Mechanism of Mesenchymal Stem Cells Preconditioned with IFN γ in the Treatment of Autoimmune Diseases

Yiru Dong [1, *]

[1] QU-Drake United College, Qingdao University, 266100 Laoshan District Qingdao, China

* Corresponding Author Email: dongyiru1@qdu.edu.cn

Abstract. Autoimmune diseases are chronic and intractable and caused by the disruption of immune tolerance mechanisms and the imbalance of immune homeostasis. Conventional treatments and targeted drugs have the limitations such as insufficient safety and low response rates. By unique advantages of immunomodulation and tissue repair, an innovative treatment, Mesenchymal stem cells (MSCs) have gradually matured. Nevertheless, they are still facing the problems like instable function and low homing efficacy in clinical translation. Interferon-γ (IFN-γ) preconditioned can effectively optimize efficacy of MSCs, with core mechanisms including:Binding to IFN-γ receptors (IFNγR) on the surface of MSCs to activate the JAK-STAT signaling pathway and trigger the transcription of immunomodulation related genes;Significantly upregulating the expression of IDO1, PD-L1, and TGF-β, which regulate the activation and function of Tregs, T cells and B cells to reestablish immune homeostasis, downregulating the expression of chemokine receptors CXCR4 and CCR7, shifting the function of MSCs from active migration to local retention and enhancing local therapeutic efficacy at lesion sites. This paper systematically organizes the activation, immunoregulatory and homing optimization mechanism of IFN-γ preconditioned MSCs, summarizes the advantages and challenges and provides a theoretical reference for basic research and clinical translation in the treatment of autoimmune diseases.

Keywords: Autoimmune diseases, Mesenchymal stem cells (MSCs), IFN-γ.

1. Introduction

Autoimmune diseases are a group of diseases caused by abnormal activation of the autoimmune system, which can lead to attacktion of healthy tissues and organs. Ultimately, they result in inflammatory damage and dysfunction. Autoimmune diseases include rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), type 1 diabetes mellitus (T1DM), multiple sclerosis (MS), Hashimoto's thyroiditis (HT), Sjögren's syndrome (SS), etc., etc.The prevalence is approximately 5%–10% and shows a sightly upwords in recent years. These diseases usually present a chronic and progressive course with high clinical heterogeneity.

They can affect multiple organs such as joints, skin, endocrine glands, and the central nervous system. Moreover, they are difficult to completely cure, severely reducing patients' quality of life.

The core mechanism is that the immune tolerance in patients is impaired, which causes abnormally activation and clonal expansion of resting or anergic T cells and B cells, followed by their differentiation into effector cells, triggering pathological immune responses against specific target organs or multiple systemic organs. Current therapeutic treatments for autoimmune diseases mainly focus on controlling inflammation and regulating immune imbalance, which can be categorized into conventional treatments, biological agents and small molecule targeted drugs, as well as emerging cellular and immunomodulatory therapies.

Conventional treatments mainly depend on glucocorticoids and anti-rheumatic drugs. These drugs can rapidly inhibit inflammation and immune activity. However, they have limitations such as infection risks caused by nonspecific immunosuppression and organ toxicity resulting from long-term use. Biological agents and small-molecule targeted drugs, like monoclonal antibodies against IL-17 and B cell surfacour

e molecules (CD20) as ll as JAK inhibitors, can achieve targeted intervention via precisely blocking key inflammatory pathways. Nevertheless, probilems such as limitied response rates,

primary or secondary drug resistance, elevated risks of severe infection and malignancy, and high treatment costs still exist [1].

Among emerging treatments, mesenchymal stem cells (MSCs) have arisen extensive attention because of their unique advantages in immune regulation, tissue repair, and induction of immune tolerance. A number of clinical studies have preliminarily verified their therapeutic potential in refractory autoimmune diseases such as SLE and RA, providing a new direction for reestablish immune homeostasis.

However, the clinical translation of this treatment still faces key issues including insufficient large scale evidence based support for long term safety and efficacy, as well as low homing efficiency, which limit its standardized promotion [2]. To address these issues, recent studies have proposed optimization via IFN γ preconditioned. It has been found that IFN γ preconditioned MSCs can be more effectively to adapt the inflammatory microenvironment after entering the body and achieve "on demand" immune regulation. Moreover, this pretreatment can also enhance the stability of immune tolerance induced by MSCs and improve their homing efficiency to target organs.

Nevertheless, the mechanisms about IFN γ preconditioned MSCs have not been systematically organized and analyzed. Therefore, this paper aims to systematically illustrate the core mechanisms about how this treatment enhance the efficacy of MSCs, with the expectation of providing guidance for further research in related fields.

