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FRONTIER

Immunomodulation by mesenchymal stem cells: Interplay between mesenchymal stem cells and regulatory lymphocytes

Oscar Ka-Fai Ma, Koon Ho Chan

Oscar Ka-Fai Ma, Koon Ho Chan, Department of Medicine, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Hong Kong, China

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Correspondence to: Dr. Koon Ho Chan, Department of Medicine, Li Ka Shing Faculty of Medicine, the University of Hong Kong, 4/F Professorial Block, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong, China. koonho@hkucc.hku.hk
Telephone: +852-22553315
Fax: +852-29741171

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Abstract

Mesenchymal stem cells (MSCs) possess immunomodulatory properties, which confer enormous potential for clinical application. Considerable evidence revealed their efficacy on various animal models of autoimmune diseases, such as multiple sclerosis, systemic lupus erythematosus and uveitis. MSCs elicit their immunomodulatory effects by inhibiting lymphocyte activation and proliferation, forbidding the secretion of proinflammatory cytokines, limiting the function of antigen presenting cells, and inducing regulatory T (T_{reg}) and B (B_{reg}) cells. The induction of T_{reg} and B_{reg} cells is of particular interest since T_{reg} and B_{reg} cells have significant roles in maintaining immune tolerance. Several mechanisms have been proposed regarding to the MSCs-mediated induction of T_{reg} and B_{reg} cells. Accordingly, MSCs induce regulatory lymphocytes through secretion of multiple pleiotropic cytokines, cell-to-cell contact with target cells and modulation of antigen-presenting cells. Here, we summarized how MSCs induce T_{reg} and B_{reg} cells to provoke immunosuppression.

Key words: Mesenchymal stem cells; Regulatory T cells; Regulatory B cells; Immunomodulation; Autoimmunity

Core tip: In this review, we summarized the mechanisms involved in regulatory T (T_{reg}) and B (B_{reg}) cell induction by mesenchymal stem cells (MSCs). In an inflammatory environment, MSCs secrete various anti-inflammatory cytokines, actively interact with immune cells and modulate them to acquire regulatory properties, thus, generate a tolerogenic environment. Particularly, by

inducing T_{reg} and B_{reg} cells, the immunomodulation of MSCs is amplified. Therefore, genetic engineered MSCs to enhance their ability to induce T_{reg} and B_{reg} cells may increase their therapeutic efficacy.

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INTRODUCTION

Mesenchymal stem cells (MSCs) are mesodermal progenitor cells that have a wide range of differentiation capacity. They can differentiate into adipocytes, osteocytes, chondrocytes, myocytes, fibroblasts and stromal cells[1]. In addition, some research studies have shown that MSCs, under certain conditions, can trans-differentiate to cells from ectodermal and endodermal lineage[2,3]. Among them, the ability of MSCs to develop into neurons is of particular interest. Considering that neural stem cells are limited in number and extremely difficult to be isolated while, comparatively, massive numbers of MSCs can be derived from numerous adult tissues, including, liver, kidney, adipose tissue, bone marrow, dental pulp, peripheral blood and umbilical cord blood. MSCs may serve as a reliable source of neural cells for potential cell replacement therapy or regenerative medicine.

Aside from its diverse differentiation capacity, their immunomodulatory properties also prompt researchers to study profoundly. MSCs are capable of regulating both innate and adaptive immunity. They secrete a large variety of soluble factors, including interleukin (IL)-6, IL-8, transforming growth factor-β1 (TGF-β1), indoleamine 2,3-dioxygenase (IDO), human leukocyte antigen-G (HLA-G) and prostaglandin E2 (PGE2)[4]. These factors allow MSCs to interact with components of the innate and adaptive immunity, subsequently modulate inflammation and immune tolerance. Monocytes, for instance, under the influence of MSCs-secreted IL-6, IDO and PGE2, tend to develop into anti-inflammatory M2 macrophages instead of proinflammatory M1 macrophages[5-9]. In addition, recent reports showed that human gingiva derived MSCs have converted M1 macrophages to M2[5]. Natural killer (NK) cells, on the other hands, express CD73 and acquires regulatory phenotype when exposed to MSCs[10,11]. Similarly, regulatory dendritic cells (DC) induced by MSCs were capable of secreting IL-10, a powerful anti-inflammatory cytokine[12-14]. Thus, MSCs are able to suppress innate immunity by skewing their differentiation into regulatory subtype (Figure 1).

