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Eph/Ephrin-mediated Mesenchymal Stem Cell Regulation of T-cell Activation and Function

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Abstract

Mesenchymal stem cells (MSC) represent a promising cellular therapy for the treatment of immune-related conditions due to their immunomodulatory properties, which include the capacity to inhibit the proliferation and function of T-cells. Despite the fact that MSC have been the subject of intense investigation as therapeutic agents for diseases in which cellular immune response is exacerbated, the underlying mechanisms of how MSC exert their Tcell suppressive properties remain to be fully understood. Eph surface tyrosine kinase receptors and their ephrin ligands are involved in T-cell development, maturation, activation and proliferation. Recent findings have demonstrated Eph/ephrin interactions as potential mechanisms mediating human MSC inhibition of activated T-cells. Here we highlight the influence of Eph and ephrin molecules in the communication between MSC and T-cells that result in T-cell suppression by MSC.

Definition and Characteristics of Mesenchymal Stem Cells

Mesenchymal stem cells (MSC) are self-renewing stem cells identified in rodent and human bone marrow aspirates based on their ability to form adherent clonogenic clusters (CFU-F; colony forming units-fibroblastic) *in vitro*, and by their capacity to differentiate into multiple specialized mesodermal cell lineages [1-4]. Similar MSC-like populations have been described in various tissues with different growth and differentiation potentials [5-7]. These MSC-like populations share a common immunophenotype based on the cell surface expression of various markers, but not limited, to STRO-1, CD73, CD105, CD106, CD90, CD146 and CD166, while lacking expression of CD34, CD3, CD14, CD19, CD31, CD34, CD45, Glycophorin-A and HLA-DR [2,3,8-10]. In the last few decades, MSC have generated considerable interest due to their production of cytokines and growth factors, which act as potent mediators of angiogenesis, regeneration of damaged tissues, hematopoiesis and immune cell responses [11-17]. In particular, the paracrine properties of MSC, makes them highly desirable as potential cellular therapies to treat a variety of immune/inflammatory based diseases and conditions.

MSC mediated regulation of T-cell proliferation and activation

The role of MSC in modulating immune responses has been demonstrated both *in vitro* and *in vivo* [13,18]. Multiple studies have shown that MSC profoundly modulate immune response through their interactions with the cellular components of the innate (natural killer cells, dendritic cells) and adaptive (B-cells, T-cells) immune system (Figure 1). MSC are known to be weakly immunogenic due to the lack of expression of MHC class II antigen and co-stimulatory molecules such as CD40, CD80, CD86 or CD40L [13]. Whilst MSC have a

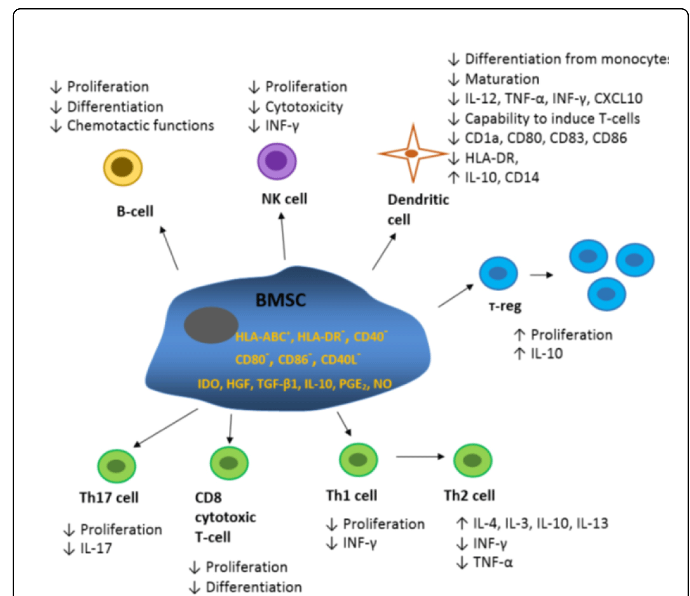

Figure 1: Immunomodulatory properties of MSC. MSC modulate the response of a broad range of immune cells including B-cells, NK cells, DC and T-cells. NK: Natural Killer cells, DC: Dendritic cells, T-reg: T-regulatory cells, Th: T-helper cells, HLA: Human leukocyte antigen; IL: Interleukin; IFNγ: Interferon-gamma; TNF-α: Tumour Necrosis Factor-alpha; IDO: Indoleamine 2,3-dioxygenase; HGF: Hepatocyte Growth Factor; TGF-β1: Transforming Growth Factor-β; IL-10: Interleukin 10; PGE2: Prostaglandin E2; NO: Nitric Oxide.

