The role of Interleukin 1 receptor antagonist in mesenchymal stem cell-based tissue repair and regeneration

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Abstract

Interleukin (IL)-1 receptor antagonist (IL-1Ra), a naturally occurring antagonist of IL-1α/IL-1β signaling pathways, has been attributed to the immunosuppressive effects of mesenchymal stem cells (MSCs). MSCs, in IL-1Ra-dependent manner, suppressed production of IL-1β in dermal macrophages, induced their polarization in anti-inflammatory M2 phenotype, attenuated antigen-presenting properties of dendritic cells (DCs), and promoted expansion of immunosuppressive T regulatory cells in the skin, which resulted in enhanced repair of the nonhealing wounds. Reduced activation of inflammasome and suppressed production of IL-1β in macrophages were mainly responsible for beneficial effects of MSC-derived IL-1Ra in alleviation of acute lung injury, dry eye syndrome, and corneal injury. Through the production of IL-1Ra, MSCs reduced migration of DCs to the draining lymph nodes and attenuated generation of inflammatory Th1 and Th17 cells that resulted in alleviation of fulminating hepatitis and rheumatoid arthritis. MSCs, in IL-1Ra-dependent manner, reduced liver fibrosis by suppressing production of Type I collagen in hepatic stellate cells. IL-1Ra was, at least partially, responsible for enhanced proliferation of hepatocytes and chondrocytes in MSC-treated animals with partial hepatectomy and osteoarthritis. Despite of these beneficial effects, IL-1Ra-dependent inhibition of IL-1α/IL-1β-signaling significantly increased risk of
infections. Therefore, future experimental and clinical studies should delineate potential side effects of MSC-derived IL-1Ra before IL-1Ra-overexpressing MSCs could be used as a potentially new therapeutic agent for the treatment of acute and chronic inflammatory diseases.

**KEYWORDS**

immunosuppression, inflammation, interleukin 1 receptor antagonist, mesenchymal stem cells, regeneration

## 1 | INTRODUCTION

Mesenchymal stem cells (MSCs) are adult, self-renewable cells that reside in all postnatal tissues. Due to easy acquisition, rapid expansion in vitro, capacity for differentiation in cells of all three germ layers under appropriate culture conditions, low surface expression of major histocompatibility complex (MHC) antigens and minor immunological rejection after transplantation in MHC-mismatched recipients, long-term coexistence in the host and immunomodulatory characteristics, MSCs represent new therapeutic agents in regenerative medicine.

MSC-based therapy of inflammatory diseases is relied on their capacity to alter detrimental innate and adaptive immune response in injured tissues. MSCs suppress innate immunity by inducing polarization of inflammatory M1 macrophages in alternatively activated, immunosuppressive M2 phenotype, by downregulating cytotoxicity of natural killer (NK) and natural killer T (NKT) cells and by inhibiting maturation and antigen-presenting properties of dendritic cell (DC). MSCs alter cytokine profile in DCs resulting in decreased production of proinflammatory, Th1-related (tumor necrosis factor alpha [TNF-α], interferon gamma [IFN-γ], interleukin [IL]-12) and Th17-related cytokines (IL-1β), IL-6, IL-23, transforming growth factor beta [TGF-β]) and increased production of anti-inflammatory IL-10, which results in the attenuated activation of naive T cells. Additionally, MSCs may directly suppress Th1 and Th17 cell-driven immune response by inhibiting expression of T-bet and RORγT transcriptional factors in CD4+ T cells and by attenuating secretion of IFN-γ and IL-17, which result in alleviation of Th1 and Th17 cell-dependent chronic inflammatory diseases. Furthermore, MSC-based suppression of acute and chronic inflammation is relied on MSC-induced enhanced generation and expansion of CD4 + CD25 + FoxP3+ T regulatory cells (Tregs) and FoxP3 + NKTrigs in injured tissues, which, in turn, create immunosuppressive microenvironment and promote tissue repair and regeneration.

Activation of toll-like receptors (TLRs) has crucially important role for the generation of immunosuppressive phenotype in MSCs. TLR-priming activates phosphoinositide 3-kinase (PI3K)/Akt pathway in MSCs which results in enhanced production of anti-inflammatory cytokines. Activated TLR-2 and TLR-4 recruit PI3K which converts phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 is involved in activation of Akt, which in turn inactivates glycogen synthase kinase 3 and promotes nuclear accumulation of cAMP response element-binding protein (CREB) which displaces p65 subunit of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) from the coactivator of transcription (CREB binding protein). An increased transcriptional activity of CREB and consequently reduced transcriptional activity of NF-κB result in increased production of immunosuppressive mediators in MSCs.