MSCs can regulate adaptive immune system by suppressing the proliferation, differentiation and activation of T cell and B cell. A number of studies have demonstrated that MSCs can inhibit the proliferation of Th1 and Th17 cell, decrease the production of interferon (IFN)-γ, IL-2, IL-6 and IL-17, and downregulate the T cell activation markers, CD38 and HLA-DR[15-19]. When MSCs were co-cultured with B cell and in the presence of different B cell trophic stimuli, B cell proliferation was inhibited and they were arrested in G_0/G_1 phase. Moreover, B cell differentiation was prohibited as indicated by limited production of IgG, IgM and IgA[20]. In addition, the regulatory-skewing propensity of MSCs observed in innate immune system also applies to T and B lymphocyte. In fact, the ability of MSCs to expand regulatory T (T_{reg}) cells and regulatory B (B_{reg}) cells have been intensively studied. However, the mechanism of how T_{reg} and B_{reg} cells are induced by MSCs has not been fully understood. Some suggest regulatory lymphocytes induction by MSCs requires mediation of other immune cells, while others propose MSCs-released cytokines are sufficient to expand T_{reg} and B_{reg} cell populations, but more and more researchers have come to the consensus that MSCs can use multiple pathways to generate regulatory lymphocytes and which pathways are more favorable is determined by the microenvironment that MSCs encounter[21]. Altogether, MSCs modulate immune cells to acquire regulatory phenotype, hence, alter the inflammatory milieu into a tolerogenic one (Figure 1).

There is another advantage of using MSCs for cellular therapy. MSCs have low immunogenicity, implying that MSCs can be used for allogeneic transplantation. This property is particularly helpful to the patient whose MSCs are compromised. Thereby, MSCs possess valuable therapeutic potential to treat immune-mediated disorders[22].

Although MSCs have demonstrated as a promising immunoregulator for clinical use, the immunomodulatory and low-immunogenicity properties of MSCs are not constitutive. The function of MSCs is based on the signals from the vicinity. MSCs, in the absence of tumor necrosis factor (TNF)-α and IFN-γ may adopt pro-inflammatory phenotype, which activate T cells to response. On the contrary, when MSCs are exposed to high level of TNF-α and IFN-γ they will behave as an anti-inflammatory regulator by producing TGF-β1, IDO, and PGE2[23]. Likewise, depending on the level of IL-6, MSCs can convert monocyte into M1 or M2 macrophages[22,24-26]. Thus, before any clinical application, the plasticity of MSCs should be carefully considered. In this review, we summarized current understandings on how MSCs interact with regulatory lymphocytes, T_{reg} and B_{reg} cells particularly, to attenuate autoimmunity, and how this knowledge can contribute to therapeutic development.

T_{reg} LYMPHOCYTE

The notion of "suppressive" T cells has long been proposed in 1970s. Due to technical limitation, their identities and phenotypic characteristics cannot be described until 1995, Sakagucho *et al*[27] isolated a unique $CD4^+$ $CD25^+$ T cells that can suppress immune responses and maintain immunologic self-tolerance[28]. Later, this subpopulation

