review

Immunomodulatory properties of stem mesenchymal cells in autoimmune diseases

Isabel Sánchez-Berná *, Carlos Santiago-Díaz, Juan Jiménez-Alonso

Unidad de Enfermedades Autoinmunes Sistémicas, Servicio de Medicina Interna, Hospital Universitario Virgen de las Nieves, Granada, Spain

ARTICLE INFO

Article history:
Received 19 November 2013
Accepted 16 January 2014
Available online 15 September 2015

Keywords:
Mesenchymal stem cells
Immunomodulation
Autoimmune diseases

ABSTRACT

Autoimmune diseases are a cluster of disorders characterised by a failure of the immune tolerance and a hyperactivation of the immune system that leads to a chronic inflammation state and the damage of several organs. The medications currently used to treat these diseases usually consist of immunosuppressive drugs that have significant systemic toxic effects and are associated with an increased risk of opportunistic infections. Recently, several studies have demonstrated that mesenchymal stem cells have immunomodulatory properties, a feature that make them candidates to be used in the treatment of autoimmune diseases. In the present study, we reviewed the role of this therapy in the treatment of systemic lupus erythematosus, Sjögren’s syndrome, systemic sclerosis, Crohn’s disease and multiple sclerosis, as well as the potential risks associated with its use.

© 2013 Elsevier España, S.L.U. All rights reserved.

INTRODUCTION

Autoimmunity is the physiological (in the context of an acute infection, active neoplasia, etc.) or pathological (in the context of paraneoplastic syndromes, autoimmune diseases, etc.) situation in which there is an innate or acquired immune response directed against self antigens. Autoimmune diseases are a group of immune disorders in which the host, previously healthy, organises a pathological immune response against himself in the absence of an evident cause. Thus, as a consequence of a genetic disposition upon which environmental and hormone factors act, there is a failure in some of the immune tolerance mechanisms, so different elements of the immune system react against their own cells and tissues.

Immunological tolerance is defined as the immune system’s recognition of its own antigens, which allows it to destroy foreign elements that invade the body without attacking it. Both B cells
and T cells submit themselves to this specific recognition process, starting in the primary organs during the foetal life (bone marrow and thymus, respectively) and continuing during postnatal life in the periphery. Autoreactive cells are eliminated at both levels through apoptosis or induction of anergy. Thus, maintenance of immune tolerance is achieved through each mechanism filtering the errors produced in the previous stages, so the simultaneous failure of all these mechanisms justifies the appearance of autoimmune diseases.3

Autoimmune diseases are one of the 10 main causes of death in women over 64 years old, and the second cause of chronic disease in U.S.A.4 Most of the current treatments of autoimmune diseases perform an immunosuppressant action, either acting specifically or over a factor (for example, anti-tumour necrosis factor), or non-specifically (for example, glucocorticoids)5 over other one. In any case, these treatments do not distinguish between pathological or protective/physiological immune response, thus increasing the risk of opportunistic infections and other adverse effects during long periods of time.4,5 Cell therapy, thanks to the immunomodulatory properties of stem cells, constitutes an alternative for the treatment of this group of diseases.

Mesenchymal stem cells (MSC), in accordance with the criteria set forth by the International Society for Cellular Therapy,6 are cells with a great capacity of adherence to surfaces (when they acquire a typical fibroblast aspect), as well as a great capacity of in vitro expansion in culture media supplemented with serum, that do not express haematopoietic or endothelial markers. They have the ability of differentiating themselves in vitro, with the appropriate stimulus, into cells of mesenchymal lineage: osteoblasts, chondrocytes and adipocytes,7 although the MSCs ability to differentiate themselves into cells of the neuroectoderm and endoderm has also been described.9 After they were initially discovered in the bone marrow, they have been found in a great number of tissues and connective organs: adipose tissue, dental pulp, placenta, umbilical cord, amniotic fluid, blood, synovial membrane, foetal tissues, etc.3,5,7 All the previously described characteristics, together with their high proliferation index and the absence of legal or ethical problems associated with their application, justify why MSCs are currently one of the main types of stem cells applied in tissue engineering and cellular therapy.7 The immunomodulatory properties of MSCs have been described in numerous studies. This function grants them great importance in transplants, in the treatment of autoimmune diseases and modulation of inflammatory responses.5,7