Among MSC-sourced factors, TGF-β, nitric oxide, indolamine 2,3-dioxygenase, IL-10, IL-6, leukocyte inhibitory factor, prostaglandin E2 (PGE2), and IL-1 receptor antagonist (IL-1Ra) have been mainly attributed to the beneficial effects of MSCs in attenuation of acute and chronic inflammation. Herewith, we summarized current knowledge regarding the therapeutic potential of MSC-derived IL-1Ra in attenuation of organ-specific and systemic inflammatory diseases. An extensive literature review was carried out in June 2019 across several databases (MEDLINE, EMBASE, Google Scholar), from 1990 to present. Keywords used in the selection were as follows: "mesenchymal stem cells," "Interleukin 1 receptor antagonist," "inflammatory diseases," "regeneration," "immunosuppression." Eligible studies had to emphasize molecular and cellular mechanisms responsible for beneficial effects of MSC-derived IL-1Ra in tissue repair and regeneration and were analyzed in this review.

## 2 | THE BIOLOGICAL BASES FOR IMMUNOSUPPRESSIVE EFFECTS OF MSC-DERIVED IL-1RA

IL-1Ra (IL1-F3) is a member of IL-1 cytokine family that consists of 11 cytokines and 10 receptors. All of IL-1 family members are potent modulators of inflammation and among them, IL-1α and IL-1β are considered as the...
most important proinflammatory cytokines while IL-1Ra, as naturally occurring antagonist of IL-1α and IL-1β signaling, has crucially important role in alleviation of IL-1α and IL-1β-driven inflammation.14

IL-1α and IL-1β signaling pathways are initiated by the binding of IL-1α and IL-1β to their cognate receptor IL-1RI.15 Upon IL-1α/IL-1β:IL-1RI binding, a coreceptor, IL-1 receptor accessory protein, is recruited to the IL-1α/IL-1β:IL-1RI complex, resulting in the creation of a ternary complex. Once this trimeric complex is formed, the cytoplasmic Toll/IL-1 receptor domains of IL-1 receptors are brought together, eliciting activation of MyD88-dependent inflammatory cascade. MyD88 links IL-1R ternary complex with IL-1R-associated kinases (IRAK) and mitogen-activated protein kinases, resulting in the activation of several transcription factors, including NF-κB and Activator protein 1.15,16 Therefore, activation of IL-1α/IL-1β:IL-1RI signaling pathways results in enhanced release of alarmins from injured cells, increased production of inflammatory mediators, and higher expression of chemokine receptors on immune cells enabling massive influx of circulating leucocytes in injured tissue and consequent development of inflammation.13

The analysis of X-ray crystal structures of IL-1 family members revealed that many of these cytokines, including IL-1α, IL-1β, and IL-1Ra, have a conserved β-trefoil conformation and a central hydrophobic core composed of 12 β-sheets, six of which (β1, β4, β5, β8, β9, and β12) form an antiparallel β-barrel.13 As IL-1α, IL-1β, and IL-1Ra share the same structural motif, all of them engage the same IL-1RI on target cells. IL-1Ra represents a typical naturally occurring antagonist that occupies the binding pocket of IL-1RI without eliciting any downstream signaling.13 Importantly, binding affinity of IL-1Ra to IL-1RI is equal to the affinity of IL-1α and IL-1β, enabling IL-1Ra-mediated competitive inhibition of IL-1RI and efficient suppression of IL-1α/IL-1β:IL-1RI-signaling. Therefore, IL-1Ra is crucially important for tissue repair and regeneration in IL-1α and IL-1β-driven inflammatory diseases.17

In addition to their effect on IL-1RI, IL-1α/IL-1β may act intracellularly as well.18 Intranuclear IL-1α and IL-1β regulate cell proliferation, migration, and production of inflammatory cytokines (TNF-α, IL-6, IL-8, and IFN-γ) by modifying chromatin structure and by acetylating transcription factors. IL-1Ra, in addition to its secreted form, exists in three intracellular forms (IcIL-1Ra1-3) which could antagonize the intranuclear actions of IL-1α/IL-1β.18 Accordingly, high mortality, due to the development of severe inflammation was observed among the infants who had a deficiency in IL-1Ra because of genetic mutations.19 On the contrary, exogenous administration of recombinant IL-1Ra significantly attenuated detrimental IL-1α- and IL-1β-dependent immune response and alleviated acute and chronic inflammatory diseases.17

MSCs, which have been used in cell-based therapy of organ-specific and systemic immune cell-mediated disorders, are valuable cellular source of IL-1Ra.20,21 Accordingly, several research groups investigated potential therapeutic use of IL-1Ra-overexpressing MSCs in the alleviation of acute and chronic skin, lung, liver, and joint inflammation (Table 1).

