THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELL-DERIVED SECRETOME IN AUTOIMMUNE PATIENTS

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Abstract. *A thorough investigation of the underlying immunopathogenetic pathways is required in order to create effective therapeutic methods for the complex clinical cases of patients with an autoreactive immune system that can harm the organism. Currently, the most commonly used therapeutic alternatives have severe side effects and reduced effectiveness after long-term treatment of patients with autoimmune diseases. The secretome of mesenchymal stem cells (MSCs-secretome) does, however, contain compounds with potential therapeutic applications, a promising new clinical strategy for treating the aforementioned patients. The data presented in this review summarizes information from experimental research and a small amount of clinical practice knowledge. It also raises fundamental questions regarding the role of modern, innovative therapies in helping autoimmune patients.*

Key words: *mesenchymal stem cells, mesenchymal stem cell-derived secretome, autoimmunity*

Introduction

Autoimmune diseases are “diseases of the new age” or “modern epidemics” due to the relatively increased number of reported autoimmune cases and their global expansion in recent years [1]. Actually, the majority of autoimmune disorders have existed since ancient times [2]. Paleopathological examinations of Egyptian mummies revealed fused vertebrae, a sign of a severe, chronic autoimmune disease known as ankylosing spondylitis (AS), which is part of the Spondyloarthropathies (SpA) group [3]. This is one of the many examples where the previously stated statement is inacurate, despite the fact that the path of autoimmune diseases from the definition of autoimmunity to diagnosis and prevention is quite long and bumpy. To be as accurate as possible, one of the reasons for this “increase” in autoimmune and inflammatory diseases is the rapidly developing science as well as methods for diagnosing patients and the modern way of life and nutrition, especially for people in developed countries [4]. The result of the incorrect lifestyle, in combination with other environmental factors and genetic predisposition, is an immune system unable to distinguish its “own” from “foreign”, whether it is a friend or foe of the organism. Patients are described as “living with the enemy”, which corresponds to the fact that their organism is attacking itself [5, 6].

Unfortunately, autoimmune diseases are both chronic and incurable. The goals set by physicians are to achieve remission and maintain it for a sufficiently long period, which is observed in a small number of autoimmune patients [7]. The therapeutic approaches applied to achieve these goals have been greatly improved and refined. These include the administration of conventional immunosuppressive drugs with or without biological treatments, aimed at more targeted action on key molecules involved in the immunopathogenesis of specific autoimmune conditions [7]. However, not all patients respond to a given conventional therapy (such as patients with refractory diseases), and they are often described as “lost cases” [8]. Thereby, this necessitates the development of new approaches to the treatment of autoimmune diseases.

The therapeutic use of stem cells in numerous pathological disorders, including hematologic, neurological, metabolic, immune-mediated, and autoimmune, has attracted significant interest in recent years [9]. However, the world of stem cells is vast, and choosing the right stem cell type for application to the target disease is crucial. Depending on their ability to differentiate, or their so-called plasticity, stem cells are totipotent, pluripotent, multipotent, oligopotent, and unipotent [10]. Most research is carried out with multipotent stem cells for a variety of ethical and efficiency reasons, as their use is inexpensive and does not violate moral and ethical rights [11]. Multipotent stem cells can differentiate into cells from a single germ layer (ectoderm, endoderm, or mesoderm) and are classified as hematopoietic (HSCs) and mesenchymal stem cells (MSCs) [10]. The most recognized multipotent stem cells are MSCs, which can be successfully multiplied in vitro to the required

dose for clinical application [12]. For this reason, in recent years, particular attention has been paid to the possible application of MSCs as an approach to therapy in many diseases, owing to their immunomodulatory properties, great plasticity, tissue repair, and regeneration [13]. All of these qualities are necessary for the fight against autoimmunity. It should also be noted that MSCs can exert the previously mentioned effects both through intercellular contact mechanisms and through the secretion of many soluble molecules with important biological actions [14]. The pool of all the molecules secreted by MSCs can be termed a "secretome".