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reduced capacity to trigger T-cell activation, they exhibit a potent capacity to inhibit T-cell proliferation and function either directly through cell–cell contact or indirectly *via* the secretion of various factors. Due to these properties, MSC are protected from natural killer cellmediated killing and have the ability to escape immunosurveillance and regulate immune cell/inflammatory responses.

Studies investigating the hypoimmunogenic properties of MSC have reported that *ex vivo* expanded third party human MSC suppress T-cell proliferation stimulated by allogeneic peripheral blood mononuclear cells (PBMNC) and allogeneic splenocytes, activated with mitogens such as, concanavalin A, anti-CD3, anti-CD28 antibodies or with IL-2, IL-7 or IL-15 *in vitro* [19,20]. It has been demonstrated that both CD4 and CD8 T-cells are equally inhibited by MSC in a dose-dependent manner. Most significantly, MSC do not induce T-cell apoptosis but rather facilitate a shutdown in proliferation/activation [21]. Indeed, the anti-proliferative effects of MSC on T-cell populations only occurs when T-cells are activated [22]. However, direct contact between MSC and activated T-cells leads to T-cell arrest in the G0 phase of the cell cycle. MSC induce T-cells to enter an anergic state, characterized by an absence of proliferation and cytokine production in response to antigenic stimulation, generally as a result of insufficient co-stimulation [23,24]. Furthermore, other studies have reported that MSC cause a reduction in the expression of the early activation markers CD25 and CD69, while others found no change in expression of these markers [20,25]. The discrepancies of these findings could potentially relate to the variations in subpopulations of MSC that were being investigated and the differences in the population of T-cell studied.

In the last decade, it has become evident that multipotent human MSC, exhibiting immunomodulatory properties, are being utilized in various clinical trials to treat various immunological conditions including steroid-resistant acute graft-versus-host disease (GvHD) following hematopoietic stem cell transplantation, inflammatory bowel diseases, encephalomyelitis and allograft rejection after solid organ transplantation [18,26,27]. To date, numerous cell based-therapies employing MSC to treat diseases in which cellular immune responses are exacerbated, have been registered at clinical-trials.gov (https://clinicaltrials.gov/ct2/results?term=%22mesenchymal+stem+cell%22+AND+%22immune+disease%22&Search=Search). Most significantly, the use of MSC to treat paediatric patients with steroid refractory acute GvHD has been approved in Japan (TEMCELL®, JCR Pharmaceuticals) [28], Canada and New Zealand (Prochymal®; Osiris Therapeutics) [29].

Despite extensive documentation of the interactions between MSC toward different Tcell subpopulations, the exact mechanisms underlying these processes remain unclear. Recent studies indicate that multiple immunosuppressive factors can be released or expressed by MSC and the nature of these factors is dependent on the type of stimuli received by MSC, such as allogeneic determinants, membrane-bound proteins, mitogens and cytokines [27,30]. Several independent studies have indicated that hepatocyte growth factor (HGF), transforming growth factor-β1 (TGF-β1), indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), HLA-G5 and IL-10 are all key players in MSC-mediated immunosuppression *in vitro* [30,31]. Other studies have suggested that contact-dependent mechanisms are involved, including the B7-H1/PD-1 pathway [13,18]. While some of these factors partially contribute to the immunomodulatory properties of MSC, the underlying mechanisms that regulate MSC mediated immune cell action are far more complex than initially thought.