3 | BENEFICIAL EFFECTS OF MSC-DERIVED IL-1RA IN THE REPAIR OF CHRONIC AND NONHEALING WOUNDS

Chronic or nonhealing wounds are defined as disruptions of the structural and functional integrity of the skin that are not progressing through the physiological wound healing process.22,23 Nonhealing wounds are usually observed in patients suffering from chronic inflammatory disorders, including diabetes, obesity, and autoimmune diseases (systemic lupus erythematosus, Crohn’s disease, systemic sclerosis).24 Cellular make-up of nonhealing wounds revealed massive intradermal accumulation of inflammatory M1 macrophages, neutrophils, DCs, Th1/Th17 lymphocytes that induce tissue breakdown and impair physiological healing processes in the skin through the production of proteases, reactive oxygen species (ROS), and proinflammatory cytokines.25

Several lines of evidence demonstrated that, among proinflammatory mediators, IL-1β had the most important pathological role in the development of chronic wounds.26–28 IL-1β induces senescence in resident fibroblasts impairing repair of injured skin. Furthermore, IL-1β promotes generation of inflammatory M1 phenotype in intradermal macrophages, resulting in the development of a persistent inflammation within the chronic wounds.26 Accordingly, increased production of IL-1β correlated with an impaired healing of skin wounds in diabetic mice, while IL-1Ra-dependent suppression of IL-1β signaling resulted in enhanced repair and regeneration of injured skin.27 Moreover, improved healing of chronic wounds were observed in IL-1R knockout mice as well as in IL-1Ra-treated animals, while genetic deletion of IL-1Ra resulted in delayed wound healing of acute wounds.27,28 Inflamasome-dependent maturation of IL-1β in dermal-infiltrated macrophages was crucially important for IL-1β-caused impaired wound healing.26 Suppression of inflamasome in dermal-infiltrated macrophages almost completely attenuated IL-1β-mediated inflammation and delayed wound healing.26 Although transient activation of the inflamasome is a prerequisite to elicit IL-1β-driven-microbial immune response in the injured skin during physiological wound healing,
permanent and enhanced activation of inflammasome results in increased secretion of mature IL-1β which consequently leads to the enhanced IL-1β-dependent activation of NF-κB.29 NF-κB transactivates IL-1β, TNF-α, IL-6, IL-12, and IL-23 genes and enhances secretion of these proinflammatory cytokines in wound infiltrated M1 macrophages, neutrophils, and DCs leading to the expansion of Th1 and Th17 lymphocytes.29 Th1 and Th17 cells, in turn, promote the production of inflammatory cytokines, ROS, and matrix metalloproteinases (MMPs) in

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Abbreviations: DCs, dendritic cells; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; MSC, mesenchymal stem cell; TNF-α, tumor necrosis factor alpha.
inflammatory M1 macrophages and neutrophils in IFN-γ, IL-17, and IL-22-dependent manner, creating an “autocrine inflammatory loop” that results in a persistent inflammation within the nonhealing wounds.25

Vander Beken and colleagues recently demonstrated that within the ATP-binding cassette Subfamily B Member 5 (ABCB5)-expressing dermal cells exists a subpopulation of MSCs (MSCs\textsuperscript{ABCB5}) which were capable to suppress proliferation of IFN-γ-producing Th1 and Th17 cells in paracrine manner.26 The analysis of MSCs\textsuperscript{ABCB5}-derived secretome revealed that IL-1Ra had crucially important role for their immunomodulatory effects. Through the production of IL-1Ra, MSCs\textsuperscript{ABCB5} suppressed IL-1β:IL-1R/NF-κB-signaling pathway and alleviated production of IL-1β, IL-6, IL-12, and IL-23 in M1 macrophages and DCs, which prevented expansion of inflammatory Th1 and Th17 cells. At the same time, MSCs\textsuperscript{ABCB5}-derived IL-1Ra induced generation of TGF-β and IL-10-producing M2 macrophages that promoted the wound healing (Figure 1).26