Overall, this review paper emphasizes the potential of the mesenchymal stem cell secretome (MSC-derived secretome) to influence immune cells and its benefits for autoimmune patients. It also raises the following questions: Is science currently able to speak authoritatively about the place of these types of stem cells and their products in the field of autoimmunity? To what extent do they have an effect after their application? Does this type of therapy meet patient safety criteria? The answers to the questions posed can be found in the lines that follow, which present literature data recorded in the last ten years in both basic research and clinical practice.

Autoimmune diseases and the therapeutic potential of MSC-derived secretome

Classic autoimmune and immune-mediated diseases with autoinflammatory characteristics are part of the so-called immune-mediated inflammatory diseases (IMIDs). Classic autoimmune disorders include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), psoriasis, and atopic dermatitis. Immune-mediated diseases with autoinflammatory characteristics include SpA, graft versus host disease (GVHD), inflammatory bowel disease (IBD), and others [15]. There are over 80 autoimmune diseases affecting approximately 4.5 percent of the human population, divided into two groups based on their target organ: organ-specific (Hashimoto's thyroiditis, Addison's disease, Graves' disease, type 1 diabetes, vitiligo, etc.) and systemic autoimmune diseases (SLE, RA, vasculitis, systemic sclerosis (SSc), MS, etc.) [4, 15]. Unfortunately, this number is rising, and the prevalence of these diseases differs between men and women, with females experiencing more cases [16]. Nevertheless, the etiology of autoimmune diseases involves many factors. The balance between pro- and anti-inflammatory immune responses has been lost, and reestablishing it calls for the use of complex yet safe approaches [7].

However, MSCs provide the possibility of a personalized approach to the therapy of incurable conditions, such as those described above. As examples of post-embryonic multipotent stem cells, MSCs are characterised by their fibroblast-like morphology, plastic adherence, and unique promise for the therapy of both immune-mediated disorders and diseases where their regenerative capacity is required [12]. MSCs have great plasticity and under the action of specific factors in vitro can undergo mesodermal differentiation into osteocytes, adipocytes, and chondrocytes [10, 12]. For many years, researchers have studied their qualities and functions as stem cells and their potential applications in the field of cell-based therapies. Indeed, the first use of MSCs in clinical practice was in 1945 in a phase I trial of human bone marrow-derived progenitor stromal cells in patients with hematological malignancies [17]. Since then, the clinical potential of MSCs has been extensively studied under various medical conditions. Some of the ability of MSCs to influence a range of immunocompetent cells is due to the formation of cell contacts and the participation of specific molecules in these tight interactions. Programmed death 1/Programmed cell death ligand 1 (PD-1-PD-L1), Fas/Fas ligand, tumor necrosis factor/tumor necrosis factor receptor (TNF-/TNF-R), HLA-G/LIRB2 (ILT4/CD85d), and KIR2DL4 (CD158d) are examples of such interactions [18]. MSCs participate in the control of cellular processes, such as apoptosis and the restriction of cellular proliferation, as a result of these juxtacrine interactions [18]. Therefore, it is important not to undervalue the direct interactions between MSCs and other immune cells, although the findings surrounding the secretome of MSCs are encouraging.

It is well known that MSCs are the source of a wide range of soluble factors, including enzymes, cytokines, a variety of proteins, growth factors, chemokines, DNA and RNA molecules, as well as a lot of secondary metabolites or end products of their metabolism (Table 1) [19, 20]. A few of the processes in which MSCs' molecules and factors, whether free-form or encapsulated, are involved include angiogenesis, apoptosis, immunomodulation, proliferation, prevention of neuronal cell death, support for HSCs, and inhibition of microbial development [19]. However, it is important to remember that the type of molecules released by MSCs is dependent on the tissue source (bone marrow, umbilical cord, adipose tissue, etc.), conditioning time, confluence level, mi-

