330-344.
181. Sanada F, J Kim, A Czarna, NYK Chan, S Signore, B Ogorek, K Isobe, E Wybieralska, G Borghetti, A Pesapane, A Sorrentino, E Mangano, D Cappetta, C Mangiaracina, M Ricciardi, M Cimini, E Ifedigbo, MA Perrella, P Goichberg, AM Choi, J Kajstura, T Hosoda, M Rota, P Anversa and A Leri. (2014). c-Kit-Positive Cardiac Stem Cells Nested in Hypoxic Niches Are Activated by Stem Cell Factor Reversing the Aging Myopathy. Circulation Research 114:41-55.
182. Rota M, T Hosoda, A De Angelis, ML Arcarese, G Esposito, R Rizzi, J Tillmanns, D Tugal, E Musso, O Rimoldi, C Bearzi, K Urbanek, P Anversa, A Leri and J Kajstura. (2007). The young mouse heart is composed of myocytes heterogeneous in age and function. Circulation Research 101:387-399.
183. Braun KM, C Niemann, UB Jensen, JP Sundberg, V Silva-Vargas and FM Watt. (2003). Manipulation of stem cell proliferation and lineage commitment: visualisation of label-retaining cells in wholemounts of mouse epidermis. Development 130:5241-5255.
184. Bearzi C, M Rota, T Hosoda, J Tillmanns, A Nascimbene, A De Angelis, S Yasuzawa-Amano, I Trofimova, RW Siggins, N Lecapitaine, S Cascapera, AP Beltrami, DA D'Alessandro, E Zias, F Quaini, K Urbanek, RE Michler, R Bolli, J Kajstura, A Leri and P Anversa. (2007). Human cardiac stem cells. Proc Natl Acad Sci U S A 104:14068-73.
185. Beltrami AP, L Barlucchi, D Torella, M Baker, F Limana, S Chimenti, H Kasahara, M Rota, E Musso, K Urbanek, A Leri, J Kajstura, B Nadal-Ginard and P Anversa. (2003). Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 114:763-776.
186. Ellison GM, C Vicinanza, AJ Smith, I Aquila, A Leone, CD Waring, BJ Henning, GG Stirparo, R Papait, M Scarfo, V Agosti, G Viglietto, G Condorelli, C Indolfi, S Ottolenghi, D Torella and B Nadal-Ginard. (2013). Adult c-kit(pos) Cardiac Stem Cells Are Necessary and Sufficient for Functional Cardiac Regeneration and Repair. Cell 154:827-842.
187. Fischer KM, CT Cottage, W Wu, S Din, NA Gude, D Avitabile, P Quijada, BL Collins, J Fransioli and MA Sussman. (2009). Enhancement of Myocardial Regeneration Through Genetic Engineering of Cardiac Progenitor Cells Expressing Pim-1 Kinase. Circulation 120:2077-U45.
188. Konstandin MH, H Toko, GM Gastelum, P Quijada, A De La Torre, M Quintana, B Collins, S Din, D Avitabile, M Volkers, N Gude, R Fassler and MA Sussman. (2013). Fibronectin Is Essential for Reparative Cardiac

Progenitor Cell Response After Myocardial Infarction. Circulation Research 113:115-125.
189. Mohsin S, M Khan, H Toko, B Bailey, CT Cottage, K Wallach, D Nag, A Lee, S Siddiqi, F Lan, KM Fischer, N Gude, P Quijada, D Avitabile, S Truffa, B Collins, W Dembitsky, JC Wu and MA Sussman. (2012). Human Cardiac Progenitor Cells Engineered With Pim-I Kinase Enhance Myocardial Repair. Journal of the American College of Cardiology 60:1278-1287.
190. Smart N, S Bollini, KN Dube, JM Vieira, B Zhou, S Davidson, D Yellon, J Riegler, AN Price, MF Lythgoe, WT Pu and PR Riley. (2011). De novo cardiomyocytes from within the activated adult heart after injury. Nature 474:640-U117.
191. D'amario D, C Fiorini, PM Campbell, P Goichberg, F Sanada, HQ Zheng, T Hosoda, M Rota, JM Connell, RP Gallegos, FG Welt, MM Givertz, RN Mitchell, A Leri, J Kajstura, MA Pfeffer and P Anversa. (2011). Functionally Competent Cardiac Stem Cells Can Be Isolated From Endomyocardial Biopsies of Patients With Advanced Cardiomyopathies. Circulation Research 108:857-U203.