As recently revealed by Kou and colleagues, MSC-derived IL-1Ra is secreted in the extracellular space associated with small extracellular vesicles (sEVs).30 Production of IL-1Ra in MSCs is regulated by the local concentration of inflammatory cytokines (particularly TNF-α and IL-1β) and Fas:Fas-associated phosphatase-1 (Fap-1):Caveolin-1 (Cav-1) complex. When MSCs engraft in the inflammatory microenvironment of the nonhealing wounds, high levels of TNF-α and IL-1β induce simultaneous upregulation of membrane-bound Fas and Fap-1

FIGURE 1 MSC-derived IL-1Ra attenuates IL-1β-driven inflammation in the skin enabling enhanced wound healing. When MSCs engraft in the inflammatory microenvironment of the nonhealing wounds, high levels of TNF-α and IL-1β induce upregulation of Fas and Fap-1 on the membrane of MSCs and induce enhanced production of IL-1Ra. Fas and Fap-1 interact with Cav-1 and from a Fas:Fap-1:Cav-1 complex which activates SNARE-mediated membrane fusion mechanism resulting in the release of sEVs. MSC-derived IL-1Ra, associated with released sEVs, in paracrine and endocrine manner, attenuates IL-1β-driven inflammation, enabling enhanced wound healing. ABCB5-expressing MSCs suppress proliferation of IFN-γ-producing Th1 and IL-1β-driven inflammation, enabling enhanced wound healing. Through the production of IL-1Ra, ABCB5 + MSCs suppressed IL-1β:IL-1R signaling pathway and activation of NF-κB in M1 macrophages and DCs, which produced alleviated production of inflammatory cytokines TNF-α, IL-1β, IL-6, IL-12, and IL-23 and prevented expansion of inflammatory Th1 and Th17 cells in the injured skin. At the same time, ABCB5 + MSCs-derived IL-1Ra induced generation of IL-10-producing immunosuppressive M2 macrophages enabling enhanced repair and regeneration of the injured skin. ABCB5, ATP-binding cassette Subfamily B Member 5; Cav-1, Caveolin-1; DC, dendritic cell; Fap-1, Fas-associated phosphatase-1; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; INF, interferon; MSC, mesenchymal stem cell; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; sEVs, small extracellular vesicles; TNF-α, tumor necrosis factor alpha
Fap-1 and enhanced production of immunosuppressive cytokines, including IL-1Ra. Fas and Fap-1 interact with Cav-1 and form a Fas:Fap-1:Cav-1 complex which activates a common soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-mediated membrane fusion mechanism resulting in the release of sEVs. MSC-derived IL-1Ra, associated with released sEVs, diffuses through the injured skin and in paracrine and endocrine manner, attenuates IL-1β-driven inflammation, enabling enhanced wound healing (Figure 1).

In similar manner as it was observed in an inflamed skin, injured corneal epithelial cells release IL-1β and, in IL-1β-dependent manner, elicit strong immune response during the early stage of corneal injury and dry eye syndrome (DED). The strongest expression of IL-1Ra was noticed in the superficial apical layer of corneal epithelium where IL-1Ra attenuates ongoing inflammation by suppressing IL-1β:IL-1RI signaling in corneal-infiltrated immune cells. Therefore, IL-1Ra represents an endogenous mechanism of protection against epithelial- and tear-derived IL-1β in the injured eyes. Jeong and colleagues recently demonstrated that MSCs, in IL-1Ra-dependent manner, suppress the production of IL-1β in macrophages and reduced the size of corneal injury and inflammation. Based on these findings, we designed MSC-based ophthalmic solution “Derived Multiple Allogeneic Proteins Paracrine Signaling (d-MAPPS)”, which activity was based on the effects of MSCs-derived immunomodulatory factors, including IL-1Ra and tested its efficacy in the treatment of corneal injury and DED. Remarkable improvement of visual acuity, relief of pain, reduced swelling, and enhanced regeneration of corneal defects were noticed in d-MAPPS-treated patients. Similarly, significantly attenuated dryness, grittiness, scratchiness, soreness, irritation, burning, watering, and eye fatigue were observed in DED patients that received IL-1Ra containing, MSC-based ophthalmic solution, indicating important protective role of MSC-derived IL-1Ra in corneal repair.

4 | THE IMPORTANT ROLE OF IL-1RA IN MSC-BASED ATTENUATION OF ACUTE AND CHRONIC LIVER DISEASES

As demonstrated by us and others, systemic administration of MSCs efficiently attenuated acute liver failure and fibrosis in mice. Importantly, similar immunosuppressive and hepatoprotective effects were observed in animals that received MSC-sourced secretome, indicating that MSC-derived soluble factors were mainly responsible for MSC-dependent beneficial effects in the liver.