croenvironmental conditions, and stimuli, physiological or pathological, that they experience following in vitro cultivation [21]. There is evidence that depending on which Toll-like receptor (TLR) expressed on their surface is triggered, MSCs can exhibit either the MSC1 or MSC2 phenotype [15]. When MSCs are stimulated with LPS, TLR4 is activated and pro-inflammatory mediators are produced. In the presence of TNF-α and interferon-γ (IFN-γ), TLR-3 is activated, and MSCs are polarized toward the MSC2 phenotype, which is responsible for immunological homeostasis via immunosuppression [15]. Thus, it is now well known that MSCs work as "sensors" and to exert their effects, MSCs must be stimulated. It follows that the possibility of manipulating the MSC-derived secretome is the reason why many researchers use in their experiments various biochemical stimuli, cytokines such as IL-1, TNF-α, IFN-γ, hypoxic preconditioning, and three-dimensional (3D) culturing, mainly to direct or support the immunosuppressive activity of stem cells [15].

The pros and cons of mesenchymal stem cells and MSC-derived secretome

Most of the secreted factors act as signaling molecules that contribute to the implementation of intercellular communication. All cells, whether inside or outside the organism from which they originate, are sensitive to environmental factors and, as a result, respond to them in a specific way [22]. MSC-generated signaling molecules also operate on this principle, inducing the so-called "signal transduction process", which results in a change in the function and behavior of a target cell, so they can switch the phenotype of immune cells very precisely. Conditionally, the secretome of MSCs can be divided into a secretory part and a vesicular part, both of which are very important for intercellular communication. The secretory part was described and tabulated in detail, but also another intriguing aspect of the MSC-derived secretome is the vesicular fraction, which includes extracellular vesicles (apoptotic bodies, microvesicles, and exosomes) [15], of which exosomes are of the greatest importance and potential in scientific research. Exosomes usually contain miRNAs, lipids, proteins, and secondary metabolites. Their size varies from 30 to 200 nm, so they are also known as nanovesicles, and finally, yet importantly, exosomes have a lipid bilayer, which makes them very suitable to release their contents directly into the cytosol of the cells [15]. Indeed, the content and function of exosomes are considered the same as those of the MSCs from which they are derived, but at the same time, they have several advantages over stem cells, such as low immunogenicity, unchanged functional activity during freezing and thawing, and a lack of tumorigenic potential. [21]. Although, the isolation and

Table 1. The proposed composition of MSC-derived secretome

Main Metabolites	Proteins, Enzymes, and Biologically active factors	Chemokines	Extracellular vesicles (EVs)
Acetate, Alanine, Choline, Ethanol, Formate, Glutamate, Glutamine, Lactate, Nicotinamide, Pyruvate, Tyrosine, L-Glucose, B-Glucose	BMP-9/GDF-2, EGF, Endogline, Endothelin-1, Eotaxin-1, FGF-1, FGF-2, Flt-3L, Follistatin, G-CSF, GM-CSF, GRO pan, HB-EGF, HGF, IFN-α2, IFN-γ, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-1B, IL-1RA, IL-12, IL-6, IL-7, IL-9, LIF, Leptin, PDGF-AA, PDGF-BB, PLGF, TGF-β1/ β2/ β3, TNF-α/β, TSG-6, VEGF-A/C/D, IDO, PGE2, MIF, KGF, IGF-1, Galectins, HO-1, NOS, ICAM, MMP-1/2/7, TIMP-1/2, LL-37, TPO	CCL2 (MCP-1), CCL3, CCL4, CCL5 (RANTES), CCL7 (MCP-3), CCL20, CCL21, CXCL1, CXCL2, CXCL5, CXCL8 (IL-8), CXCL12, CXCL16, CX3CL1 (Fractalkine)	Apoptotic Bodies (> 1000 nm) Microvesicles (200-1000 nm) Exosomes (30-200 nm) Part of the MSC-Exosome content: tetraspanins (CD9, CD63, CD81), RNA (mRNA, miRNA, Pre-mi-RNA, si-RNA, t-RNA, sn-RNA), DNA (mt-DNA, ds-DNA, ssDNA), ATPase, PGK1, GAPDH, Aldolase, G protein, syntetin, HSP60, HSP70, HSP90, RTK, annexins, GTPases, RAB protein

Note: The composition and amount of released factors, molecules, and EVs differ between MSCs isolated from different tissues, as well as cultured under different conditions [19, 20].

