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REGULAR ARTICLES

Introducing the immunomodulatory effects of mesenchymal stem cells in an experimental model of Behçet's disease

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Abstract Behçet's disease (BD) is a systemic vasculitis which is characterised by oral, aphthous ulcers, genital ulcers, skin lesions and ocular manifestations. Although the aetiopathogenesis of BD is still unknown, the critical role of Th1 immune responses, neutrophil hyperactivation alongside overproduction of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-8, tumour necrosis factor-alpha (TNFα) and particularly IL-17 have been demonstrated in the immunopathogenesis of the disease. Despite significant progress in understanding of the aetiology of the disease, its treatment remains intricate, and is still treated with immune-suppressive drugs and biological agents with probable systemic side effects. Accordingly, there is a necessity to establish the more efficient and less toxic therapeutic methods which may offer a long-time remission of BD.

Mesenchymal stem cells (MSCs) are non-haematopoietic and multipotential stem cells with immunosuppressive capacities in innate and acquired immune systems. MSCs can migrate to damaged tissues and prevent secretion of proinflammatory cytokines and other immunomodulatory effectors, increasing the survival of damaged cells, although the exact underlying mechanisms are still unknown. For this purpose, numerous herpes simplex viruses are injected into C57BL/6 mice to produce Behçet's mouse model and transferring a certain number of MSCs may have therapeutic

Abbreviations: BD, Behçet's disease; Th1, T lymphocyte helper 1; Treg, T regulatory; IL, interleukin; TNF-α, tumour necrosis factor alpha; IFN-γ, interferon gamma; TGF-β, tumour growth factor beta; NK, natural killer cell; HLA, human leucocyte antigen; MHC, major histocompatibility complex; APCs, antigen presenting-cells; MSCs, mesenchymal stem cells; pfu, plaque-forming unit; DMEM, Dulbecco's modified Eagle's medium; FBS, foetal bovine serum; CD, cluster of differentiation; Sca-1, stem cell antigen-1; Vcam-1, vimentin cell adhesion molecule-1; HSV, herpes simplex virus; PBS, phosphate-buffered saline; IHC, i; RT-PCR, reverse transcriptase–PCR.

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value for control of Behçet's animal model, so researchers could deliberate the function of MSCs and proinflammatory cytokines particularly IL-17A-F, TNF-α, interferon gamma (IFN-γ), IL-2, IL-6 and IL-8 in an experimental model.

The aim of this hypothesis is to evaluate immunosuppressive and immunomodulatory properties of MSCs in syngeneic animal model for BD, in order to clarify the mechanisms of MSCs in BD management, as a broad and more confident treatment in clinical application.

Introduction

Behçet's disease (BD) is an immune-mediated, chronic and relapsing disorder which is currently thought to be an autoimmune/autoinflammatory syndrome [1,2]. This multisystem disease is mostly common among young men and women in the Far East, Mediterranean countries and Middle East including Iran [3]. BD is characterised by oral aphthosis, genital ulcers, skin lesions and ocular manifestations. Vascular, cardiopulmonary and neurological involvements are some minor manifestations of the disease which are rare but important because they could have irreversible complications [3,4].

The exact details about the aetiopathogenesis of BD are still unknown, although it has been well established that a combination of genetic predisposition, (the most described gene in this regard is human leucocyte antigen-B51 (HLA-B51) [5], environmental factors and infectious agents could lead to disturbances in immune responses and thereby participate in the pathogenesis of BD [6,7]. Dysfunction of hypothalamo–pituitary–adrenal axis, modified levels of prolactin, sex hormones and some neuropeptides with main biological effect on both innate and acquired immune system are also associated with long-term stress in BD [4]. The immunopathogenesis is shown in Fig. 1. Initially, internal antigens such as organ-specific proteins and external antigens such as viruses and micro-organisms encounter the T cells (αβ-T cells and δγ-T cells) through antigen-presenting cells (APCs) which leads to lymphocyte activation. Hyperactivated T lymphocyte helper 1 (Th1) produces proinflammatory cytokines such as interleukin 2 (IL-2), IL-6, IL-8, IL-17, tumour necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ). These cytokines stimulate neutrophils and activate monocytes. Activated monocytes promote Th1 differentiation via release of IL-12 and hyperfunction of neutrophils causes tissue injury. As a whole, association between APCs, hypersensitivity of T lymphocytes and hyperactivity of neutrophils could be the main reason for immune responses in BD [4,7–9].