2. Immune cell regulatory mechanisms in autoimmune diseases

The occurrence and progression of autoimmune diseases are essentially attributed to the collapse of immune tolerance and imbalance of immune homeostasis, in which the abnormal quantity and dysfunction of Treg, T cells and B cells exert critical roles in disease pathogenesis.

2.1. Reduction and functional deficiency of treg

Tregs are a core cell subset in maintaining immune tolerance. Through the secreting of IL 10 and transforming TGF-β, they negatively regulate excessive activation of effector T cells through direct contact mediated suppression. In various autoimmune diseases, Tregs exhibit characteristics such as reduced number, imbalanced proportion, or impaired suppressive function. For instance, the percentage of Tregs is significantly diminished in the peripheral blood from SLE patients, and their suppressive function exhibits a negative correlation with disease activity. Although Tregs can be locally richened in the synovial fluid of patients with RA, their immunosuppressive function is weakened by the inflammatory microenvironment, failing to control abnormal proliferation of effector T cells. Insufficient number and dysfunction of Tregs reduce immune tolerance to self antigens, becoming a key factor in the occurrence and progression of diseases [3].

2.2. Imbalance and abnormal activation of T cell subsets

Differentiation imbalance of T cells, especially T helper (Th) cell subsets, is important in autoimmune diseases. Under normal circumstances, subsets like Th1, Th2, Th17 and Treg maintain a dynamic balance. Under pathological circumstances, however, pathogenic T cell subsets are excessively activated, leading to a proinflammatory shift in the immune response. Among them, Th17 cells and IL 17 secreted by them are significantly increasing in autoimmune diseases such as MS and RA, which act as the key effector cells mediating inflammatory tissue damage. Abnormally enhanced cellular immune responses mediated by Th1 cells are also widely involved in various organ specific autoimmune diseases, such as HT and T1DM.

2.3. B cell excessive activation and autoantibody production

In autoimmune diseases, B cells act a "double pathogenic" role. They not only can mediate humoral immune damage by differentiating themselves into plasma cells, which produce numbers of autoantibodies, but also activate T cells through antigen presentation and cytokine secretion. In

diseases such as SLE and SS, the excessive activation of B cells and significantly level of autoantibody are iconic features. Among these, the abnormal expansion of autoreactive B cell clones, sustained activation of germinal center reactions, and long-term survival of plasma cells lead to immune complex deposition and target organ damage together. In clinical studies, the appearance and titer changes of autoantibodies such as antinuclear antibodies, rheumatoid factor, and anti-dsDNA antibodies are closely associated with disease activity and prognosis. Furthermore, abnormal differentiation of B cell subsets, such as the increased of memory B cells and insufficient function of regulatory B cells (Bregs), weakens the immune negative regulatory mechanism, contributing to disease persistence and relapse [4].

3. Mechanism of IFN-γ mediated activation of MSCs

There are IFN-γ receptors (IFNγR) on the surface of MSCs. When IFN-γ binds to IFNγR on resting MSCs, the conformation of IFNγR changes. This change brings the inactive Janus kinase 1 (JAK1) and Janus kinase 2 (JAK2) into proximity, which are associated with the intracellular domains of IFNγR1 and IFNγR2, respectively, leading to transphosphorylation and activating the JAKs.

In resting MSCs, activated JAKs can induce the phosphorylation of signal transducers and activators of transcription (STATs). Studies have proofed that JAKs can phosphorylate STAT1 and STAT3..Furthermore, activated JAKs can catalyze the phosphorylation of STAT1 at Tyr701 and STAT3 at Tyr705. Such phosphorylation triggers a conformational shift in STAT monomers, allowing their Src homology 2 (SH2) domains to recognize and bind to phosphorylated tyrosine residues on adjacent STAT proteins, thereby forming parallel dimers. This dimerization masks the original nuclear export sequence (NES) and exposes the nuclear localization signal (NLS), driving the translocation of STAT complexes into the nucleus to initiate the transcription of immunoregulatory genes. After entering the nucleus, STAT complexes bind to γ-activated sequences (GAS) or interferon-stimulated response elements (ISRE) in the promoter regions of interferon-stimulated genes (ISGs), recruit co-activators and RNA polymerase II to form the transcription initiation complex, and trigger the transcription of a series of ISGs with broad-spectrum immune defense functions [5].

4. Immunoregulatory Mechanism of MSCs Preconditioned with IFN-γ

As a key proinflammatory cytokine, IFN-γ exerts a pronounced effect on increase the expression of multiple immunomodulatory molecules in MSCs after preconditioning. Among these, IDO1, PD-L1, and TGF-β serve as the core effector molecules mediating immunosuppressive functions. Through the cooperation of these three molecules, IFN-γ preconditioned MSCs can multidimensionally regulate the activated status and functions of Tregs, T cells, and B cells, thereby reestablishing the imbalanced immune homeostasis in autoimmune diseases.