Abreviations: growth and differentiation factor 2 (BMP-9/GDF-2), epidermal growth factor (EGF), fibroblast growth factors (FGFs), Fms-related tyrosine kinase 3 ligand (Flt-3L),granulocyte/granulocyte-macrophage colony-stimulating factor (G-CSF, GM-CSF), heparin-binding EGF-like growth factor (HB-EGF), hepatocyte growth factor (HGF), interferons (IFNs), interleukins (ILs), platelet-derived growth factors (PDGFs), placental growth factor (PlGF), transforming growth factors (TGFs), tumor necrosis factor (TNF), tumor necrosis factor- (TNF) stimulated gene-6 (TSG-6), vascular endothelial growth factor (VEGF), indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), macrophage migration inhibitory factor (MIF), keratinocyte growth factor (KGF), Insulin-like growth factor 1 (IGF-1), heme oxygenase-1 (HO-1), nitric oxide synthase (NOS), intercellular adhesion molecule-1 (ICAM-1), matrix metalloproteinases (MMPs), tissue inhibitor of metalloproteinase (TIMP), human cathelicidin (LL-37), thrombopoietin (TPO), monocyte chemoattractant protein (MCP), phosphoglycerate kinase 1 (PGK1),Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), heat shock proteins (HSP), receptor tyrosine kinase (RTK), Ras-associated binding (Rab) proteins, Leukemia inhibitory factor (LIF)

separation of exosomes is a time-consuming and costly process without a standardized protocol [23].

In contrast, MSCs are very easy to isolate, have rapid growth rates under laboratory conditions, and the required number of cells for a given therapy can be easily manipulated [12]. The use of patient-derived stem cells (autologous transplantation) is becoming more and more popular for personalized therapy applications. However, the question of whether autologous or allogeneic stem cells should be used remains [24]. It has been assumed that MSCs from autoimmune patients would be "primed" to produce more immunosuppressive molecules because they resided in a potentially inflammatory environment of the organism from which they originated, and the risk of immunological transplant rejection was also prevented. This proved to be false, however, as evidenced by data showing the defective or deficient function of MSCs obtained from patients with autoimmune disorders [25, 26]. In general, MSCs are poorly immunogenic, with low expression of co-stimulatory molecules (CD80, CD86, CD40, and CD40L), lack of HLA class II expression, and absence or weak expression of HLA class I molecules [19, 27]. This suggests that allogeneic transplantation is an additional possibility. Unfortunately, not everything in science is clear-cut and follows a logical path. MSCs administration is considered safe; however, there are rare risks of pulmonary embolism, tumors, and ectopic tissue formation, as well as the possibility of an HLA mismatch [21, 28]. Furthermore, even though the route of administration, whether local or systemic, is important for infusion efficacy [29, 30], we shouldn't disregard the discouraging evidence that MSCs are short-lived following in vivo infusion due to macrophage clearance [29].

Some researchers have suggested using MSCs-produced molecules because of the potential drawbacks and limitations of MSCs that have been documented in the literature. The application includes either a conditioned medium obtained after culturing the cells in vitro for a specific period of time or individual products of MSCs' conditioned medium, such as extracellular vesicles (EVs), obtained through specific separation methods. According to field data [25, 31], cell-free therapy is an alternative therapy that, if it does not help patients, will not harm them. There are several advantages to the application of the secretome derived from MSCs, as opposed to cells. For example, there is no need for a specific solution to isolate the cells, there is no danger of overdose and safe testing, there is convenience in application, and there is the ability to successfully manipulate the composition of the secretome produced by stem cells [15]. To be fully honest, there aren't many studies that show the secretome of MSCs is connected to any negative impacts in the literature [15, 32]. It should also be taken into account that the various researchers, in their experiments, did not rely on a single, commonly accepted protocol for producing and studying the effect of the MSC-derived secretome. The profile of secreted factors depends on the conditions and time at which the cells are cultured. The development of a standardized methodology for the receiving of MSCs' secretory products as well as further research into the subject of MSCs and their secretome are thus required.