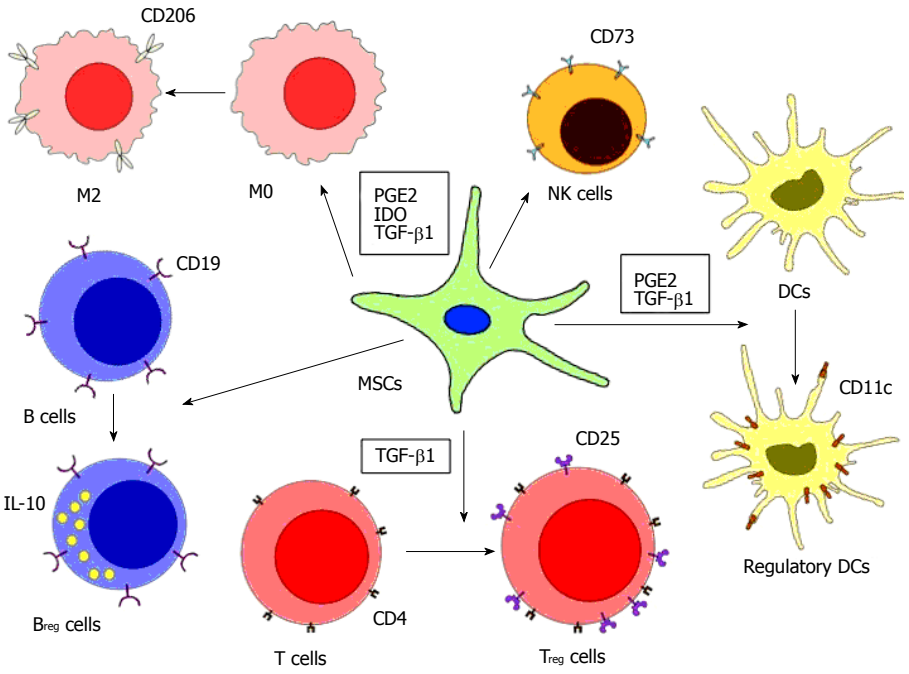

Figure 1 Immunosuppression by mesenchymal stem cells. MSCs suppress innate and adaptive immune responses by enhancing regulatory immune cells with tolerogenic properties. MSCs suppress macrophages by favoring monocyte polarization to anti-inflammatory M2 macrophages, increasing the production of IL-10, and decreasing the production TNF-α and IL-12. MSCs can also regulate DCs by downregulating the expression of MHC, CD40, CD80, CD83 and CD86, thus, diminishing their antigen presenting ability, while upregulating the expression of IL-10. MSCs can reduce the NK cell cytotoxicity and decrease their production of TNF-α and IFN-γ. T_{reg} and B_{reg} cells can be induced by MSCs, further increase the production of anti-inflammatory cytokines (IL-10 and TGF-β1). However, the mechanisms of how B_{reg} cells are induced by MSCs are still not clear. MSCs: Mesenchymal stem cells; TNF: Tumor necrosis factor; IL: Interleukin; NK: Natural killer; DCs: Dendritic cells; IFN-γ: Interferon-γ; T_{reg}: Regulatory T; B_{reg}: Regulatory B; TGF: Transforming growth factor; PGE2: Prostaglandin E2; IDO: Indoleamine 2,3-dioxygenase.

of T cells was named as T_{reg} cells. For those T_{reg} cells that undergo maturation in thymus, are referred to as thymus-dervied T_{reg} (tT_{reg}) cells. Three days post-maturation, tT_{reg} cells will relocate from thymus to periphery[29]. Surprisingly, tT_{reg} cells only comprise 5%-10% of peripheral T cells, but they are the critical regulator of autoimmunity. This is evidenced in mice lacking peripheral T_{reg} cells. They were lethal due to various autoimmunity enhancements[29,30].

Apart from tT_{reg} cells, T_{reg} cells can also be generated in periphery[31,32]. Periphery-derived T_{reg} (pT_{reg}) cells are converted from naïve T cells ($CD4^+CD25^-Foxp3^-CD45RB^{hi}$). Upon activation of naive T cells and in the presence of particular cytokines, two main types of T_{reg} cells can be differentiated in the periphery and *in vitro*, namely, T helper 3 (Th3) cells and type 1 regulatory T (Tr1) cells. Th3 cell and Tr1 cell differentiation are promoted by TGF-β and IL-10, respectively[33-35]. Both Th3 and Tr1 cells are suppressive to effector and memory T cells, and they are able to secrete cytokine for self-activation. However, one distinct phenotypical difference is Th3 cells are $Foxp3^+$ whereas Tr1 cells are $Foxp3^-$.

Forkhead box P3 (Foxp3) is a transcription factor that constitutively express in tT_{reg} cells and some types of pT_{reg} cells. It has been recognized as the master regulator of T_{reg} cells. Scurfy, a *Foxp3* gene mutated mouse, is lethal by one month after birth, displays hyperactivation of $CD4^+$ T cells and overproduction of proinflammatory cytokines[36]. In human, immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is X-linked recessive disorder caused by mutation in *Foxp3* gene[37]. T_{reg} cells from the patients with IPEX are either dysfunction or completely vanished. As a result, IPEX patients are afflicted with various autoimmune diseases, allergy and/or inflammatory bowel disease[38]. The provoked inflammation on IPEX patients indicates the failure of immune tolerance. Foxp3 promotes its regulatory effect by enhancing the expression of IL-2 receptor (CD25), cytotoxic T cell-associated antigen-4 (CTLA-4), and glucocorticoid-induced TNF receptor family-related protein (GITR), meanwhile suppressing the production IL-2, IL-4 and IFN-γ[39]. T_{reg} cells monitor the inflammatory status by the exogenous level of IL-2. Binding of IL-2 to CD25 would enhance the expression of T_{reg}-cell associated genes and regulate the inflammation by suppressing effector T cell proliferation or by altering the function of antigen presenting cells[40]. Retroviral transfer of *Foxp3* to naïve T cells ($CD4^+CD25^-Foxp3^-$) can upregulate the expression of some T_{reg} cell-associated genes, including CD25, CTLA-4, GITR and CD103, and the *Foxp3*-transduced T cells were shown to be suppressive[41]. Altogether, Foxp3 is critical to the function and the development of T_{reg} cells and to a greater extent, the maintenance of immune homeostasis[42,43].