IL-1Ra has been delineated as the most important MSC-sourced anti-inflammatory mediator for suppression of macrophage-driven inflammation in injured livers. Lee and colleagues showed that MSCs in IL-1Ra-dependent manner induced differentiation of macrophages toward immunosuppressive M2 phenotype which resulted in alleviation of acute liver failure. MSC-derived IL-1Ra promoted phosphorylation of STAT3 and production of IL-10 in macrophages, but reduced phosphorylation of STAT1 in naïve T cells resulting in attenuation of Th1- and IFN-γ-dependent activation of liver macrophages. Significantly higher levels of IL-10 and increased number of alternatively activated M2 macrophages created immunosuppressive microenvironment in the inflamed livers which resulted in the enhanced regeneration of injured hepatocytes. Similarly, Zheng and coworkers demonstrated therapeutic potential of IL-1Ra-overexpressing amniotic fluid-derived MSCs (AF-MSCs) in alleviation of fulminant hepatitis. AF-MSCs genetically modified to overexpress IL-1Ra, successfully engrafted in the injured livers and suppressed the production of proinflammatory cytokines (IL-1β and IL-6) in liver macrophages. Accordingly, significantly improved liver function and increased survival rates were noticed in animals that received IL-1Ra overexpressing AF-MSCs.

IL-1Ra was mainly responsible for beneficial effects of MSC-sourced secretome in the attenuation of liver fibrosis as well. Through the secretion of IL-1Ra, MSCs downregulated the secretion of inflammatory cytokines (IL-β, TNF-α) in liver macrophages and suppressed the production of Type I collagen in hepatic stellate cells that led to the alleviation of chronic liver inflammation and fibrosis.

As suggested by Cosgrove and colleagues, IL-1Ra is a member of TNF-α-inducible self-antagonizing TGF-α-IL-1α/β-IL-1ra autocrine cascade that enhances TNF-α-stimulated hepatocyte proliferation. Therefore, MSC-derived IL-1Ra has been attributed to the enhanced hepatocyte proliferation that was observed in regenerating livers of MSC-treated hepatotomized mice.

5 | IL-1RA-EXPRESSING MSCS AS A NEW THERAPEUTIC AGENT IN THE CELL-BASED THERAPY OF LUNG DISEASES

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), attributable to the acute inflammation and dysfunction of alveolar–epithelial membrane, are usually characterized by pulmonary edema and manifested by severe respiratory dysfunction. A large
number of experimental and clinical studies suggested that MSCs, due to their capacity to regenerate alveolar-epithelial barrier and to suppress detrimental immune response in the lungs, represent potentially new remedy in cell-based therapy of ALI and ARDS.\textsuperscript{49–51}

IL-1β has important pathogenic role in the development and progression of ALI and ARDS.\textsuperscript{52} An increased concentration of IL-1β in lung airways is mainly responsible for the enhanced accumulation of neutrophils in the lungs following ALI.\textsuperscript{52} Accordingly, several lines of evidence demonstrated crucial importance of IL-1Ra for MSCs-based treatment of ALI and ARDS.\textsuperscript{53–55} Ortiz and colleagues identified IL-1Ra-producing subpopulation of lungs following ALI.\textsuperscript{52} Intravenous injection of IL-1Ra-producing MSCs completely attenuated lung inflammation in mice by suppressing the production of TNF-α, IL-1α, and IL-1β in alveolar macrophages resulting in alleviated influx of circulating neutrophils and lymphocytes in BLM-injured lungs.\textsuperscript{54} The highest production of IL-1Ra by lung-infiltrated MSCs was observed 72 hr after lung injury and was mainly triggered by IL-6, released from lung-infiltrated neutrophils and alveolar macrophages.\textsuperscript{53,56,57} Importantly, as revealed by Xu and colleagues, MSC-derived IL-1Ra creates “positive autocrine loop” in population of lung-infiltrated MSCs by promoting their expansion and capacity for neovascularization, bacterial clearance, and immunosuppression.\textsuperscript{53,55} Under the influence of IL-1Ra, MSCs upregulate the expression of vascular endothelial growth factor (VEGF) that prevents the apoptosis of endothelial cells, induces neovascularization, and promotes regeneration of injured alveolar Type II epithelial cells.\textsuperscript{53,58} IL-1Ra regulates TLR-4 signaling pathway in MSCs, leucocytes, and nonimmune cells, having important role in the modulation of immune response against bacterial antigens and allergens in the lungs.\textsuperscript{21,58,59} The effects of MSC-derived IL-1Ra on TLR-4 axis depend on the role that TLR-4-expressing cells play in the immune response to the inhaled antigens.\textsuperscript{12,21} During the onset of bacterial pneumonia, under the influence of low concentration of inflammatory cytokines, MSCs obtain proinflammatory phenotype and cooperate with lung resident immune cells (macrophages, DCs) in the elimination of bacterial pathogens.\textsuperscript{5} For that purpose, through the secretion of IL-1Ra, MSCs in an autocrine manner, upregulate expression of TLR-4 that has crucially important role in bacterial clearance.\textsuperscript{59} Lipopolysaccharide, released from the cell wall of Gram-negative bacteria, binds to TLR-4 in MSCs and induces enhanced secretion of lipocalin-2 that, in turn, binds bacterial ferric siderophores, reduces the uptake of iron, and suppresses bacterial growth.\textsuperscript{58} On the other hand, upon the elimination of bacterial pathogens and under the influence of high concentration of released inflammatory cytokines, MSCs obtain anti-inflammatory phenotype and, through the production of immunosuppressive factors (IL-1Ra, IL-10, and PGE2) inhibit production of proinflammatory cytokines (IL-1β, TNF-α) in alveolar macrophages and attenuate ongoing inflammation in the lungs (Figure 2).\textsuperscript{5,53,54}