Experimental *in vitro* and *in vivo* studies with MSC-derived secretome

In addition to what has already been stated, more serious consideration should be given to the potential role of therapeutic application of the conditioned medium, which includes secretory MSCs molecules and extracellular vesicles. Indeed, an ever-increasing body of literature in the last decade shows interesting data on MSC-secreted factors' potential to influence the immune cells of patients diagnosed with autoimmune disorders [15]. An important aspect of the adaptive immune system is that T and B lymphocytes, together with other innate immune cells, participate in various mechanisms as part of the immunopathogenesis of autoimmune diseases. In reference to that, it should be noted that the secretome of MSCs is associated with suppressed expression of key participants in programmed cell death in natural killer T-cells (NKT), such as FASL and tumor necrosis factor-alpha-related apoptosis-inducing ligand (TRAIL) [33] as well as the transformation of NKT cells into immunosuppressive cells [34]. Simultaneously, it was demonstrated that conditioned medium from adipose tissue-derived MSCs (AT-MSCs) has an immunosuppressive effect on the Th17/Treg axis in RA patients *in vitro* [35]. IDO, NO, HO-1, and PGE2 all inhibited T-cell proliferation in a variety of ways, including phosphorylation of STAT-5, inhibition of the IL-2 signaling pathway, and activation of the cyclin-dependent kinase inhibitor p27kip1 [36-38]. However, MSCs cannot always suppress immune cells' proliferation, and regarding that of B cells, the data are quite contradictory [39]. IDO produced by MSCs has different effects on B-cells: it can induce a pro-survival effect on CD5+ B-cells, which could trigger T-cell differentiation into regulatory T cells (Tregs) via IL-10 production [40];

moreover, IDO, together with CCL2, is associated with attenuated antibody production [36]. *Gazdic M* et al. (2018), on the other hand, demonstrated that NO and kynurenine had the potential to reduce IL-6 and TNF-α production from B cells while increasing the regulatory phenotype of marginal zone (MZ) B cells (CD23-CD21+IgM+) in the liver [33, 41]. And that‘s not all, because the effect of MSCs-secreted factors is not limited to cells of adaptive immune responses.

Biologically active molecules and factors like IL-8, MIF, PGE2, TSG-6, and IL-6 stimulate the function of phagocytic cells like neutrophils and macrophages, as well as their ability to fight pathogenic microorganisms [42, 43]. The unique ability of MSCs to shift the phenotypic polarization of macrophages from M1 (pro-inflammatory) to M2 (anti-inflammatory) macrophages, with the aid of substances like PGE2, TSG-6, IL-6, KGF, and IGF-1, is frequently highlighted in the literature [43]. We couldn‘t help but point out that MSC-derived exosomes can even affect the transformation of dendritic cells (DCs), which are known as the bridge between innate and adaptive immune responses, into tolerogenic ones [44]. The fact that MSCs factors also appear to suppress the proliferation, activation, and production of co-stimulatory molecules in DCs is also significant. All of this leads to impaired antigen presentation and naive T lymphocyte activation [45]. Finally, it‘s interesting to note that MSCs molecules can inhibit the functional activity of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells, as well as the expression of activation receptors by NK cells (NKp30, NKp44, and NKG2D) [36, 45]. These findings are only a small part of the *in vitro* studies following the effect of secreted factors from MSCs on some immune cells, but they are fundamental for the subsequent pre-clinical studies in the field.