More recently, the tyrosine kinase receptors, Eph (Erythropoietin-producing hepatocyte kinases) and their ligands, ephrin (Eph receptor interaction proteins) have been reported to be involved in MSC attachment, migration, differentiation, bone remodeling, fracture repair and hematopoietic support [11,32-35]. Studies have also demonstrated that Eph/ephrin tyrosine kinase molecules play an important role in the development and function of immune cells [36-39], including, T-cell populations [40-42].

Eph and ephrin molecules

Eph receptors and their ephrin ligands represent the largest group of receptor tyrosine kinases [43] and are known to be involved in the regulation of numerous biological systems in which cell–to–cell interactions are particularly relevant [44,45]. The Eph receptors are membrane bound proteins and are divided into EphA (10 members) and EphB (6 members) subclasses based on their binding affinity to either ephrinA (6 members) or ephrinB (3 members) ligands (Figure 2). The EphA and EphB receptors share similar structural homology

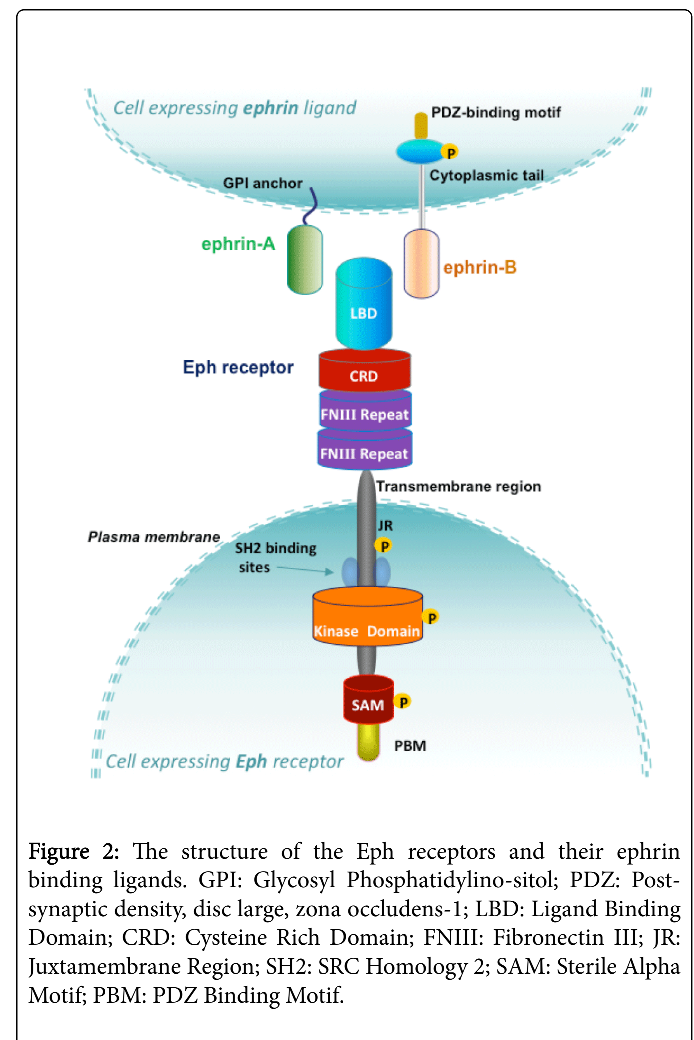

Figure 2: The structure of the Eph receptors and their ephrin binding ligands. GPI: Glycosyl Phosphatidylino-sitol; PDZ: Post-synaptic density, disc large, zona occludens-1; LBD: Ligand Binding Domain; CRD: Cysteine Rich Domain; FNIII: Fibronectin III; JR: Juxtamembrane Region; SH2: SRC Homology 2; SAM: Sterile Alpha Motif; PBM: PDZ Binding Motif.