In line with these findings, Bustos and colleagues used serum samples of ARDS patients, which contained high concentration of IL-1Ra and IL-10, to “prime” MSCs prior to their transplantation in ARDS animals. Serum sample of ARDS patients remarkably increased the expression of IL-1Ra and IL-10 in MSCs and enhanced their immunosuppressive and regenerative potential after engraftment in the injured lungs. Significantly decreased lung injury score, reduced pulmonary edema, down-regulated serum levels of inflammatory cytokines, and lower number of inflammatory cells in bronchoalveolar lavage were noticed in ARDS animals that received ARDS patients’ primed MSCs compared to ARDS animals that were transplanted with nonprimed MSCs.\textsuperscript{55}

In addition to its potential to suppress acute inflammation in the lungs, IL-1Ra was also responsible for the beneficial effects of MSCs in the treatment of chronic airway inflammation.\textsuperscript{58,60} As suggested by Duong and coworkers, MSC-derived IL-1Ra was crucially important for the attenuation of house dust mite (HDM)-induced chronic allergic inflammatory response in mice.\textsuperscript{60} Inhaled HDM activates TLR-4 in the airway epithelial cells (AECs) and initiates production of IL-1α that, in turn, acts in an autocrine loop to amplify itself and promote nuclear-to-cytoplasmic translocation of high-mobility group box 1 (HMGB1) enabling its later release in airway lumen.\textsuperscript{61} HMGB1 acts as alarmin in the lungs. Increased secretion of HMGB1 results in enhanced production of pro-Th2 cytokines IL-25 and IL-33 that promote generation of Type 2 innate lymphoid cells (ILC2), which are involved in progression of chronic airway inflammation.\textsuperscript{61} Through the secretion of IL-4, IL-5, and IL-13, ILC2 promote generation of CD4 + Th2 cells, stimulate production of matrix-degrading enzymes in eosinophils, induce IgE-dependent activation of basophils and mast cells, and massive secretion of histamine, prostaglandins, leukotriens, and inflammatory cytokines (IL-1β, TNF-α, IL-4, and IL-6). MSC-derived IL-1Ra regulates ILC2-driven immune response in the lungs by suppressing TLR-4-signaling in AECs.\textsuperscript{21} Through the production of IL-1Ra, MSCs attenuate TLR-4:IL-1α-dependent release of alarmins in injured AECs and inhibit generation and expansion of ILC2.\textsuperscript{12,21}