T_{reg} LYMPHOCTYE INDUCTION BY MSCs

MSCs are able to induce $Foxp3^+$ T_{reg} cell population *in vitro* and *in vivo*. So far, several mechanisms have been proposed, including: (1) secretion of soluble mediators; (2) cell-cell interaction; and (3) modulation of antigen

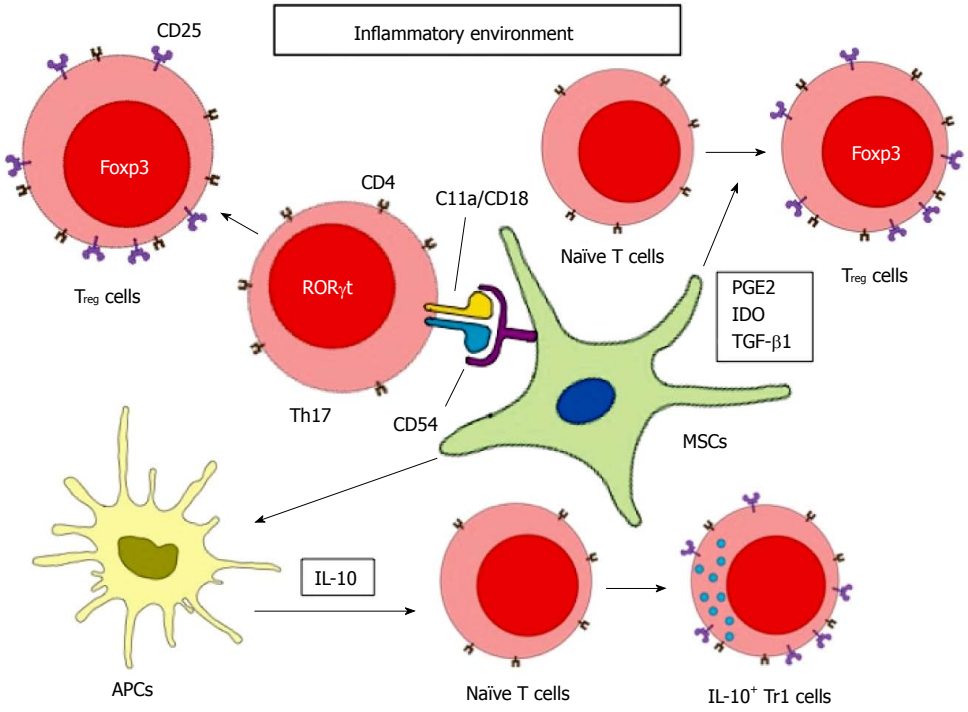

Figure 2 Mesenchymal stem cells-mediated regulatory T cell induction. MSCs induce T_{reg} cells through soluble mediators stimulation, cell-cell interaction, and modulation of antigen-presenting cells. Under inflammatory environment, MSCs secretes TGF-β1, PGE2 and IDO to facilitate the differentiation of naïve T cells to $Foxp3^+T_{reg}$ cells. MSCs can also interact with Th17 cells by direct contact *via* CD54 and C11a/CD18. With the presence of PGE2, differentiated Th17 cells can be converted to functional $Foxp3^+T_{reg}$ cells. MSCs can increase the secretion of IL-10 by antigen presenting cells, which will then induce Tr1 cells differentiation. MSCs: Mesenchymal stem cells; IL: Interleukin; T_{reg}: Regulatory T; TGF: Transforming growth factor; PGE2: Prostaglandin E2; IDO: Indoleamine 2,3-dioxygenase.

presenting cells (Figure 2).