**Immunomodulatory function of mesenchymal stem cells**

In their surface, MSCs express major histocompatibility complex class I molecules, but not class II or co-stimulation molecules (CD40, CD40-binding, CD80, CD86),3,5,7 characteristics that are conserved when the maturation of MSCs is completed.7 The phenotype described provides de MSCs, in general, with an immunoprivilege since they are unperceived by the immune system and, in case they are recognised, enables them to induce cellular anergy upon the absence of co-stimulatory molecules in their surface.4,7,8 These characteristics justify the low risk of rejection related with its application, regardless of whether their origin is autogenous, syngeneic, allogeneic or xenogeneic.2

MSCs are capable of regulating the action of most of the cells implicated in the immune system, generally inhibiting their proliferation and the production of proinflammatory cytokines, antibodies and cytotoxic mediators, as well as stimulating an anti-inflammatory response and immunological tolerance.2

**Application of mesenchymal stem cells in autoimmune diseases**

As stated before, the current treatment of autoimmune diseases consists of the use, in a chronic manner, of drugs with significant adverse effects that condition a situation that favours the development of opportunistic infections. Thanks to their significant proliferation potential, their immunosuppressant properties and the easy access to the tissues in which we find these stem cells, the therapeutic utility of MSCs is being studied in different immune-mediated diseases.4

**Systemic lupus erythematosus**

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterised by the existence of autoreactive T and B cells, with inhibition of regulatory T cells, which gives rise to an uncontrolled polyclonal response with great production of antibodies.9,10 Among the antibodies produced, some perform a specific direct action on an antigen (anti-red blood cells, antiplatelets, antiphospholipids, etc.), others strengthen the immunoregulation disorder (anti-lymphocytes), and others give rise to the formation and deposit of immunocomplexes in different tissues, which causes inflammation and multi-organ damage.9,10 Their prevalence ranges between 4 and 250 cases per every 100,000 inhabitants-year and its morbidity and mortality are significant. It is more frequent in women of childbearing age, and in Black and Latin races.8,9,10 The current treatment for SLE, in addition to hygienic-dietary measures, consists in the staggered application, depending on the patient’s severity and frequently combined, of non-steroidal anti-inflammatory drugs (NSAIDs), antimalarial drugs, glucocorticosteroids, immunosuppressants (azathioprine, methotrexate, mycophenolate mofetil, cyclophosphamide, among others) and biological therapies (rituximab, belimumab, etc.).9,10

MRL/lpr mice have a mutation in the gene *Fas*, in charge of the induction of the apoptosis, which leads to the proliferation of autoreactive lymphocytes that trigger an immune response and consequences similar to those observed in SLE in the human being, which is the reason why they are used in SLE studies in mice. Several studies have proved how the intravenous transplant of human MSCs into these mice achieves the decrease, or even the normalisation, of the titres of autoantibodies, the decrease of urine protein, renal damage improvement and prolongation of survival times.11 In contrast, in NZB/W F1 mice, another group of murines that develop an autoimmune disease similar to the human SLE, contradicting results have been obtained regarding the action of MSCs on the renal function and the titres of autoantibodies.11

Regarding the results obtained in mice, in 2009, Sun et al. treated 4 patients with SLE with systemic activity and lupus nephritis with scarce response to cyclophosphamide and prednisone with an intravenous MSC infusion at the beginning of the study. As a result, the disease activity index (Systemic Lupus Disease Activity Index [SLEDAI]) improved at 1, 6 and 12 months, urine protein decreased, CD4+ CD25+ FoxP3+ regulatory T cells increased at 3 months, and the required doses of drugs decreased without complications during the 12–18 months after transplant.4,11 In 2010, Liang et al. expanded the study to 15 patients with active and refractory SLE, with lupus nephritis and other extrarenal manifestations (arthritis, severe cutaneous condition, thrombocytopenia), observing a significant SLEDAI improvement, a decrease of urine protein and an increase of the proportion of CD4+ CD25+ FoxP3+ regulatory T cells in the first week of the treatment, even before the decrease of anti-DNA antibodies, which occurred between the first and third month. No adverse effects, deaths or graft-versus-host disease were detected during the mean follow-up period (17 months).4 These encouraging results were confirmed by Sun et al.4
in 2010 in a group of 16 patients with serious SLE after a mean follow-up of 8.25 months: improvement of SLEDAI score, serum albumin, urine protein, serum creatinine, serum supplement and anti-DNA antibodies. Finally, it is worth mentioning how this work team treated a 19-year-old female patient with a recent diagnosis of SLE who developed a refractory and massive diffuse alveolar haemorrhage to corticosteroids and immunoglobulins with intravenous MSC infusion, obtaining a radiological and clinical control of the condition between the fifth and ninth day after the cellular transplant.11