[46], composed of extracellular and cytoplasmic components. The extracellular portion of the Eph receptor consists of an amino terminal ligand binding domain, a cysteine rich region and two fibronectin type III repeats [43]. The cytoplasmic region of the Eph receptor contains SRC homology 2 binding sites, a juxtamembrane region, a tyrosine

kinase domain, a sterile alpha motif (SAM) proteinprotein interaction domain, and a post-synaptic density, disc large, zona occludens-1 (PDZ) binding motif [47]. Each region is important for signalling in different situations, the juxtamembrane domain contains two conserved tyrosine residues (tyrosine and serine) which become phosphorylated upon the binding of ephrin ligand, allowing the intracellular tyrosine kinase domain to convert into its active form and activate or repress downstream signalling cascades, whereas the role of the SAM domain is in protein–protein interactions. The PDZ domain consists of a binding sequence which has a hydrophobic residue (generally valine or isoleucine) at the carboxyl terminus, that also functions in protein–protein interactions [48]. Both subclasses of the ephrin ligands consist of an extracellular Eph-receptor binding domain, however, the structure of the ligands differs between subclasses [49]. The ephrinA ligands are GPI linked to the membrane, where the GPI anchor is composed of one molecule of phosphatidylinositol, which has a carbohydrate chain link through the C-6 hydroxyl of the inositol. The GPI anchor functions to attach the ephrinA ligand to the exoplasmic leaflet of the membrane and to other specific domains inside the cell [48]. Conversely, ephrinB ligands are transmembrane proteins with a short cytoplasmic tail, which contains functional tyrosine kinase phosphorylation sites and an intracellular PDZ binding motif [49].

Eph/ephrin signalling is highly complex due to the high number of members and promiscuity of individual Eph receptors binding to multiple ligands and vice versa, albeit with distinct affinities [43]. While the Eph molecules are regarded as the receptor and the ephrin molecules as the ligand, Eph/ephrin-mediated interactions can result in both *forward* signalling through the Eph receptor expressing cell and *reverse* signalling through the ephrin-expressing cell, and/or bidirectional signalling in both Eph and ephrin expressing cells. As a result, Eph/ephrin interactions often provide different cell responses depending on the multiple combinations and directions of signalling. In the context of T-cell biology, Eph/ephrin interactions activate numerous signalling pathways that regulate T-cell progenitor recruitment to the thymus [50], thymocyte development and maturation within the thymic microenvironment [51], T-cell co-stimulation, activation and proliferation (Table 1). More recently, members of the EphB subclass are involved in MSC-mediated suppression of activated T-cells [52].

Cell population	Eph/ephrin involvement	Biological Function	Refs
Mouse T-cells	ephrinB1, ephrinB2	suppressed murine Tcell proliferation	[44]
	ephrinB1, ephrinB2	regulated T cell chemotaxis and migration in encephalomyelitis and multiple sclerosis	[45]
	ephrinB1, ephrinB2	double knockout ephrinB1 and ephrinB2 mice specifically in the T-cell compartment resulted in reduced thymus and spleen size and cellularity, and compromised T-cells homeostatic expansion	[36]
	EphB2	EphB2deficient mice exhibited thymic hypo-cellularity, altered survival and proliferation of the differentiating lymphocytes, decreased double positive and single positive thymocyte subpopulations and mature CD4^{+} and CD8^{+} Tcell populations	[54]
	EphB6	EphB6 knockout mice displayed compromised T-cell response to TCR stimulation *in vitro* and *in vivo*	[55]
	ephrinB1	is important in T-cell–to–T-cell co-operation in response to antigen stimulation	[56]
	ephrinB2, ephrinB3	is crucial in T-cell co-stimulation to enhance T-cell signalling	
Human T-cells	EphB4, ephrinB1	EphB4 and ephrinB1, expressed by human T-cells, interact with ephrinB2 and EphB2 respectively, expressed by human MSC, result in suppression of activated T-cells.	[52]
	ephrinB1	enhanced lymphocyte migration and cytokine production in patients with rheumatoid arthritis	[59]
Human MSC	EphB2, ephrinB2	EphB2/ephrinB1 and ephrinB2/EphB4 communication between human MSC and T-cells inhibit T-cell proliferation	[52]
MSC: Mesenchymal Stem Cells			

Table 1: Regulation of T-cell development and function by Eph/ephrin interactions.