Secretion of soluble mediators

TGF-β1: MSCs can secrete TGF-β1 to promote T_{reg} cell differentiation, especially when MSCs are placed in an inflammatory environment[21]. TGF-β1 is a potent immunosuppressor secreted by every leukocyte lineages, including macrophages, DCs, NK cells, T cells and B cells. Both TGF-β1 knockout mice and T-cell specific TGF-β receptor Ⅱ knockout mice develop severe autoimmunity, leading to multiple organs failure and death, suggesting the importance of TGF-β1 in regulating peripheral tolerance[44,45]. Generally, TGF-β1 can suppress the proliferation of T cells, the activation of B cells, the maturation and antigen presentation of DCs, the cytotoxicity of NK cells, and phagocytic effect of macrophages[46]. Moreover, as mentioned earlier, TGF-β1 is able to convert naïve T cells to $Foxp3^+$ Th3 cells, although such conversion seems to be concentration-dependent. High concentrations of TGF-β1 suppresses the expression of IL-23R and shifts the conversion to $Foxp3^+$ Th3 cells, whereas at lower concentrations and in the presence of IL-6 and IL-21, the expression of IL-23R is enhanced and results in $RORγt^+$ Th17 differentiation[47]. In addition, neutralizing TGF-β1 reduced mRNA and protein level of Foxp3 and CD25, further confirms its essential role in promoting T_{reg} cell differentiation[48]. In conclusion, MSCs-secreted TGF-β1 not only acts as a suppressor of innate and adaptive immune response, it can also induce development of T_{reg} cells from naive T cells, which further enhance the regulatory effects.

PGE2: MSCs can also secrete PGE2 to induce T_{reg} cells. PGE2 plays a major role in suppressing chronic inflammation. PGE2 can reduce IFN-γ production of NK cells, limit the phagocytic ability of macrophages and interfere early activation of B cells[49-52]. Although PEG2 can suppress early development of DCs, it is surprising that PGE2 also stabilize matured DCs and enhance its antigen presenting capacity[53-55]. Moreover, despite PGE2 is able to shift the differentiation of naïve T cells from Th1 to Th2 cells, PGE2 also promote proinflammatory Th17 cell development by elevating IL-23 production[56]. Thereby, PEG2 is not exclusively anti-inflammatory. It also possesses the ability to provoke inflammation. Nevertheless, like TGF-β1, PGE2 can induce $Foxp3^+T_{reg}$ cell differentiation and it is one of many soluble mediators that produce by MSCs. Diminishing PGE2 signaling when co-culture $CD4^+$ T cells with MSCs by antagonist indomethacin fail to upregulate Foxp3 and CD25 expression. In fact, when inhibiting both TGF-β1 and PGE2 signaling, the expression of Foxp3 and CD25 further decreased[48]. Furthermore, after transferring adipose tissue-derived MSCs in asthmatic mice, the number of infiltrated inflammatory cells was significantly reduced and no obvious goblet cell hyperplasia was found in the lung. Meanwhile, the number of T_{reg} cells was elevated. When TGF-β1 neutralizing antibodies or indomethacin was added to MSCs-treated asthmatic mice, the anti-

inflammatory effects promoted by MSCs as well as the T_{reg} cell expansion. These results demonstrated the necessity of TGF-β1 and PGE2 for T_{reg} cell induction as well as the anti-inflammatory effect of MSCs[57].

IDO: IDO is a rate-limiting enzyme that catalyzes the degradation of tryptophan *via* kynurenine pathway. IDO is expressed in various cell types, including macrophages, DC and MSCs. Interestingly, IDO expression can be induced by IFN-γ and other proinflammatory cytokines. Munn *et al*[58] treated pregnant mice carrying allogeneic or syngeneic fetus with 1-methyltryptophan, an IDO inhibitor. As a result, allogeneic, but not syngeneic, fetuses provoked severe immune rejection[58]. Also, some studies suggested the association of tryptophan catabolism with inhibition of T cell proliferation, emphasizing its tolerogenic potential[59,60]. In addition, kynurenines, a tryptophan catabolite, can promote T_{reg} cell induction[61]. Infusion of MSCs to kidney allograft murine model prevented graft rejection, and the T_{reg} cell population was elevated. In contrast, allograft tolerance and T_{reg} cell expansion diminished when the recipients were treated with IDO-deficient MSCs. These results demonstrated the importance of IDO in MSCs-mediated Treg cell induction and graft tolerance[62]. Other soluble factors, like human leukocyte antigen-G5 and haem oxygenase 1, are also shown to be involved in MSCs-mediated T_{reg} cell induction[63,64]. However, the underlying mechanisms are not clear. More studies need to be done in order to further increase the efficacy of MSCs-based therapy and to reveal the potential risk that could cause to the patients.