As MSCs may attenuate allergic inflammation by suppressing nuclear-to-cytoplasmic translocation of HMGB1 in alveolar epithelial cells and IL-1Ra may
suppress maturation, migratory, and antigen-presenting properties of DCs. Duong and colleagues investigated therapeutic potential of IL-1Ra-producing MSCs in alleviation of HMD-allergic inflammation. In similar manner as AECs, lung DCs also capture HDM in TLR-4-dependent manner. Activation of TLR-4 leads to the engagement of intracellular IL-1β which acts synergistically with TNF-α to enhance migration of DCs to the regional lymph nodes where DCs activate HDM-specific naive CD4 + T cells and induce their differentiation in IL-4-, IL-5-, and IL-13-producing, effector Th2 cells. CD4 + Th2 cells, in similar manner as ILC2, induce activation of mast cells, basophils, and eosinophils in the lungs and promote progression of chronic airway inflammation. By suppressing TLR-4:IL-1β signaling pathway in lung DCs, MSC-derived IL-1Ra reduces DCs migration to the draining lymph nodes, prevents generation of CD4 + Th2 cells, and attenuates IL-4-, IL-5-, and IL-13-driven inflammation in the lungs. Accordingly, significantly reduced number of activated HDM-loaded DCs were observed in the mediastinal lymph nodes of MSC-treated animals. MSCs isolated from the IL-1Ra-deficient mice did not manage to protect against HDM-induced inflammation, suggesting crucially important role of IL-1Ra for beneficial effects of MSCs in alleviation of chronic allergic inflammation.
Rheumatoid arthritis (RA) is characterized by TNF-α and IL-1β-driven synovial inflammation that results in the bone and cartilage destruction. Despite the fact that TNF-α is a pivotal inflammatory cytokine in acute joint swelling, IL-1β is considered as the dominant cartilage destructive cytokine and its production may occur in TNF-α-independent manner. Therefore, IL-1Ra-mediated inhibition of IL-1β-driven synovial inflammation has crucially important protective role in the pathogenesis of RA. A large number of experimental and clinical studies documented beneficial effects of IL-1Ra-based therapy in attenuation of RA symptoms, while IL-1Ra knockout (IL-1RaKO) mice, which spontaneously develop RA-like disease by 20 weeks of age, have been most usually used in RA-related experimental studies.

Osteoarthritis (OA), characterized by progressive cartilage degradation, subchondral bone remodeling, meniscal damage, and synovitis, represents a chronic inflammatory disease driven by IL-1β and TNF-α-producing macrophages and IFN-γ-producing CD4 + Th1 cells. During the early phase of inflammation, through the secretion of IL-1β and TNF-α, macrophages shift synovial tissue homeostasis toward catabolism, significantly increasing bone and cartilage resorption. Additionally, in IL-1β and TNF-α-dependent manner, synovial macrophages promotes expression of adhesion molecules (E and P selectins) on endothelial cells, facilitating influx of circulating leucocytes in injured joints. T-bet-expressing CXCR3 + CD4 + Th1 cells massively migrate in inflamed synovial tissue and in IFN-γ-dependent manner promote polarization of resident macrophages toward inflammatory (IL-1β and TNF-α-secreting) M1 phenotype, creating a “positive inflammatory loop” in inflamed synovia that results in OA progression.

MSCs spontaneously differentiate into chondrocytes and osteocytes and, at the same time, are able to suppress pathogenic immune response in inflamed synovial tissue. Therefore, during the last decade, therapeutic potential of MSCs in cell-based therapy of RA and OA have been intensively explored in animal and clinical trials. Several lines of evidence suggested crucially important role of MSC-derived IL-1Ra for chondrogenic and osteogenic potential of MSCs and for MSC-based alleviation of joint inflammation. Lee and coworkers demonstrated that MSCs (5 × 10^6 cells, intraperitoneally injected on Days 0 and 43) significantly alleviated synovial inflammation and cartilage destruction in IL-1RaKO mice. Remarkably reduced number of inflammatory cells was observed in synovial tissue of MSC-treated animals suggesting that MSCs attenuated influx of circulating leucocytes in inflamed joints. Cellular makeup of the spleens revealed significantly reduced Th17:Tregs ratio in MSC-treated IL-1RaKO mice compared to MSC-nontreated IL-1Ra deficient animals. Through the production of IL-1Ra, MSCs inhibited IL-1β:IL-1-dependent production of IL-1β, IL-6, and IL-23 in DCs, impairing capacity of DCs to induce differentiation of naïve CD4 + T cells in effector, IL-17- and IL-22-producing RORγT-expressing, inflammatory Th17 cells. On the other hand, administration of MSCs induced expansion of immunosuppressive CD4 + CD25 + FoxP3 + Tregs in the spleens of IL-1RaKO mice and enabled Treg-dependent suppression of Th17 cell-driven inflammation. Accordingly, significantly lower levels of inflammatory Th17-related cytokines (IL-1β, IL-6, and IL-17) were noticed in serum samples of MSCs-treated IL-1RaKO mice compared to MSC-nontreated IL-1Ra deficient animals. Human MSCs:CD4 + T cell coculture experiments confirmed that MSC-derived IL-1Ra, in a dose-dependent manner, could decrease generation of Th17 cells, suggesting potential clinical application of IL-1Ra-producing MSCs in cell-based therapy of IL-17-driven inflammatory diseases, including RA.