Eph/ephrin-mediated MSC suppression of activated T-cells

Cell–to–cell contact represents one of the main mechanisms involved in T-cell suppression by MSC [16]. Various studies exploring the dynamics of MSC and T-cell interactions reported that cell–to–cell contact produced a stronger MSC-mediated suppressive effect on T-cell proliferation/activation [17,53]. Indeed, a number of MSC expressing membrane molecules such as integrins (α1, α2, α3, α5, α6, αv, β1, β3 and β4), intercellular adhesion molecules (ICAM-1, ICAM-2), vascular cell adhesion protein (VCAM-1), CD72 and CD58 (LFA-3) that bind to T-cells with high affinity. In addition, a number of membrane molecules participate in MSC-mediated immunoregulation include the B7-H1/PD-1 pathway, HLA-G1 and Jagged-1 [16,17].

Eph and ephrin, contact-dependent molecules involved in T-cell biology and MSC regulation, have only recently been demonstrated to play an important role in MSCmediated suppression of T-cell proliferation. Human primary T-cells express high levels of ephrinB1 and EphB4 [52], which bind with highest affinity to EphB2 and ephrinB2, respectively, which are highly expressed by human MSC [32], emphasizing a role of EphB2/ephrinB1 and EphB4/ephrinB2 interactions in MSC–T-cell communication. This is in accord with previous studies demonstrating that ephrinB1 and ephrinB2 suppressed murine Tcell proliferation [42], and that EphB2 and ephrinB2 interactions significantly suppressed human T-cell proliferation, in allogeneic mixed lymphocyte cultures [52]. Of note, the EphB2 and ephrinB2-mediated T-cell suppression of MSC was reversed in experiments using EphB2 and EphB4 blocking peptides, to disrupt the EphB2/ephrinB1 and ephrinB2/EphB4 interactions, respectively, between MSC and T-cell populations. The functional role of EphB2 and ephrinB2 in T-cell suppression of MSC was further confirmed in shRNA knockdown studies of either EphB2 or ephrinB2 in human MSC, which resulted in a decreased capacity to suppress T-cell proliferation, compared with non-silencing shRNA scrambled control MSC [52]. These findings are supported by investigations

utilizing double knockout ephrinB1 and ephrinB2 mice specifically in the T-cell compartment (through loxPmediated gene deletion), which resulted in reduced thymus and spleen size and cellularity, and compromised T-cells expansion [36]. Furthermore, EphB2-deficient mice revealed that thymic hypo-cellularity was associated with altered survival and proliferation of the differentiating lymphocytes, evident by a decrease in the double positive and single positive thymocyte subpopulations and mature $CD4^{+}$ and $CD8^{+}$ Tcell populations [54].

Interestingly, contradictory studies reported that members of the EphB-subclass act as co-stimulatory molecules for murine T-cell proliferation [42,55-57]. The ephrinB2 ligand was shown to stimulate T-cell function by enhancing T-cell response to antigens by *in vitro* TCR stimulation [56]. In EphB6 knockout studies, T-cells were reported to be defective in their response to TCR stimulation *in vitro* and *in vivo* [57]. Furthermore, ephrinB1 was found to be crucial in T-cell–to–T-cell co-operation in response to antigen stimulation, while ephrinB2 and ephrinB3 were found to play a role in T-cell co-stimulation to enhance T-cell signaling [58]. In rheumatoid arthritis, EphB1/ephrinB1 interactions were demonstrated to enhance T-cell migration [59]. Therefore, the contribution of Eph/ephrin molecules during T-cell proliferation remains controversial. Nevertheless, it must be highlighted that whilst Eph/ephrin molecules constitute a large family with many members that bind promiscuously to each other, both Eph receptors and ephrin ligands have their own signalling mechanisms that interact with many other signalling pathways (such as MAPK, PI3K, Src, Pyk2) adding to the variability of biological responses. Therefore, Eph/ephrin signalling is associated with a complicated network of agonistic or antagonistic cross-talk, even between the same Eph/ephrin pairings, and is highly dependent on the cell types and their state of differentiation.