In addition to the considerable impression of Th1 and Th2, recent studies illustrate that IL-17 has a consequential and exclusive role in BD pathogenesis [10,11]. IL-17 mainly produces a high value of IL-22, IL-17A-F and TNF-α. As reported in previous studies, IL-23, IL-6 and tumour growth factor beta (TGF-β) trigger Th-1 differentiation from naive T cells. Additionally, IL-21, which is the major increased cytokine in active phase, involved in Th17/(regulatory T lymphocyte) Treg equilibrium regulating and decreased Treg cell frequency [12].

Additionally, humoral immunity system is also involved with a minor role, in the pathogenesis of BD. Enhancing of autoantibodies such as cardiolipin and immune complexes (antigen–antibody complex) has been reported in more than half of patients with BD, specifically in the active phase of the disease [8] which probably is associated with severity of the disease [13].

BD is treated with immunosuppressant drugs and biological agents such as monoclonal antibodies which are associated with severe systemic side effects [14,15]. Unfortunately, there are still cases of severe and life-threatening BD, refractory to conventional medications and biological agents [3]. Less toxic and more effective therapeutic options are needed for BD patients, especially with devastating complications.

Non-haematopoietic MSCs are precursor multipotential cells which are being obtained from many tissues, particularly bone marrow. In addition to the capacity of differentiation into mesodermal cell line, these cells can operate as modulators of immune system [16]. MSCs have the ability to inhibit T lymphocyte cell cluster of differentiation (CD4 and CD8), activation and proliferation [17,18].

Furthermore, MSCs reduce secretion of IL-2, IL-12, INF-γ and TNF-α and induce production of IL-4 and IL-10. Recent studies have demonstrated that MCSs probably have this potency to secrete cytokines, hence could switch equivalence between Th_2/Th_1 towards Th_2 [19–21]. In fact, MSCs, on the one hand, could repress development of proinflammatory Th1 and natural killer cell (NK) signals and, on the other hand, expand the range of anti-inflammatory Th2 and T repressor cells [22].

Recent studies exhibited that MSCs inhibit TH17 differentiation from naive T cells [23]. MSCs can also decrease the expression of major histocompatibility complex class *E* (MHC class *E*) [24].

Generally, *in vivo* and *in vitro* immunosuppressive effect of MSCs have been demonstrated for autoimmune diseases such as diabetes [25], lupus erythematosus [26], rheumatoid arthritis [27,28] and autoimmune encephalomyelitis [29] with an effective role in treatment of animal models. Furthermore, transplantation of MSCs in patients with autoimmune disorders, such as multiple sclerosis [30], scleroderma [31] and systemic lupus erythematosus [32], has encouraging results.

Although the use of MSCs has been established as a non-toxic, available therapeutic procedure with poor immunogenicity [33], there are, nevertheless, many ambiguities in this area. Despite frequent efforts, the exact mechanisms underlying immunomodulatory effects of MSCs for its therapeutic application remain unclear [34]. Certainly, more studies are necessary to determine the immunosuppressive mechanisms of MSCs for broad application in treatment of patients.

In this article, we proposed that MSCs could have an important role in inhibiting cytokines such as IL-17, INF-γ and TNF-α genes via post-transcriptional and post-translational regulations of gene expression in Behçet's experimental model.

The hypotheses

Clinicians still face severe and life-threatening cases of BD resistant to conventional immune-suppressive medications