In line with these findings, Hu and colleagues transfected IL-1Ra gene in MSCs, encapsulated them in alginate-poly-L-lysine microcapsules (in order to enable persistent delivery of IL-1Ra) and evaluated their efficacy in RA treatment. Obtained results revealed that encapsulated, nonautologous, IL-1Ra gene-transfected MSCs continuously delivered IL-1Ra for at least 30 days and significantly attenuated collagen-induced arthritis in rats in IL-1Ra-dependent manner.

Similar findings were recently reported by Gabner and coworkers who designed IL-1Ra-overexpressing MSCs and evaluated their therapeutic potential in cell-based therapy of OA by using in vitro model. For that purpose, Gabner and colleagues cocultured IL-1Ra-overexpressing MSCs with osteoarthritic chondrocyte spheroids. As the expression of IL-1Ra in MSCs was under the control of an inducible NF-κB-responsive promoter, MSCs produced large amount of IL-1Ra upon priming with inflammatory cytokines (TNF-α or IL-1β). MSC-derived IL-1Ra significantly attenuated expression of inflammatory mediators (MMP-1, MMP-13, and IL-6) in osteoarthritic chondrocyte spheroids. Additionally, significantly enhanced expression of cartilage specific genes (aggrecan and alpha 2 chain of Type II collagen) were noticed in osteoarthritic chondrocyte spheroids after their coculture with IL-1Ra-overexpressing MSCs, suggesting potential therapeutic use of IL-1Ra-overexpressing MSCs in cell-based therapy of OA.
Furthermore, MSC-derived IL-1Ra promotes production of IL-1Ra in injured and proliferating chondrocytes within OA joints and in autocrine manner induces creation of anti-inflammatory microenvironment in inflamed joints. As observed by van Buul and coworkers, synovial and cartilage explants exhibited a higher expression of IL-1Ra gene after treatment with MSC-derived, IL-1Ra-containing secretome.69,78

In addition to cartilage regeneration, MSC-derived IL-1Ra also participates in bone and ligament healing.79 As demonstrated by Mohanty and colleagues, IL-1Ra-dependent signaling maintains pool of self-renewable MSCs in the bone marrow (BM) and regulates differentiation of BM-derived MSCs into functional osteoblasts.69 Genetic deletion of IL-1Ra significantly affected clonality of BM-derived MSCs and impaired their capacity for osteogenesis. Remarkably lower number of mesenchymal progenitors was noticed in BM of IL-1RaKO mice. IL-1Ra-deficient BM-derived MSCs were not able to optimally proliferate and had reduced potential for osteogenic differentiation, which led to osteoporosis in IL-1RaKO mice due to the accelerated bone loss.80 By using rat model of bilateral ligament injury, Seather and colleagues demonstrated that MSCs, through the production of IL-1Ra, induced generation of anti-inflammatory M2 phenotype in macrophages and attenuated ongoing inflammation in injured ligaments, resulting in improved ligament healing that was manifested by increased ligament strength.79

7 | CONCLUSIONS

Through the secretion of IL-1Ra, MSCs suppress IL-1α and IL-1β-driven inflammation, induce anti-inflammatory phenotype in macrophages, attenuate antigen-presenting properties of DCs and increase generation of Tregs enabling generation of immunosuppressive milieu in inflamed tissue that results in alleviation of Th1/Th17 cell-dependent immune response and promotes enhanced tissue repair and regeneration (Figure 3). Despite of these beneficial effects, results obtained in several clinical studies revealed that IL-1Ra-based inhibition of IL-1α/IL-1β:IL-1RI signaling significantly increases risk of infections.80–82 Additionally, several lines of evidence indicated that combined treatment of IL-1Ra and biological agents should be applied with extreme caution.83 Leucopenia was observed more frequently in patients that received combined treatment of IL-1Ra and TNF-α inhibitors and some of these patients (with neutrophil counts below 1,000/mm³), developed serious, life-threatening infections.83 As MSCs produce...
large amount of immunosuppressive factors that may act as TNF-α inhibitors,\textsuperscript{21} transplantation of IL-1Ra-producing MSCs should be contraindicated in immunocompromised patients and in patients with a history of recurrent infections. Future experimental and clinical studies should determine the exact dose of IL-1Ra-producing MSCs and should delineate potential side effects of MSC-derived IL-1Ra before IL-1Ra-overexpressing MSCs could be used as a potentially new remedy for the treatment of acute and chronic inflammatory diseases.

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