Cell-cell interaction

Apart from soluble mediators, cell-cell interaction is also important to the modulatory function of MSCs and T_{reg} cell induction. MSCs are known to express adhesion molecules on their surface, although only low level of expression can be detected in normal condition. However, after placing MSCs in inflammatory conditions, adhesion molecules, ICAM-1 and VCAM-1, chemokine ligands of CCR5 and CXCR3 are upregulated. Through these molecules, T cells are attracted and anchored to MSCs. With close proximity, adhesion molecules co-operate with IDO and NO, suppress T cell activity by inducing their apoptosis or cell arrest[65-68]. It is also worth to note that MSCs can inhibit the expression of ICAM-1, CXCR3 and α-integrin on $CD3^+$ T cell, reduced the interaction between T cells and endothelial cells, thus, disrupted T cells from infiltrating into CNS[69]. On the other hand, MSCs can attach to Th17 cells *via* CCR6 and CD11a/CD18 and facilitate Th17 to adopt regulatory phenotype[70]. Moreover, when co-culture MSCs with $CD4^+$ T cells in transwell system; T_{reg} cells cannot be induced, even in the presence of PGE2 and TGF-β[48]. These results further confirmed cell-cell interaction is essential to the overall suppressive effect of MSCs. However, T_{reg} cell induction ability was recovered if MSCs were co-cultured with peripheral blood mononuclear cells instead of isolated $CD4^+$ T cells, suggesting there is an alternative pathway that does not require cell-cell contact, and it is likely, through soluble mediators in peripheral blood mononuclear cells[48].

Modulation of antigen presenting cells

Increasing evidence has indicated MSCs are able to shift macrophages, DCs and NK cells to a regulatory phenotype and alter their cytokines production. For example, MSCs skew monocyte toward M2 macrophage differentiation. Subsequently, M2 macrophages secrete CCL18 and IL-10 to exert suppressive response and induce T_{reg} cell differentiation[26]. As discussed above, IL-10 is able to induce naïve T cell to $Foxp3^-$ Tr1 cell, which secrete high level of IL-10 and TGF-β to modulate the inflammatory microenvironment. Interestingly, although MSCs express neither IL-10 nor its receptor, MSCs are able to induce NK cells, DCs, macrophages, T cells and B cells to produce IL-10[5,10-12,17]. In addition, IL-10 is a powerful anti-inflammatory cytokine that suppresses antigen-specific immune responses, reduces pathological immune responses and promotes allograft tolerance.

In conclusion, the mechanisms underlying MSCs-mediated T_{reg} cell development are complicated, which involve synthesis and secretion of multiple mediators, direct interaction with target cells and modulation of certain antigen-presenting cells. Apparently, there is no single pathway that governs the whole induction process, indicating that MSCs possess certain degree of plasticity. Regardless of how T_{reg} cells are enhanced by MSCs, MSCs-activated T_{reg} cells play a significant role on immunoregulation and affect a wide spectrum of immune responses[43,71,72]. Certainly, T_{reg} cells can massively amplify the immunomodulatory effect of MSCs. However, the mechanism in regard to T_{reg} cell induction is far from elaborate and additional researches are required.

B_{reg} LYMPHOCYTE

In recent decade, B_{reg} cells were being intensively investigated due to its immunosuppressive effect on excessive inflammation. Like T_{reg} cells, B_{reg} cells can produce anti-inflammatory cytokines, like TGF-β and IL-10. Among these, IL-10 is strongly associated with B_{reg} cells since depleting IL-10-producing B cells result in chronic inflammation, outgrowth of proinflammatory T cell after autoimmune induction[73-75]. But unlike T_{reg} cells, there is no "master regulator" being identified in B_{reg} cells, which complicated the process of B_{reg} cell classification. So far, there are several B cell subsets have been identified as B_{reg} cells in mice. They are $CD5^+CD1d^{hi}$ B (B10) cells and $Tim1^+$ B cells[76-78]. In human, there is $CD19^+CD24^{hi}CD38^+CD1d^{hi}$ B cells and $CD19^+CD24^{hi}CD27^+$ B cells[79,80]. B_{reg} cells control inflammation by suppressing IL-12 secretion from DCs, thus inhibiting Th1 and Th17 differentiation[81]. Through