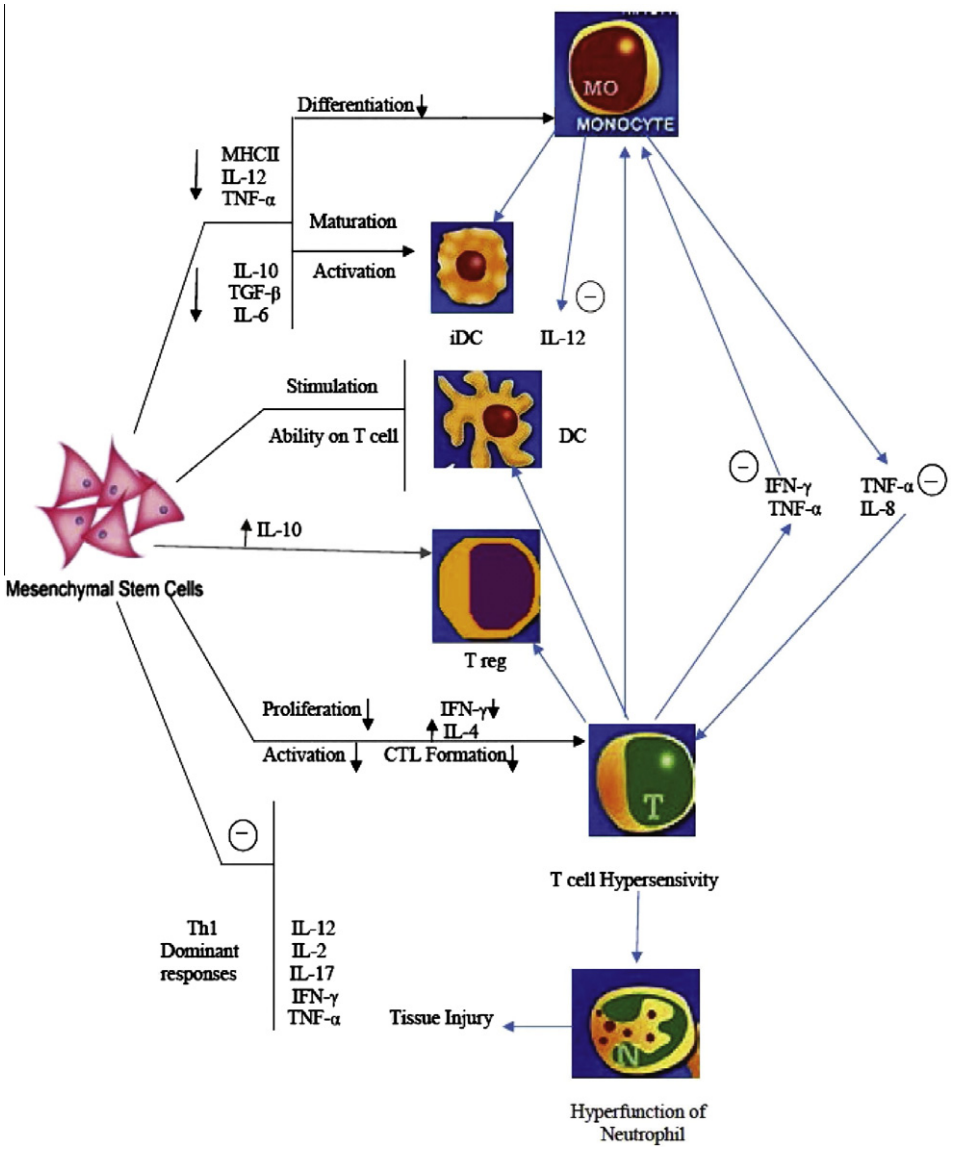

Figure 1 Immunomodulatory property of MSCs and BD immunopathogenesis.

and biological agents. Regarding the key role of cellular and humoral immunity in pathogenesis of BD, we hypothesize that usage of immune-modifying properties of MSCs could be more efficient and competent in the control of induced BD in animal model. The use of MSCs in clinical application is associated with special restrictions, because the exact underlying mechanisms are not well established. The aim of this hypothesis is to evaluate MSCs' molecular and cellular mechanisms, involved in BD control, in an experimental model. It is obvious that the more investigators study about MSCs' underlying mechanisms, the affected individuals will be treated with the more efficacy and reliability.

Evaluation of hypotheses

We suggest a syngeneic model of BD to evaluate the immunomodulatory effects of non-haematopoietic MSCs in control or full recovery of disease by improving clinical symptoms, behavioural changes of models and investigating the role of MSCs in inhibiting cytokines such as IFN-γ, TNF-α and IL-17 genes via post-transcriptional and post-translational regulations of gene expression in Behcet's syngeneic animal model.

Research design and methods

1. Injection of 1.0×10^6 plaque forming units (pfu ml–1) herpes simplex virus type 1 (KOS strain) to earlobes of 20 C57BL/6 mice (considering that C57BL/6 is the only instance in Iran); after 4 weeks the second injection is administered to produce Behçet's mouse model according to the Hirata et al. method [35]. As controls 10 mice are inoculated in the same site with culture medium. The induced BD-like major symptoms are included, eye syndrome, genital, oral and other skin ulcers. Gastrointestinal ulcers, arthritis and neurological involvement are defined as minor symptoms (two or more symptoms in mice are considered as a BD-like syndrome) [36].