4.1. IDO1 mediated tryptophan metabolic regulation

IDO1 is the most significantly upregulated key enzyme in MSCs after IFN-γ precondition ($P < 0.05$). It makes a double immunosuppression effects by catalyzing the degradation of tryptophan via the kynurenine pathway. On the one hand, the local depletion of tryptophan can suppresses the activation and proliferation of effector T cells by triggering the GCN2 stress pathway. On the other hand, kynurenine and its metabolites can induce the shift of T cells to the Treg phenotype and promote apoptosis of B cells. In autoimmune disease models, IFN-γ preconditioned MSCs effectively inhibited the differentiation and expansion of The Th1 and Th17 cells and promote the production of $Foxp3^+$ Tregs via high-level expression of IDO1. Furthermore, IDO1 dependent mechanisms also inhibit the viability of autoreactive B cells and reduce the generation of autoantibodies. Notably,the IDO1 expression level is tightly correlated with the IFN-γ concentration in the micro-environment, which reflects the on-demand immunomodulation property of MSCs [6].

4.2. PD-L1 mediated co-inhibitory signaling pathway

PD-L1 is a key co-inhibitory molecule which is highly expressed on the surface of MSCs preconditioned with IFN-γ. By binding to PD-1 on the surface of T cells, it can deliver negative co-stimulatory signals, thereby inducing T-cell anergy, apoptosis, or differentiation toward a regulatory phenotype. Specifically, activation of the PD-L1/PD-1 signaling pathway suppresses downstream signal transduction of the T-cell receptor (TCR), reduces IL-2 secretion ($p<0.0001$), and limits clonal expansion of effector T cells. During the therapeutic intervention of autoimmune diseases, IFN-γ preconditioned MSCs remarkably inhibit the infiltration and function of pathogenic Th1 and Th17 cells via a PD-L1 dependent mechanism, while promoting the stable maintaining of Tregs. In addition, PD-L1 signaling can also directly work on B cells to inhibit their activation and antibody class switching, thereby reducing autoantibody production. Similar to IDO1, the expression of PD-L1 is also controlled via IFN-γ concentration, enabling MSCs to dynamically adjust their immunosuppressive potency in inflammatory environments.

4.3. TGF-β-mediated induction of immune tolerance

TGF-β is an important anti-inflammatory cytokine ($p<0.05$) secreted by IFN-γ preconditioned MSCs and plays a central role in inducing immune tolerance. Through Smad-dependent signaling pathways, TGF-β directly promotes the differentiation of naive T cells into $Foxp3^+$ Tregs while sustaining the stability and suppressive function of Tregs. Simultaneously, TGF-β has the capability of inhibiting the switch of Th1 and Th17 cells, particularly through the downregulating RORγt expression, thereby blocking Th17 mediated inflammatory tissue damage. In terms of B-cell regulation, TGF-β can induce the shift of B cells to Bregs and promote the secretion of LI-10, thereby enhancing negative immune regulatory effects. Additionally, in regulating the immunomodulatory properties of MSCs, TGF-β also plays an important role, forming a cooperative network with IDO1 and PD-L1 to construct a multilayered immunosuppressive barrier [7].

In studies on metabolic regulation of $CD8^+$ T cells, quantitative Seahorse glycolysis stress test proofed that the extracellular acidification rate of the TGF-β-treated group showed a limited increase after the injection of glucose, and the oligomycin induced extracellular acidification rate peak was significantly reduced. This indicates that cells not only are impaired basal glycolytic capacity but also exhausted their maximal glycolytic reserve. This complete chain of evidence clearly confirmed that TGF-β is the key factor in the treatment of autoimmune diseases [8].

4.4. Integrated mechanism of cooperative regulation of T cells, tregs and B cells by this three factors

IDO1, PD-L1, and TGF-β, which are upregulated in MSCs after IFN-γ preconditioned, do not work independently. Instead, they achieve precise regulation of the immune cell network through a cooperative and complementary integrated mechanism. For T cells, IDO1 inhibits the proliferation of Th1 and Th17 cells via tryptophan exhaustion and accumulation of metabolites. PD-L1 induces T cells anergy through co-inhibitory signaling. TGF-β directly suppresses Th17 differentiation. Together, these three factors effectively restrain the abnormal activation of pathogenic T cells. For Tregs, metabolites of IDO1 promote Tregs differentiation, PD-L1 signaling contributes to Tregs stability, and TGF-β acts as an indispensable inducer of Tregs differentiation. Synergy of the three factors shifts immune balance from a pro-inflammatory condition into an immune-tolerant phenotype. For B cells, IDO1 induces the cell apoptosis of autoreactive B cells through metabolic mechanisms, PD-L1 signaling inhibits B cells activation and antibody production, and TGF-β promotes Bregs differentiation. Collectively, the three molecules suppress aberrant hyperactivation of humoral immunity.