the secretion of TGF-β, B_{reg} cells can induce $CD4^+$ T cell apoptosis and anergy in $CD8^+$ cytotoxic T cells[82,83]. Recent studies indicated that B_{reg} cells play a role in T_{reg} cell development and function. As B_{reg} cells are one of the major sources of IL-10, which drive Tr1 differentiation, it is not surprising that B_{reg} cells can expand T_{reg} cell population during inflammation. Additionally, when B cell specific IL-10 defective mice (DBA/1IL-10 $KO^{-/-}$ mice) were induced with arthritis, the percentage of Tr1 was significantly decreased, indicating effects of IL-10^+B_{reg} cells on T_{reg} cell formation[75]. Besides TGF-β and IL-10, recent studies reported that IL-35 is another pleiotropic cytokine that regulate overwhelming inflammation and autoimmunity[84,85]. Antigen-driven proliferation assay revealed that IL-35 was able to suppress $CD4^+$ T cell proliferation[86]. Treatment with IL-35 ameliorated disease severity and reduced Th1 and Th17 cells in mice with experimental autoimmune uveoretinitis (EAU)[85]. More importantly, IL-35 can increase T_{reg} and B_{reg} cell populations. Similar to IL-10, IL-35-induced T_{reg} (iTr35) cells are $Foxp3^-$. However, adoptive transfer of iTr35 cells to various autoimmune disease animal models has sufficiently alleviated their clinical severity, and the effect was comparable to tT_{reg} cells-treated mice[35]. On the other hand, when recombinant IL-35 was injected into the EAU mice, the frequency of $B220^+$ IL-10^+B_{reg} cells, IL-35^+B_{reg} cells and B10 cells were upregulated in the spleen and draining lymph nodes[85]. Collectively, B_{reg} cells exhibit anti-inflammatory and immunoregulatory effects, at least in part, by secreting multiple anti-inflammatory cytokines (TGF-β, IL-10 and IL-35), promoting differentiation of other regulatory cells, and inhibiting the proliferation and function of effector T cells.

B_{reg} LYMPHOCYTE INDUCTION BY MSCs

Although MSCs do not constitutively express IL-10, and currently there is no evidence to indicate that MSCs produce IL-35, several studies have reported that MSCs induce IL-10^+B_{reg} cell differentiation in mouse model[87-89]. Our group studied the effects of human bone marrow-derived MSCs in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, and observed attenuation of clinical severity and neuroinflammation; and excitingly, these were associated with expansion of $CD1d^{hi}$ $CD5^+$ B_{reg} cells after MSCs administration[87]. Subsequently, another study demonstrated intravenous infusion of adipose tissue-derived MSCs to $Roquin^{san/san}$ mice, an animal model of systemic lupus erythmatosus (SLE), lead to increased numbers of B10, B10pro and naïve T_{reg} cells[89]. Moreover, the MSCs-mediated B_{reg} cell induction is not restricted to murine models. Administrating MSCs into refractory chronic graft *vs* host disease (cGvHD) patients have improved patients' overall clinical conditions. Consistent with murine models, MSCs increased the frequency and the function of $CD5^+$ IL-10^+B_{reg} cells by enhancing their proliferation and survival[88]. Momentarily, we are still not clear about the mechanism regarding to MSCs-mediated B_{reg} cell induction. It is worthwhile to ask whether the induction is IL-35 or IL-10-dependent since MSCs can induce IL-10 production by T_{reg} cells, DCs, and M2 macrophages, implying the possibility of creating a positive feedback loop for B_{reg} cell generation. Further understanding the mechanisms of how MSCs induce T_{reg} and B_{reg} cells can definitely contribute to the therapeutic development of MSCs and further improve their potential therapeutic efficacy.

THERAPEUTIC POTENTIAL OF GENETIC ENGINEERED MSCs

MSCs contain multiple properties that are suitable for therapeutical use. Wide-spectrum of differentiation capacity made it a perfect candidate for regenerative medicine. MSCs have been used to generate cartilage, bone, liver, intervertebral disc, and cardiac tissue[90]. Recent reports have suggested using MSCs for neural cell replacement. However, rather than direct neural differentiation, MSCs tend to recruit neural progenitor cells (NPCs) to the injury sites and support NPCs proliferation and differentiation[91]; Immunomodulatory properties of MSCs are potentially useful for the treatment of autoimmune diseases and GvHD. Transplanted MSCs suppressed the proliferation and activation of T cells and NK cells in type 1 diabetes animal model. Also, the level of IFN-γ and TNF-α were reduced. When MSCs were co-transplanted with pancreatic islets, MSCs protected grafted islets from immunorejection and secreted various trophic factors to promote graft vascular network[92,93]. Another intriguing advantage of using MSCs to treat immune diseases is that, unlike traditional immunotherapy in which a certain modulator act on a particular pathway, MSCs elicit their suppression on multiple immune cell types *via* various mechanisms. Although the immunosuppressive effects of MSCs appear very promising, further investigations are required to elucidate the underlying mechanisms, so as to prevent complications and maximize the therapeutic efficacy.