In addition to the findings described above, there are quite significant experimental data obtained from in vivo studies with experimental animal models and autoimmune patients. One of the classical autoimmune diseases whose treatment has been the subject of intense interest and increased research in the field of MSC-based therapies is SLE. Several authors have proposed that MSCs and their exosomes induce M2 polarization of macrophages [46], inhibit T-cell differentiation to Th1 via glycolysis and cytokine pathway regulation [47], inhibit Th17, and, most importantly, stimulate Treg development [48]. Summary data from an extensive meta-analysis conducted in 2020 shows that MSCs treatment of lupus nephritis in animal models resulted in lower levels of ds-DNA antibodies, antinuclear antibodies (ANA), serum creatinine (Scr), blood urea nitrogen (BUN), proteinuria, and renal sclerosis score [49]. This knowledge demonstrates the anti-inflammatory and beneficial effects of MSCs-application in lupus models and the ability of the MSC-derived secretome to regulate chronic inflammation, a very characteristic feature of the disease. However, the role of MSCs in the immunopathogenesis of SLE should be highlighted, not only because of the known deficiency or defective function of MSCs isolated from lupus patients [50], but also because of the involvement of micro-RNA (miRNAs), non-coding RNA (ncRNAs), and circular RNA (circRNAs) molecules in driving the autoimmune process itself [51, 52]. Due to these discouragingly conflicting results, further experimental and clinical studies with MSCs and their products in SLE are needed.

But despite that, MSCs‘ exosomes have been shown to have favorable effects in numerous other autoimmune animal models. For instance, systemic administration of MSCs‘ exosomes has hepatoprotective effects in animal models of autoimmune hepatitis. According to two independent studies from 2017 and 2019, these effects were attributed to suppressed caspase-3-induced apoptosis and caspase-1-induced pyroptosis of hepatocytes [53, 54]. Furthermore, in the course of research for the treatment of MS, the most common inflammatory disease of the central nervous system (CNS), various studies in animal models of experimental autoimmune encephalomyelitis (EAE) have demonstrated improvement in neurobehavioral symptoms, a decrease in CNS inflammation, and demyelination. The results in experimental autoimmune uveitis [55] and non-obese diabetic (NOD) mouse models were also encouraging, with decreased blood glucose, recovered pancreatic islets, and increased quantities of insulin-producing cells [56, 57].

In rheumatic diseases, the results are at times conflicting, perhaps due to the large involvement of MSCs in the immunopathogenesis of these diseases. Different types of miRNA from MSC‘ exosomes are involved in the suppression of arthritis and bone damage by affecting two types of cells involved in the immunopathogenesis of RA, namely the so-called fibroblast-like synoviocytes and antibody-producing B lymphocytes [58]. These findings are hopeful for people diagnosed with RA. At the same time, similar to SLE, SpA-MSCs may have both beneficial and harmful effects. In a TNF transgenic mouse model of Bechterew’s disease MSCs participate in joint inflammation, proof of which is an expression of

TNF receptor I and the presence of enthesitis [15]. Another important discovery made in a study on experimental Sjogren's syndrome showed that IL-6 released by olfactory ecto-mesenchymal stem cells (OE-MSCs) exosomes leads to the suppression of disease progression by stimulating the expression of arginase and the subsequent increase in reactive oxygen species (ROS) and NO levels [59]. Studies by B Hai et al. (2018) that showed reduced lymphocyte infiltration in the salivary glands and reduced serum levels of autoantibodies after infusion of EVs produced from induced pluripotent MSCs (iPSC-MSCs) [60] provided support for this investigation.

For chronic illnesses like IBD, particularly Crohn's disease and ulcerative colitis, for which a rise has been seen globally in recent years, there is no shortage of pre-clinical evidence. Studies on experimental colitis using MSCs-exosomes have revealed decreased mucosal inflammation, clinical relief, and decreased mortality [61-63]. Metallothionein 2, miRNA 146, TSG-6, and other yet-unidentified molecules are among the factors responsible for those favorable results. According to a very recent study by Li-Li Qi et al. (2021) [64], activated conditioned media (CM-AcMSCs), produced from pre-treated adipose-derived MSCs with the serum from rats with dextran sodium sulfate (DSS) – induced colitis models, lead to significantly increased expression of mucin 2 (MUC2) and tight junctions, and suppressed production of pro-inflammatory cytokines in the colonic tissues. This work is encouraging because it not only demonstrates a novel method for recharging MSCs but also shows a way to improve the effectiveness of the products they produce.