In the context of T-cell proliferation, Kawano and colleagues have demonstrated a switch from T-cell stimulation to suppression of proliferative responses in the presence of ephrinB1-Fc and ephrinB2-Fc fusion molecules by varying the concentrations [42], indicating EphB/ephrinB signalling in T-cell activation involves a concentration-dependent switch from co-stimulation to inhibition. Furthermore, the findings that MSC expressing EphB2 or ephrinB2 inhibits T-cell proliferation are in agreement with other studies, which found that EphA/ephrinA interactions inhibit activated $CD4^{+}$ T-cell proliferation and T-helper-2-cell activation [60], while ephrinB1 and ephrinB2 compromise STAT3 activation leading to a decreased T-cell proliferation [36]. Notably, the immunosuppressive effects of MSC mediated by EphB2 and ephrinB2 was independent of T-cell apoptosis, consistent to other findings that MSC do not appear to induce T-cell cell death [17,53]. Therefore, EphB2/ephrinB1 and ephrinB2/EphB4 interactions can be considered as additional mechanisms of cross-talk between MSC and T-cells, revealing the importance of cell adherence in the context of immunomodulation by MSC.

The role of ephrinB1 and ephrinB2 in homeostatic expansion of T-cells have also been reported [36]. Mice with double conditional knockout of both ephrinB1 and ephrinB2 (through loxP-mediated gene deletion) presented failed homeostatic expansion in sub-lethally irradiated recipients. This effect was thought to be mediated by reduced IL-6 signalling in the T-cell compartment. These findings were consistent to another study showing that IL-6 is required for the survival of naïve T-cells during homeostatic expansion [61].

Mechanisms of Eph/ephrin-mediated MSC suppression of activated T-cells

In addition to cell–to–cell contact, MSC exert their immunomodulatory effects by releasing soluble factors [13,15]. The production of suppressive soluble factors is dependent on cross-talk between MSC and activated T-cells since the use of conditioned supernatant from MSC cultures is not sufficient to elicit T-cell suppression, in contrast to condition media from MSC co-cultured with activated T-cells. Similar levels of activated T-cell suppression can also be seen using conditioned media from MSC cultures, following pre-stimulated with T-cell derived factors such as IFNγ [18]. Treatment of MSC with IFNγ have been reported to stimulate MSC production of IDO [18,21,27], where MSC lacking the IFN-γ receptor lose their capacity to suppress T-cell proliferation [21]. Recent findings have shown that EphB2 and ephrinB2 mediate MSC suppression of T-cells by their capacity to secrete elevated levels of IDO expression following activation of EphB4 or ephrinB1 expressing T-cells in the presence of IFN-γ [52]. In addition, the expression of other immunomodulatory factors, TGF-β1 and iNOS, were also reported to be up-regulated following stimulation with EphB4 expressing human T-cells [52]. Induction of iNOS expression correlated with elevated levels of NO, as iNOS is induced by cytokines for the synthesis of NO [62]. Other soluble factors produced by T-cells, such as TNF-α, IL-2, IL-17 and IL-4 are vital for T-cell activation and proliferation [63]. While MSC have been demonstrated to suppress Tcell proliferation by reducing IL-17 production [64], TNF-α, IL-2 and IL-17, but not IL-4, expression levels were down-regulated in human T-cells following stimulation with EphB2 and ephrinB2, expressed by human MSC [52]. Moreover, stimulation of murine T-cells with ephrinB2 suppressed their proliferation associated with a reduction of TNF-α, IL-2 and IL-17 [42]. However, the expression levels of HGF and IL-10, also known to be involved in MSC mediated immunosuppression of T-cells, were not affected by EphB4 or ephrinB1 stimulation, indicating a level of specificity in EphB/ephrinB signalling during MSC and T-cell interactions.