In contract of untreated MSCs, since the expression of IDO1, PD-L1, and TGF β is all positively regulated by IFN γ levels in the inflammatory microenvironment, preconditioned MSCs can rapidly establish potent immunosuppressive function at inflammatory sites (eg. reducing level of IgE levels, $p<0.005$), while maintaining low immunosuppressive activity in non-inflamed tissues. This

mechanism achieves spatiotemporally precise immune regulation and avoids the infection risk associated with systemic immunosuppression [9].

5. Mechanisms by which IFN γ preconditioned MSCs overcome impaired homing ability

Efficient homing of MSCs to sites of injury or inflammation in vivo is a critical prerequisite for their therapeutic effects, and this process highly depends on precise guidance by chemokine receptors on the cell surface. Studies have shown that after precondition with IFN-γ, the expression levels of CXCR4 and CCR7, two key homing receptors on MSCs, are both significantly decreased.

Among them, CXCR4 functions as a specific receptor for stromal cell-derived factor-1 (SDF-1/CXCL12). The SDF-1/CXCR4 axis is widely recognized as one of the most important pathways in MSC homing. SDF-1 is abundantly expressed in bone marrow, injured tissues, and inflammatory sites, and by attracting CXCR4-positive MSCs to achieve their proper migration to target regions. CCR7 acts as the shared receptor for chemokines CCL19 and CCL21, both of which exhibit a high expression levels in secondary lymphoid organs and inflammation associated lymphoid tissues. Activation of CCR7 helps the migration of MSCs toward these immune organs or inflammatory lymphoid tissues, participating in immune regulation.

IFN γ precondition which leads to the downregulation of these two receptors indicates reduced chemotactic sensitivity of MSCs to SDF-1 and CCL19/CCL21, and their ability to migrate toward corresponding chemokine gradients is conventionally considered to be potentially inhibited. Nevertheless, this change seemingly is unfavorable to homing,but it actually reveals a more fine-tuned adaptive regulatory mechanism of MSCs in the inflammatory microenvironment.

When MSCs are exposed to the inflammatory microenvironment with a high concentration of IFN γ, which indicates that they have reached or are close to lesion sites with active immune responses.Under such case, maintaining high expression of homing receptors and responding to distant chemotactic signals may cause MSCs to leave the effective action sites that they have reached and migrate further away, which in turn reduces their local retention time at the lesion and therapeutic efficacy.Therefore, by downregulating the expression of CXCR4 and CCR7, IFN γ decreases the responding of MSCs to distant chemotactic signals, making them tend to stay in local inflammatory tissues.

This functional shift from active migration to local retention is crucial for MSCs to contribute to immunomodulatory and tissue repair.MSCs stayed in inflamed lesions can continuously secrete various soluble chemokines, cytokines, and growth factors, achieving sustained suppression of local immune cells and promoting the repair of damaged tissues.Meanwhile, the long-term retention of MSCs also helps to establish a stable immunomodulatory microenvironment, preventing the recurrence and exacerbation of inflammatory responses [10].

6. Conclusion

In summary, autoimmune diseases have long been a major challenge in clinical and basic research due to their complex pathogenesis and difficult long-term treatment. Although conventional and targeted therapies can control disease progression, they fail to fundamentally restore immune homeostasis and face issues such as safety concerns and individual difference. MSCs offer a novel approach for reestablishing self-tolerance because of their unique immunomodulatory and repaired ability of tissue. However, their clinical translation is limited by inherent heterogeneity and unstable functional activity in vivo. As a key proinflammatory cytokine, IFN γ can activate signaling pathways including JAK–STAT in MSCs and induce the expression of IDO, PD-L1, and multiple chemokines. This markedly enhances the immunosuppressive capacity and directional migration of MSCs, effectively addressing obstacles to their clinical translation.

By precondition of MSCs with IFN γ, which simulates the inflammatory microenvironment in vivo to preactivate their immunoregulatory potential, provides a key strategy to overcome the above bottlenecks. It not only enhances the stability of their immunosuppressive function and the homing ability of MSCs, but also improves the controllability and standardization of therapy. However, issues such as the optimal induction concentration and duration of IFN γ precondition, consistency of cell quality, and standardization of large scale production remain to be clarified, and its clinical translation efficacy requires further validation through large scale randomized controlled trials. With the standardization of precondition protocols, the maturation of large-scale production technologies, and the accumulation of more high-ourquality clinical evidence, preconditioned MSCs therapy is expected to break through the current therapeutic limitations in the future. Such a treatment will enable a shift in treating autoimmune diseases, from symptom mitigation to immune homeostasis remodeling, thus delivering renewed hope for many affected individuals.

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