**Sjögren’s syndrome**

Sjögren’s syndrome is a chronic systemic autoimmune disease characterised by the lymphocytic infiltration of exocrine glands, mainly lachrymal and salivary, whose characteristic symptomatology is the appearance of xerostomy and xerofthalm, associated with B cell hyperactivity, with production of autoantibodies (antinuclear antibodies, rheumatoid factor, anti-Ro(SS-A), anti-La(SS-B)).12,13 It may be primary or it may be associated with other autoimmune diseases. Although it is believed to be an under-diagnosed disease, its prevalence ranges between 0.1 and 1% of the general population, being more frequent in women from 40 to 50 years old.12 The SS treatment is divided between those symptomatic measures addressed to alleviate mucosal dryness and the treatment of the extraglandular affection with NSAID, antimalarial drugs, corticosteroids, immunosuppressants (methotrexate, cyclophosphamide, cyclosporine, among others) and biological therapies (infliximab, etanercept, etc.). These last 2 treatments are unable to modify the evolution of the disease.12

There are only a few published studies evaluating the utility of MSCs in the treatment of SS. In 2012, Khalili et al.13 came to the conclusion, after a study with NOD mice (similar to SS), that the combined use of MSCs and Freund’s co-adjuvant immunopotentiating improved salivary secretion and decreased the lymphocytic infiltration in the salivary glands. In 2013, Xu et al. proved how the accurate intravenous MSC infusion to 24 patients with serious primary SS improved the disease activity (Sjögren’s Syndrome Disease Activity Index) progressively throughout the 12 months of follow-up, as well as organ dysfunction at different levels, including salivary hypofunction. Moreover, an increase of regulatory T cells, an increase of Th2 anti-inflammatory response and a decrease of Th1 proinflammatory response were observed.8

**Systemic sclerosis**

Systemic sclerosis (SS) is a chronic and systemic autoimmune disease in which there is an excessive deposit of connective tissue at different levels, giving rise to tissue fibrosis, as well as microvascular damage that leads to the appearance of ischaemic complications, being the most affected organs the skin, the digestive tract, the lung, the heart and the kidney.4,14 The incidence is 4–12 new cases per million inhabitants-year, and it usually affects middle-aged women.14 Currently, no pharmacological treatment has demonstrated to significantly modify the course of the disease, including NSAID, antifibrictic drugs (o-penicillamine, colchicine, among others), vasodilators (calcium antagonists, endothelin receptor antagonists, etc.), glucocorticosteroids and immunosuppressants (methotrexate, mycophenolate, cyclophosphamide, etc.). Some published studies have proved how, after their in vitro processing, MSCs behave as pericytes in the SS, performing a proangiogenic action.15 Moreover, their intravenous application induces apoptosis of T cells and the proliferation of regulatory T cells, improving cutaneous induration and the complications associated with the condition, as well as decreasing the titre of circulating autoantibodies.4

**Crohn’s disease**

Crohn’s disease (CD) is a chronic granulomatous inflammatory condition that may affect any portion of the digestive tract (mainly, intestinal segments surrounding the ileocaecal valve) in a segmental manner and with typically cross-sectional involvement, frequently giving rise to the appearance of fistulas.16,17 Its evolutionary course is recurrent and its aetiology is unknown. Serological markers associated with specific forms of inflammatory bowel disease and with prognostic value, such as anti-Saccharomyces cerevisiae antibodies have been identified, with a sensitivity of 45–60% and a specificity of 85–95%, and others are still being studied, like anti-L, anti-C, ACCA, ALCA, etc.17 The incidence of CD is one to 10 cases per 100,000 inhabitants-year. It usually appears in young people of 20–30 years old, without a clear sex predominance.16 The treatment may be topical or systemic, depending on the extension and location of the condition, most frequently used drugs being—based on severity—aminsalicylates, corticosteroids, immunosuppressants (methotrexate, azathioprine, among others, etc.) and biological therapies (infliximab, adalimumab, etc.), reserv- ing surgery for complications and refractory cases.16