While Eph/ephrin interactions trigger numerous signalling pathways to mediate T-cell migration, maturation, proliferation and differentiation, it appears that the underlying mechanisms of Eph/ephrin-mediated T-cell suppression by human MSC were through EphB2induced ephrinB1 reverse signalling and ephrinB2-induced EphB4 forward signalling in activated T-cells[52]. Experiments employing signalling inhibitors showed that inhibition of T-cell proliferation is mediated through activation of Src family kinase, PI3 Kinase, JNK and Abl kinases signalling pathways. Conversely, inhibitors to the MEK, p38 MAPK pathways failed to elicit any significant changes in EphB4 forward signalling or ephrinB1 reverse signalling mediated suppression of T-cell proliferation. Whilst ephrinB reverse signalling has previously been described to be mediated via the Src molecule, Grb4, the PDZ domain or through the JNK pathway independent of tyrosine kinase phosphorylation [65,66], recent findings have demonstrated that reverse ephrinB signalling inhibited MSC attachment and spreading by activating Src, PI3 Kinase and JNK-dependent signalling pathways [32]. In addition, the Eph family are mediated predominantly through their tyrosine kinase activity and receptor phosphorylation [67], where ephrinB2-mediated suppression of T-cell proliferation was promoted by phosphorylation of EphB4 in T-cells activated by allogeneic MLR. Similarly, EphB2-mediated suppression of T-cell proliferation was also dependent on tyrosine phosphorylation during ephrinB1 reverse signalling. This is consistent with the finding of Kawano et al. describing that ephrinB2 inhibits

primary murine T-cell proliferation by inducing EphB4 phosphorylation [42].

The role of EphB2 and ephrinB2 interactions in MSC mediated suppression of activated T-cells appears to be quite specific. In addition to EphB2 and ephrinB2, MSC also express EphB4, ephrinB1, EphA2 and ephrinA5 that can promiscuously bind to various Eph and ephrin molecules expressed by T-cells. Studies examining the functional role of these Eph/ephrin molecules in Tcell proliferation demonstrated that T-cell proliferation was unaffected in the presence of these molecules [52]. This work confirmed the high affinity binding of MSC expressing EphB2 and ephrinB2 to ephrinB1 and EphB4 respectively, which are highly expressed by human T-cells. Furthermore, T-cells were found to express high gene expression levels of EphA4, which is known to promiscuously bind to ephrinA5 expressed by MSC [48,49]. However, functional studies showed no difference in T-cell proliferation in the presence of inhibitor to EphA4 receptor, supporting the notion that EphA and ephrinA molecules, are not directly involved in MSC-mediated suppression of activated T-cells. Human T-cells also express ephrinB3, which is known to bind to EphB2 [46]. However, it appears that ephrinB3 is unlikely to be involved in EphB2 mediated inhibition of Tcell proliferation. This is consistent with a previous report suggesting that ephrinB3 plays a stimulatory role in murine T-cell proliferation induced by anti-CD3, while ephrinB1 and ephrinB2 strongly inhibit murine activated T-cell proliferation [42]. Collectively, these findings suggest a role for EphB/ephrinB molecules in mediating MSC suppression of activated T-cells (Figure 3).

In addition to cell–to–cell contact and release of soluble factors, generation of regulatory T-cells has also been one of the main mechanisms involved in T-cell suppression by MSC [13]. Regulatory T-cells (Tregs), also known as suppressor T-cells, express the immunophenotype, $CD4^+CD25^+FoxP3^+$, and play an important role in the maintenance of immunological tolerance to suppress auto-immunity and graft rejection [24]. Co-culture of MSC with naïve T-cells in mixed allogeneic lymphocyte reactions results in an increase in the number of Treg cells [68,69]. Similarly, in an *in vivo* setting, it was reported that circulating Tregs increased in number when MSC were used in the treatment of systemic lupus erythematosus or in a kidney allograft in mice [69,70]. In these studies, MSC contributed to the expansion of the existing Treg populations. It appears that MSC act as a "homeostatic niche" for Tregs, to recruit, regulate and maintain Treg phenotype and function. While the interactions of EphB2/ephrinB1 and ephrinB2/EphB4 between MSC and T-cells play an important role in MSC-mediated Tcell suppression, their role in MSC stimulation of Tregs have yet to be identified. Studies have demonstrated that T-cell-specific ephrinB1 or ephrinB2 gene knockout (KO) mice have naive $CD4^+$ cells exhibiting similar ability to differentiate into Th1, Th2, Th17 and Treg cells to wildtype (WT) control $CD4^+$ cells [40,41]. However, these studies found that ephrinB1 or ephrinB2 KO mice displayed normal thymus and spleen weight, size and cellularity and normal T-cell subpopulations in these organs. In addition, the T-cell progenitors from KO mice repopulated host spleen T-cell pool similar to WT control mice. T-cell activation, proliferation and differentiation potential were also similar to that of WT control mice. These observations suggest that the function of ephrinB1 or ephrinB2 in the T-cell compartment, could be compensated by other members of the Eph family. These studies also indicate that ephrinB1 and ephrinB2 are unlikely to affect peripheral tolerance due to the absence of an autoimmune phenotype. Collectively, the role of ephrinB1 or ephrinB2 in the regulation of Treg population is not conclusive and further investigations are required to fully establish the contribution of Eph/ephrin interactions in Treg activation and function.