192. Bolli R, AR Chugh, D D'Amario, JH Loughran, MF Stoddard, S Ikram, GM Beache, SG Wagner, A Leri, T Hosoda, F Sanada, JB Elmore, P Goichberg, D Cappetta, NK Solankhi, I Fahsah, DG Rokosh, MS Slaughter, J Kajstura and P Anversa. (2011). Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet 378:1847-57.
193. Chugh AR, GM Beache, JH Loughran, N Mewton, JB Elmore, J Kajstura, P Pappas, A Tatooles, MF Stoddard, JA Lima, MS Slaughter, P Anversa and R Bolli. (2012). Administration of cardiac stem cells in patients with ischemic cardiomyopathy: the SCIPIO trial: surgical aspects and interim analysis of myocardial function and viability by magnetic resonance. Circulation 126:S54-64.
194. Kajstura J, M Rota, SR Hall, T Hosoda, D D'Amario, F Sanada, H Zheng, B Ogorek, C Rondon-Clavo, J Ferreira-Martins, A Matsuda, C Arranto, P Goichberg, G Giordano, KJ Haley, S Bardelli, H Rayatzadeh, X Liu, F Quaini, R Liao, A Leri, MA Perrella, J Loscalzo and P Anversa. (2011). Evidence for human lung stem cells. N Engl J Med 364:1795-806.
195. Kotton DN and A Fine. (2008). Lung stem cells. Cell and Tissue Research 331:145-156.
196. Anversa P, MA Perrella, S Kourembanas, AM Choi and J Loscalzo. (2012). Regenerative pulmonary medicine: potential and promise, pitfalls and challenges. Eur J Clin Invest 42:900-13.
197. Brody JS and MC Williams. (1992). Pulmonary Alveolar Epithelial-Cell Differentiation. Annual Review of Physiology 54:351-371.
198. Hong KU, SD Reynolds, S Watkins, E Fuchs and BR Stripp. (2004). Basal cells are a multipotent progenitor capable of renewing the bronchial epithelium. American Journal of Pathology 164:577-588.
199. Morrisey EE and BLM Hogan. (2010). Preparing for the First Breath: Genetic and Cellular Mechanisms in Lung Development. Developmental Cell 18:8-23.

200. DelaRosa O and E Lombardo. (2010). Modulation of adult mesenchymal stem cells activity by toll-like receptors: implications on therapeutic potential. Mediators Inflamm 2010:865601.
201. Gonzalez-Rey E, P Anderson, MA Gonzalez, L Rico, D Buscher and M Delgado. (2009). Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut 58:929-39.
202. Zappia E, S Casazza, E Pedemonte, F Benvenuto, I Bonanni, E Gerdoni, D Giunti, A Ceravolo, F Cazzanti, F Frassoni, G Mancardi and A Uccelli. (2005). Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 106:1755-61.
203. Okita K, N Nagata and S Yamanaka. (2011). Immunogenicity of induced pluripotent stem cells. Circ Res 109:720-1.
204. Uccelli A, L Moretta and V Pistoia. (2008). Mesenchymal stem cells in health and disease. Nat Rev Immunol 8:726-36.
205. Uccelli A, G Mancardi and S Chiesa. (2008). Is there a role for mesenchymal stem cells in autoimmune diseases? Autoimmunity 41:592-5.
206. Menard C, L Pacelli, G Bassi, J Dulong, F Bifari, I Bezier, J Zanoncello, M Ricciardi, M Latour, P Bourin, H Schrezenmeier, L Sensebe, K Tarte and M Krampera. (2013). Clinical-grade mesenchymal stromal cells produced under various GMP processes differ in their immunomodulatory properties: Standardization of immune quality controls. Stem Cells Dev.
207. Liu Y, L Wang, T Kikuiri, K Akiyama, C Chen, X Xu, R Yang, W Chen, S Wang and S Shi. (2011). Mesenchymal stem cell-based tissue regeneration is governed by recipient T lymphocytes via IFN-gamma and TNF-alpha. Nat Med 17:1594-601.
208. de Hoon MJ, S Imoto, J Nolan and S Miyano. (2004). Open source clustering software. Bioinformatics 20:1453-4.