MSC-derived secretome, autoimmune diseases, and clinical practice

The increasing diversity of experimental methods and the body of knowledge on the ability of stem cells to secrete substances that can affect the immune system are proof of the scientific community's strong desire to help treat and better understand incurable autoimmune diseases. However, it should be noted that these experimental approaches in mice, for example, were performed under highly controlled sterile conditions in a pathogen-free environment. This is not possible in human clinical trials [25]. Furthermore, even in remission, patients with various autoimmune diseases must take the necessary medications for their treatment, whereas mouse models frequently allow experimental studies to be conducted on untreated cohorts [25]. However, the direction of medical-biological knowledge starts from the laboratories and reaches hospital facilities, where created therapeutic products find invaluable applications. Because of this, the preclinical, in vivo, and in vitro studies are promising and shouldn't be underestimated, but it's important to keep in mind the aforementioned differences.

The ability of a therapy to benefit patients is the most crucial factor determining its efficacy. This can only be proven when conducting clinical trials with a large number of patients. There is a variety of data regarding the clinical use of MSCs [25, 27, 45], their potential for transplantation, and their ability to correct the immunological imbalance seen in autoimmune disorders, according to NIH U.S. National Library of Medicine (ClinicalTrials.gov). The effect of MSC-derived secretome was observed in registered clinical trials on alveolar bone atrophy, hair loss, alopecia, polycystic ovary syndrome (PCOS), ocular surface disease, regeneration of posterior cruciate ligament injury, ischemic stroke, bone defects, and eight clinical trials at different stages in severe cases of COVID-19 (ClinicalTrials.gov). In the field of autoimmunity and conditions with an overactive immune response, there are only four clinical trials, as described in detail in Table 2. Unfortunately, comprehensive information and the findings of these investigations are not yet available. There is a clinical case report of a patient with psoriasis who received AT-MSC-conditioned media and showed total regression within a treatment duration of only one month [65], which is a modest glimmer of optimism. Indeed, the rapid developments in science and medicine will lead to the accumulation of large amounts of data in the field and contribute to a better understanding of the advantages and disadvantages of this therapeutic approach. Until now, the necessary data have not been accumulated to provide a clear answer to the possibility that the MSC-derived secretome favorably influences immunopathogenesis and the overall condition of patients with autoimmune diseases. As a final remark, it is also vital to mention that MSCs and their secretory products have shown tremendous potential in research trials to be good candidates for autoimmune disease therapies but have yet to be seen in clinical practice.

Conclusion

According to the literature included in this review article, the use of MSC-derived secretome as a cell-free alternative biological therapy in clinical practice is in its early stages, and its potential in the

Table 2. MSC-derived secretome – based clinical trials in conditions with an overactive immune system

Title of the study	Location	Trial Stage	Recruitment Status	Study Start Date	Estimated Study Completion Date	Identification Number ClinicalTrials.gov
Effect of Microvesicles and Exosomes Therapy on β-cell Mass in Type I Diabetes Mellitus (T1DM)	Sahel Teaching Hospital - General Committee of Teaching Hospitals and Institutes	Phase I	Unknown	April 2014	September 2014	NCT02138331
Safety and Feasibility Study of Intranasal Mesenchymal Trophic Factor (MTF) for Treatment of Asthma	Punta Pacifica Hospital Panama City, Panama	Phase I Phase II	Unknown	July 2014	October 2020	NCT02192736
Safety of Injection of Placental Mesenchymal Stem Cell Derived Exosomes for Treatment of Resistant Perianal Fistula in Crohn's Patients	Division of Colorectal Surgery, Department of Surgery, Tehran University of Medical Sciences, Tehran, Iran	Phase I Phase II	Active, not recruiting	January, 2022	September 2022	NCT05499156
Safety and Tolerability Study of MSC Exosome Ointment (in Psoriasis)	National University Hospital, Singapore	Phase I	Completed	March, 2022	April 2022	NCT05523011

Note: Clinicaltrials.gov (accessed November 11th, 2022)

field of autoimmunity remains unclear. Future studies should focus on further examining the efficacy and safety of this next-generation therapeutic tool to close this gap in the literature. However, preliminary studies in this area and good outcomes are bringing science and medical practices closer together. There is a chance of victory over the internal foe in severe, refractory autoimmune diseases, and the future will show the way.

All authors declare that the material has not been published before.

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