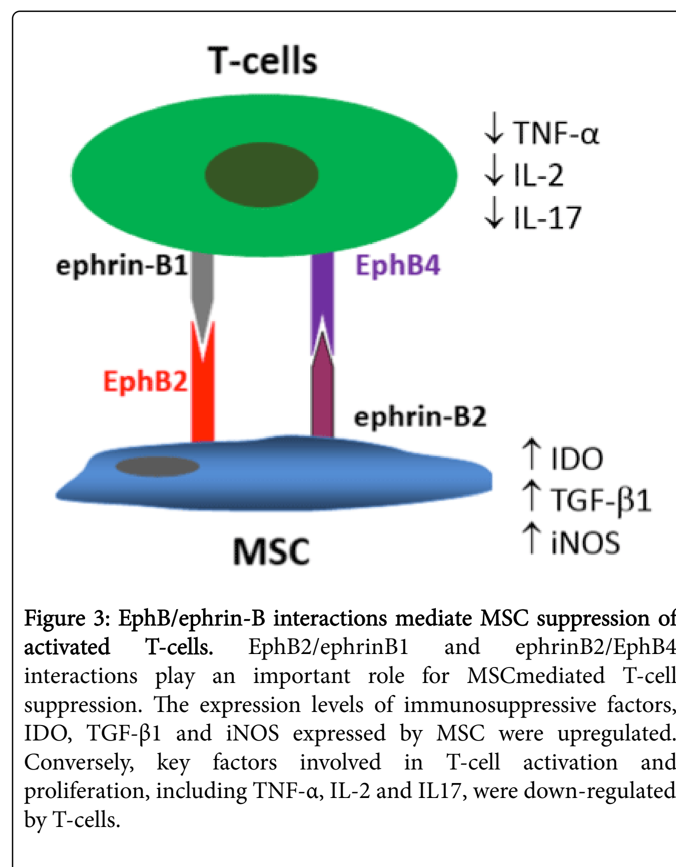

Figure 3: EphB/ephrin-B interactions mediate MSC suppression of activated T-cells. EphB2/ephrinB1 and ephrinB2/EphB4 interactions play an important role for MSCmediated T-cell suppression. The expression levels of immunosuppressive factors, IDO, TGF-β1 and iNOS expressed by MSC were upregulated. Conversely, key factors involved in T-cell activation and proliferation, including TNF-α, IL-2 and IL17, were down-regulated by T-cells.

Concluding Remarks

In the last few decades, there has been increasing interest in MSC and their potential use in modulating immune/ inflammatory responses. However, limited information has been available concerning the molecular mechanisms responsible for the action of MSC in these processes. The finding that EphB/ephrinB interactions contribute to MSCmediated Tcell suppression, has extended our current understanding of how MSC exert their immunosuppressive effects on activated T-cells. While MSC have been shown to modulate the responses of T-cell subsets such as $CD4^+$ T-helper cells, $CD8^+$ cytotoxic T-cells and promote the generation of $CD4^+CD25^+$Foxp3 regulatory T-cells [17], further investigations into the role of EphB/ephrinB interactions in the communication of MSC and these T-cell populations would provide better therapeutic strategies utilizing Eph and ephrin molecules to facilitate the therapeutic use of MSC in the clinic.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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