209. Saldanha AJ. (2004). Java Treeview--extensible visualization of microarray data. Bioinformatics 20:3246-8.
210. Brooke G, H Tong, JP Levesque and K Atkinson. (2008). Molecular trafficking mechanisms of multipotent mesenchymal stem cells derived from human bone marrow and placenta. Stem Cells Dev 17:929-40.
211. De Ugarte DA, Z Alfonso, PA Zuk, A Elbarbary, M Zhu, P Ashjian, P Benhaim, MH Hedrick and JK Fraser. (2003). Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. Immunol Lett 89:267-70.
212. Augello A, R Tasso, SM Negrini, A Amateis, F Indiveri, R Cancedda and G Pennesi. (2005). Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol 35:1482-90.
213. Luz-Crawford P, D Noel, X Fernandez, M Khoury, F Figueroa, F Carrion, C Jorgensen and F Djouad. (2012). Mesenchymal stem cells repress Th17 molecular program through the PD-1 pathway. PLoS One 7:e45272.
214. Najar M, G Raicevic, HF Kazan, C De Bruyn, D Bron, M Toungouz and L Lagneaux. (2012). Immune-related antigens, surface molecules and regulatory factors in human-derived mesenchymal stromal cells: the expression and impact of inflammatory priming. Stem Cell Rev 8:1188-98.
215. Bottino C, R Castriconi, L Moretta and A Moretta. (2005). Cellular ligands of activating NK receptors. Trends Immunol 26:221-6.

216. Poggi A, C Prevosto, AM Massaro, S Negrini, S Urbani, I Pierri, R Saccardi, M Gobbi and MR Zocchi. (2005). Interaction between human NK cells and bone marrow stromal cells induces NK cell triggering: role of NKp30 and NKG2D receptors. J Immunol 175:6352-60.
217. Benvenuto F, S Ferrari, E Gerdoni, F Gualandi, F Frassoni, V Pistoia, G Mancardi and A Uccelli. (2007). Human mesenchymal stem cells promote survival of T cells in a quiescent state. Stem Cells 25:1753-60.
218. Caplan AI. (2007). Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol 213:341-7.
219. Eming SA, T Krieg and JM Davidson. (2007). Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol 127:514-25.
220. Krampera M, A Pasini, G Pizzolo, L Cosmi, S Romagnani and F Annunziato. (2006). Regenerative and immunomodulatory potential of mesenchymal stem cells. Curr Opin Pharmacol 6:435-41.
221. Lukacs-Kornek V, D Malhotra, AL Fletcher, SE Acton, KG Elpek, P Tayalia, AR Collier and SJ Turley. (2011). Regulated release of nitric oxide by nonhematopoietic stroma controls expansion of the activated T cell pool in lymph nodes. Nat Immunol 12:1096-104.
222. Mellor AL and DH Munn. (2004). IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol 4:762-74.
223. Mougiakakos D, R Jitschin, CC Johansson, R Okita, R Kiessling and K Le Blanc. (2011). The impact of inflammatory licensing on heme oxygenase-1-mediated induction of regulatory T cells by human mesenchymal stem cells. Blood 117:4826-35.
224. Ren G, J Su, L Zhang, X Zhao, W Ling, A L'Huillie, J Zhang, Y Lu, AI Roberts, W Ji, H Zhang, AB Rabson and Y Shi. (2009). Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. Stem Cells 27:1954-62.
225. Chen Y, LX Xiang, JZ Shao, RL Pan, YX Wang, XJ Dong and GR Zhang. (2010). Recruitment of endogenous bone marrow mesenchymal stem cells towards injured liver. J Cell Mol Med 14:1494-508.
226. Sasaki M, R Abe, Y Fujita, S Ando, D Inokuma and H Shimizu. (2008). Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. J Immunol 180:2581-7.
227. Wu Y, L Chen, PG Scott and EE Tredget. (2007). Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 25:2648-59.
228. Anversa P, J Kajstura, M Rota and A Leri. (2013). Regenerating new heart with stem cells. J Clin Invest 123:62-70.
229. Krampera M, J Galipeau, Y Shi, K Tarte, L Sensebe and MSCCotISfCT On behalf of the. (2013). Immunological characterization of multipotent mesenchymal stromal cells-The International Society for Cellular Therapy (ISCT) working proposal. Cytotherapy.