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REVIEW ARTICLE

Regulatory perspective on *in vitro* potency assays for human mesenchymal stromal cells used in immunotherapy

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Abstract

Mesenchymal stromal cells (MSCs) are multipotent cells derived from various tissues that can differentiate into several cell types. MSCs are able to modulate the response of immune cells of the innate and adaptive immune system. Because of these multimodal properties, the potential use of MSCs for immunotherapies is currently explored in various clinical indications. Due to the diversity of potential MSC medicinal products at the level of cell source, manufacturing process and indication, distinct functionality tests may be needed to ensure the quality for each of the different products. In this review, we focus on *in vitro* potency assays proposed for characterization and release of different MSC medicinal products. We discuss the most used functional assays, as presented in scientific advices and literature, highlighting specific advantages and limitations of the various assays. Currently, the most proposed and accepted potency assay for release is based on *in vitro* inhibition of T cell proliferation or other functionalities. However, for some products, assays based on other MSC or responder cell properties may be more appropriate. In all cases, the biological relevance of the proposed assay for the intended clinical activity should be substantiated with appropriate product-specific (non-)clinical data. In case practical considerations prevent the use of the ideal potency assay at release, use of a surrogate marker or test could be considered if correlation with functionality has been demonstrated. Nevertheless, as the field of MSC immunology is evolving, improvements can be expected in relevant assays and consequently in guidance related to potency testing.

Key Words: *cell-based therapy, government regulation, immunomodulation,* in vitro *potency assays, mesenchymal stromal cells, quality control*

Introduction

Mesenchymal stromal cells (MSCs) are multipotent cells that can differentiate into several cell types. MSCs can be isolated from various tissues and although their numbers are low and decrease with age, their potential to expand *ex vivo* may allow production of sufficient amounts for therapeutic use [1]. Human tissues from which MSCs have been isolated include bone marrow, adipose tissue, placental tissue, umbilical cords and embryonic stem cells [1–12]. Importantly, these MSCs differ in their expression of surface markers and in their functional capacity after stimulation with proinflammatory mediators [2,13]. As a consequence, comparison of the clinical effects of the various MSC products is hampered. To create a broader consensus for more uniform characterization of MSCs, the International Society for Cellular Therapy (ISCT) proposed three minimal criteria to define human MSCs: (i) MSCs should be plastic-adherent when maintained in standard culture conditions; (ii) MSCs should express CD105, CD73 and CD90 and should lack CD45, CD34, CD14/CD11b, CD79α/CD19 and human leukocyte antigen (HLA)-DR expression; and (iii) MSCs should be able to differentiate (*in vitro*) into osteoblasts, adipocytes and chondroblasts [14].

However, the nonspecificity of these criteria has been a topic of discussion ever since [15,16]. One should at least bear in mind that, in spite of above minimal criteria, MSCs remain heterologous populations of cells with a variety of gene expression profiles, differentiation and expansion potential and phenotype, which are influenced by tissue origin, cell isolation and expansion procedures [14,17–19].

In vivo, MSCs play a crucial role in peripheral tissue homeostasis (including blood vessels) and maintenance

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of the (hematopoietic) stem cell niche, mainly due to their potential to differentiate into various cell types and their secretion of several growth-promoting factors [20,21]. It has been shown that MSCs preferentially home to damaged tissue, where they are thought to exert their biological action by direct cell-cell interactions through their surface receptors, but also by producing soluble factors [18,22–24].

Apart from their tissue homeostasis and regeneration capacities, MSCs also have immunomodulatory abilities with potential therapeutic applications. This review article focuses on the *in vitro* testing of the functionality of these MSC medicinal products.

Immunomodulation by MSCs

The general immunomodulatory role of MSCs involves the orchestration of immunologic tolerance, next to the role of regulatory T and B cells and innate suppressor cells [25]. MSCs function via direct suppression of the activation, proliferation and effector functions of proinflammatory cells and the stimulation of various anti-inflammatory cell types to indirectly augment immune response regulation [20]. The most prominent MSC functions are exerted locally, although also some systemic effects have been found, e.g., via the induction of a more tolerogenic immune profile with an anti-inflammatory and T helper (Th)2-biased response [26–31].

To be able to exert immunosuppression, MSCs need to be preliminarily activated by proinflammatory cytokines like interferon gamma (IFN-γ) [32]. Inflammatory monocytes, which are initiators of inflammation, produce a proinflammatory milieu. Within this inflammatory environment, MSCs acquire an immunosuppressive phenotype and augment expression of their receptors and adhesion molecules (e.g., several integrins, pattern recognition receptors and cytokine receptors) to enhance interaction with other immune cells or even pathogens, contributing to a more efficient immunosuppression [1,6,19,21]. The suppression of cellular immunity by MSCs appears to be mainly based on paracrine effects via soluble mediators such as indoleamine 2,3-dioxygenase (IDO), prostaglandin E_2 (PGE_2), transforming growth factor beta (TGF-β), nitric oxide, HLA-G5 and several interleukins (ILs), which are released after cross-talk with activated immune cells [19,33–35]. MSC-mediated modulation of the (local) immune response is the result of cumulative action of several of these soluble mediators, because none of these factors alone can completely abrogate lymphocyte proliferation for example [32].

MSC-mediated modulatory action on specific cells of the immune system has been extensively investigated and described [6,13,18,19,21,26,35–41]. They can exert this effect both on the innate (a.o. natural killer cells, neutrophils, monocytes, macrophages and dendritic cells [DCs]) and on the adaptive (T and B cells) immune system. The effects of MSCs on innate cells includes inhibition of their maturation, changes in cytokine secretion profiles and differentiation toward a more tolerant or regulatory phenotype.

The consequence of MSC actions on the function of T cells is somewhat controversial and conflicting results have been reported, possibly due to differences in the MSC culture method and tissue of origin. Effects of MSCs on $CD4^+$ T cells mainly involve inhibition of proliferation via cell cycle arrest in the G_0/G_1 phase, alterations in Th subtype proportions and induction of regulatory T cells (Tregs) to tip the balance toward a more anti-inflammatory response [19,37,39]. Importantly, most studies only determined the effect on cytokines produced by T cells as parameter for MSC functionality, whereas other T cell properties (e.g., chemotactic potential) have only incidentally been analysed [42]. For $CD8^+$ T cells, the effect on functionality (among which is cytotoxicity) is more clear; MSCs can only suppress the stimulation of antigen-specific cytotoxic T cells, but they do not inhibit previously activated (memory) cells and inhibition of the effector functions of $CD8^+$ T cells is only possible when they are not yet in their cytotoxic phase [18,39,43].

Conflicting results have also been described for the modulation of B cell responses by MSCs. Some authors have shown inhibition of proliferation, impairment of antibody secretion and changes in chemotactic properties, whereas others have found opposite results [18,19,21,32]. As with data obtained with T cells, these contradictory results are most likely the consequence of differences in the specific MSCs and/or in experimental conditions used.

Overall, MSCs skew the inflammatory environment into an anti-inflammatory one, both directly and indirectly, through immunoregulatory circuits involving a.o. monocytes and Tregs [35,36]. The potential of this immunomodulatory capacity is considerable, as MSC-mediated immune modulation can even cross species barriers, although the main mechanisms of action may differ between species [18,44].

MSC product development

Clinical application

As said, MSCs have a diversity of physiological functions including the ability to migrate to inflamed tissue, differentiate into various cell types and secrete anti-inflammatory and tissue-renewing factors. Due to their broad immunoregulatory potential, clinical application of these cells is explored for a wide range of disorders with high immune activation, including graft-versus-host disease (GvHD), transplant rejection and