The application of MSCs in fistulising CD with bad response to medical treatment (including biological agents) via their injection at perianal fistulas level has been studied, achieving healing in 71% of the patients treated with MSCs, with no cases of incontinence, and with a recurrence frequency of 17.6% after the first year of follow-up, better results than those of the control group.4,8,19 So far, there are contradicting studies regarding the consequences of the intravenous MSC infusion in the control of the disease activity.4

**Multiple sclerosis**

Multiple sclerosis (MS) is the most frequent demyelinating inflammatory autoimmune disease of the central nervous system4 whose evolutionary course is usually chronic and frequently recurring. Demyelination alters the typical saltatory conduction of the normal myelinated pathways, which justifies a slowdown of the axonal conduction and, therefore, the appearance of a very varied neurological symptomatology.20 It is the most frequent chronic neurological disease in young adults in Europe and U.S.A., with prevalence in near 80 cases per every 100,000 inhabitants. It usually appears in women of 20–40 years old.20

It has been proved in vitro how stem cells perform in the nervous system, not only an immunomodulatory function, but also a myelin regenerative and neuroprotective action by directly stimulating oligodendrocytes.21 In accordance with this, the response after the intrathecal administration of MSCs has been analysed in small series of patients, observing an improvement in the severity of the paralysis and a decrease of demyelination, although no statistically significant results were detected and there was absence of systemic adverse effects. Moreover, a certain increase in the proportion of CD4+ CD25+ regulatory T cells, a decrease in lymphocytes proliferation and an activation of dendritic cells have been detected a couple of hours after the MSC infusion.4 Furthermore, in some studies in which the intravenous infusion of autogenous MSCs has been performed, an improvement in the visual function and a certain improvement in the evolution of the disability have been observed, without evidencing the appearance of adverse effects.4

**Conclusion**

The morbidity and mortality of autoimmune diseases, their difficult therapeutic management and the great incidence of adverse
effects, as well as the immunomodulatory capacity of the MSCs justify the existing interest in the last couple of years in promoting numerous lines of research in this field, with promising initial results and a low incidence of associated complications. There are contradicting data regarding their potential capacity to trigger a systemic immunosuppression. For this reason, since MSCs play an unspecified immunomodulatory role, the secondary effects derived from their application must be thoroughly analysed and compared with others caused by pharmacological treatments currently available.

1. Immunogenicity: MSCs are considered hypoimmunogenic due to the low HLA class I expression and the absence of HLA class II and co-stimulation molecules. Although it was initially thought that this allowed them to escape immunological vigilance and be, therefore, immunoprivileged; it has been proved how in certain circumstances they are capable of triggering an immune response in immunocompetent hosts. Nevertheless, the rejection of allogeneic MSCs could have profitable aspects since the temporary inhibition of the immune system reduces the risk of infection, malignant disruption and appearance of a graft-versus-host reaction.

2. Genesis of ectopic tissue: MSCs have the ability of differentiating themselves into several mesenchymal cellular types, although it has always been considered that they do it in a favourable microenvironment. However, there are studies in which the appearance of characteristics typical of a different cellular type than the one initially thought of has been observed in vivo in the transplanted cells, as in the Breitbach et al. study from 2007, where the appearance of calcifications in the infarcted hearts in which intrasosional MSCs had been injected was observed.

3. Malignant disruption of MSCs: the immortality and potential transformation of MSCs into different cellular types, together with the susceptibility of appearance of chromosome aberrations evidenced in murine cultures after successive passages, justify the hypothesis that there is a potential risk of neoplastic transformation of MSCs. For this reason, we are postulating the need to prove the karyotypic normality of MSCs before their administration.

4. Opportunistic infections: the unspecified systemic immunosuppression exerted by MSCs suggests the possibility that their application might lead to a risk of opportunistic infections more or less important, although the studies performed so far have not proved this hypothesis.

A better understanding of this population of stem cells might offer a new therapeutic strategy to modulate immune responses in immunomediated diseases.

Conflict of interest
The authors declare that there are no conflicts of interest.

References