One current issue on immunotherapy is that a particular modulator or antibody may be seemingly effective, however, the therapeutic efficacy is limited since such modulator may also compromise certain cells or mediators beneficial to the disease recovery. Rituximab, for example, is a CD20 neutralizing antibody and it is believed to be an effective treatment for B and T-cell-mediated diseases, such as rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus[94-96]. Rituximab-induced B-cell depletion depends on the expression of CD20 on the cell surface, but the expression of CD20 gradually disappeared upon plasma cell differentiation[97,98]. Moreover, B_{reg} cells were also depleted, thus, exacerbates the disease symptoms[73]. In EAE, B10 cells play an important regulatory role during the initiative phase whereas they are less involved at the late phase of the disease[99,100]. Therefore, depleting B cells by rituximab at the early phase have a potential risk of worsening the

clinical conditions. As a consequence, it is necessary to develop an alternative strategy.

The immunosuppressive properties of MSCs on different murine autoimmune disease animal models support its potential clinical application. However, the immunomodulatory secretome of MSCs vary and greatly rely on the host inflammatory environment[21]. To minimize this uncertainty, a novel therapeutic strategy, in which MSCs are genetically engineered with defined immunoregulatory cytokines, has been developed. Transplantation of IL-10-engineered adipose-derived MSCs attenuated EAE by reducing the number of immune cell infiltration to the CNS, decreasing the secretion level of IL-17A, TNF-α and IL-2, and inhibiting antigen-presenting function of DC[101]. Since the immunosuppressive effect of MSCs is enhanced if they are placed proximal to the inflammatory area, Liao *et al*[102] engineered MSCs with CNS homing ligand genes, P-selectin glycoprotein (PSGL-1) and Sialyl-Lewis^x (SLeX), along with IL-10 to EAE model. Consequently, EAE was attenuated, CNS homing ability was enhanced and their therapeutic efficacy was increased[102]. Genetic engineering of MSCs has been well studied in regenerative medicine. Different combination of treatments is documented and aims to redirect the MSCs differentiation propensity. Comparatively, genetic modification of MSCs for the treatment of autoimmune diseases is currently under development. Considering that the effect of MSCs may vary between patients with different severity of neuroinflammation, information on the clinical condition and pathology of the individual patient will probably help to predict treatment efficacy. Moreover, questions like in what phase of a particular disease introducing MSCs can improve the clinical outcome, or to what extent MSCs can elicit their suppressive effect and meanwhile, does not compromise the immunity in response to pathogens or infectious agents, are worthwhile to explore in order to safely use in human patients.

SAFETY AND CONCERNS OF MSCs AS CELLULAR THERAPIES IN PATIENTS

To date, there are nearly 500 ongoing MSC-based clinical trials. They aim to investigate the effectiveness of MSCs on treating different diseases, including GvHD, diabetes, cardiovascular diseases, hematological diseases and neurological diseases[103]. Although most of these clinical trials reported the patients were well tolerated to the MSC infusion and administration, there are some safety concerns requiring caution[104]. During *in vitro* expansion, MSCs can give rise to replicative senescence, which may affect the activity of surrounding healthy cells and therefore, reduce the clinical efficacy[105]. Moreover, although MSCs have low immunogenicity due to the reduced expression of co-stimulatory receptors and major histocompatibility complex (MHC) class Ⅱ antigens, *in vitro* stimulation of pro-inflammatory cytokines on MSCs can upregulate MHC class Ⅰ and MHC class Ⅱ expression, compromising the hypo-immunogenicity property of MSCs.

CONCLUSION

The immunomodulatory properties of MSCs have been massively studied due to its intriguing suppressive effects on various immunological diseases. Broad-range of immune cells can be regulated by MSCs through a series of soluble mediators stimulation, chemokine attraction, and cell-to-cell interaction. MSCs-induced T_{reg} and B_{reg} cells enhance the immunosuppressive capacity and generate a tolerogenic microenvironment against overwhelmed inflammation. This hypothesis supports the observation that infused MSCs can only survive in the recipient for a short period of time, however, the regulatory effects of MSCs are long lasting, suggesting MSCs may act as an activator or a switcher that initiate certain cells, possibly T_{reg} and B_{reg} cells, to react to the inflammation and at the same time, alter the microenvironment for those cells to sustain their immunosuppressive effects. Although MSCs appear very promising as treatment in experimental models of autoimmune diseases, there are still many challenges need to overcome before MSCs can be widely use in clinical medicine.

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