2. Isolation of MSCs from 6–8-week-old C57BL/6 mice. C57BL/6 mice are euthanised by cervical dislocation. Bone marrow is collected from tibia and femur of mice. 70×10^6 bone marrow cells from one donor are obtained [37,38]. Then 25×10^6 cells are prepared [38] and suspended in Dulbecco's modified Eagle's medium (DMEM) containing 15% foetal bovine serum (FBS), penicillin and streptomycin. After 24 h, cells are washed and non-adherent cells, which mostly are haematopoietic stem cells, removed. Adherent cells are cultured in complete medium for 1 week until they covered 80–90% of the bottom of the culture bottle. MSCs from passages 4 are used in the subsequent experiments. Mature MSCs are determined by their ability to differentiate into adipocytes and osteocytes. Further properties are based on the expression of surface markers such as CD34. CD44, stem cell antigen-1 (Sca-1) and vimentin cell adhesion molecule-1 (Vcam-1) [37,38].
3. Transferring 5×10^6 syngeneic MSCs (isolated from C57BL/6 mice) to both BD-induced models [33], and the affected group with herpes simplex virus (HSV-1), without any clinical manifestations, to study the therapeutic effects of MSCs. An equal volume of phosphate-buffered saline (PBS) is used in the control group [33].
4. Comparing clinical manifestations of MSCs treated with the control group and evaluating probable cellular and molecular mechanisms of MSCs, which is shown in Fig. 1, by means of RT-PCR, flow cytometry and immunohistochemistry (IHC) in models.
5. All assessments were conducted in four separate groups: (1) Control: normal mice C57BL/6. (2) Control model group, without MSC transplantation (BD is induced in these mice). (3) BD-induced mice model that have received the MSCs which is divided into three categories: (1) MSCs will be inoculated into mouse models before injection of HSV-1 in the first group. (2) In the second group, MSCs will be inoculated simultaneously with HSV-1 into animal models. (3) Eventually, in the third group, MSCs will be transferred after HSV-1 induction, when clinical manifestations have been stabilised in models. (4) The fourth group containing mice did not show any clinical symptoms, despite inoculation of HSV-1. This group is also treated with MSCs. The fourth group of this experiment probably will be advantageous, because different individuals are exposed to variable environmental factors such as viruses.

Conclusion and discussion

Nowadays, it has been established that MSCs have the potential of suppressing the immune response via inhibiting the maturation of dendritic cells and suppressing the function of T lymphocytes, B lymphocytes and NK cells. A proper animal model of BD can be useful to evaluate the immune suppressor effects of MSCs in control or full recovery of disease by improving clinical symptoms, behavioural changes of model and investigating probable cellular and molecular mechanisms of MSCs such as TNFα and IFN- γ and IL-17 gene expression.

The use of of animal model helps to clarify the cellular and molecular principles involved in BD pathogenesis and exacerbation of inflammation, and also provides the possibility of creating novel drugs, for preventive, controlling and therapeutic objects, with less toxicity and side effects. In cell therapy, immunologic responses in recipients are detected as a major obstacle, which is eliminated with the use of syngeneic system. Achievement of these mechanisms in the future, in addition to the use of MSCs as a cell therapy strategy, could lead to transfected MSCs as a vector, in order to candidate gene delivery for disease management.

Conflicts of interest statement

The authors have no conflicts of interest to declare.

Overview Box

First Question: What do we already know about the subject?
BD is still associated with devastating complications despite current treatments. Critical role of cellular and humoral immunity is proved in the pathogenesis of the disease. The immune-modulator functions of MSCs have been described.

Second Question: What does your proposed theory add to the current knowledge available, and what benefits does it have?
We hypothesised that, using immunomodulatory properties of MSCs in disease control in an experimental model of BD, and also evaluating its appropriate cellular and molecular mechanisms could be effective for BD patients not responding to common treatments.

Third question: Among numerous available studies, what special further study is proposed for testing the idea?
We induce Behçet's animal model with injection of HSV-1 in C57BL/6 mice and transmission of MSCs into syngeneic mouse model, to evaluate the effects of MSCs in clinical symptom improvement and identification of its cellular and molecular mechanisms compared to the controls.

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