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IL-2/IL-2R axis modulation by mesenchymal stromal cells: interaction with immunosuppressive drugs?

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Summary sentence: Discussion on the damper effect of mesenchymal stromal cells on immunosuppressive drugs

Running title: Interactions between MSCs and immunosuppressive drugs

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Abbreviations

CsA: Cyclosporin A
DMSCs: decidua-derived mesenchymal stem cells
DSCs: decidual stromal cells
FKBP: FK506 binding protein
GVHD: graft versus host disease
IDO: indoleamine-pyrrole 2-3-dioxygenase
IS: immunosuppressive
MLR: mixed lymphocyte reactions
MSCs: mesenchymal stromal cells
SRL: Sirolimus

In this issue of the Journal of Leukocyte Biology, Erkers and colleagues offer new insights into the immune suppressive properties of placenta-derived decidual stromal cells [1]. Mesenchymal stromal cells (MSCs) are a cell population which was first identified in bone marrow (BM-MSCs) and contains a significant number of stem cells with ability to differentiate into different tissues. It was soon noted that MSCs display, not only regenerative properties, but also immunomodulatory features which could be exploited for therapy in different immune disorders. In addition, these cells are considered to be relatively immune privileged and their use as therapy has been shown to be safe in several animal models and clinical trials. In recent years, therefore, MSCs have come to be regarded as a potential therapeutic choice for several pathologies, whether hematologic, neurologic or cardiovascular diseases, diabetes, diseases of the lung, liver and kidney, steroid-resistant graft versus host disease (GVHD), and cancer, or autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus, reviewed in [2]. The tissue most extensively used as an MSC source is bone marrow, inasmuch as it was the first to be reported as a supplier of these multipotent cells. However, several other tissues have been shown to be suitable sources of MSCs, including adipose tissue, synovial membranes, umbilical cord, endometrium, and placenta. Among these, human decidua is a promising candidate as a source of MSCs for use in cell-based therapies, thanks to easy cell isolation without the need for any invasive methods and high cell proliferation rates in vitro. Decidua-derived MSCs obtained in different laboratories seem to have different differentiation potential yet similar immunomodulating activity. Macias et al. [3] isolated multipotent decidua-derived MSCs from decidua parietalis, denoted by the authors as DMSCs. DMSCs are able to differentiate into derivatives of all germ layers, and their immunomodulatory properties have been demonstrated in an animal model of multiple sclerosis [4]. Erkers et al. described another cell population from decidua parietalis, called decidual stromal cells (DSCs), with more limited differentiation potential but with effective capability to suppress alloreactivity of T lymphocytes.

Erkers et al. [1] define a new immunomodulatory mechanism which could collaborate in the therapeutic effect of MSCs on GVHD as well as on other pathologies. They demonstrate that DSCs increase IL-2 production by alloantigen-stimulated T cells, while reducing the functional IL-2R. The authors used mixed lymphocyte reactions (MLR) to analyze DSC effects on T cell activation by alloantigen. Uptake of exogenously added IL-2 and Stat5 phosphorylation assays showed impaired IL-2 signaling in T cells from MLR cultures in the presence of DSCs. Such impairment could be related to the anti-proliferative power of DSCs on T lymphocytes. Most of the previous reports exploring MSC influence on IL-2R have been focused on IL-2Rα (CD25). The study by Erkers et al. analyzes the T cell responses to IL-15 and IL-7, and determines that the limiting subunit in the DSC-mediated impairment expression of the full IL-2R is the IL-2Rβ subunit (CD122). On the basis of the results with exogenous IL-2, down modulation of IL-2R by DSCs is most probably a consequence of increased IL-2 levels in DSC-MLR cocultures, thus for the first time identifying a role for IL-2 in the immune suppression mediated by stromal cells.

A second important contribution of the work by Erkers et al. is the interaction of DSCs with the efficacy of the immunosuppressive (IS) drugs, cyclosporine A (CsA), a calcineurin inhibitor, and sirolimus (SRL), an mTOR inhibitor also known as rapamycin. Previous antagonisms with IS compounds have been reported for BM and heart tissue-derived MSCs [5; 6]. The DSC results reported by Erkers et al. support the idea that these types of interactions are a general feature of MSCs. Despite the importance that such findings can have for the use of MSCs as a treatment for acute GVHD, little research has as yet focused on the possible mechanisms for MSCs and IS drug interactions. Although different hypotheses have been proposed, none of them can fully explain the data obtained by different laboratories.

Erkers et al. consider the possibility of drug absorption by MSCs limiting their availability to be uptaken by immune cells, as an explanation for the adverse effect of DSCs on CsA and SRL action (Fig. 1A-1). Conversely, the same process of drug absorption by MSCs was put forward by Girdlestone et al. in an attempt to account for the potentiation of the immunosuppression exerted by MSCs when these are pretreated with each of these two drugs [7]. If both seemingly contradictory results can be explained by MSC drug absorption, this would need to be analyzed in depth. Considering the results obtained by Erkers et al. with the combination of CsA and DSCs, it might also be thought that the DSC-mediated increase of

IL-2 production could counteract the CsA-mediated inhibition of IL-2 transcription, thereby avoiding the effect of the calcineurin inhibitor (Fig. 1A-2). However, this mechanism does not wholly explain the detrimental effect of DSCs on SRL action, since the latter mainly act through IL-2 signal transduction, rather than through IL-2 production. In addition, it has been recently proposed that MSCs can also mitigate mTOR-signaling [8]. Therefore some synergism, or at least no damaging effect of DSCs on SRL action, should be expected.

Another suggested explanation for the interaction between IS drugs and MSCs was proposed by Buron et al [6], and envisages the involvement of IFNγ. The report by Erkers et al. includes this as another possibility to explain their results. Indeed calcineurin and mTOR inhibition converge in the control of the action of several inflammation-related factors apart from IL-2. One of these factors is IFNγ, with a controversial role in several inflammatory pathologies. Furthermore, IFNγ has long been known to be an important stimulus for the immunoregulatory action of MSCs, probably owing to an IFNγ-mediated increase in MSC-derived indoleamine-pyrrole 2-3-dioxygenase (IDO), a main player in the IS activity of MSCs. IFNγ down-regulation would result in an impairment of IDO production by MSCs, and might explain the lack of synergism between MSC and calcineurin or mTOR inhibitors (Fig. 1A-3). Once again, however, the unfavorable effect of *in vitro* combination of MSCs and IS drugs under this hypothesis it is not fully understood. Nevertheless, this assumption is not in contradiction with the proposal made by Hoogdujin et al. [5], who reported adverse effects of MSCs on the IS ability of tacrolimus (another calcineurin inhibitor) and SRL. These authors speculated that the dual action of MSCs on activated versus quiescent immune cell states, inhibiting or favoring proliferation respectively, might be behind their data (Fig. 1A-4). Indeed, it is known that MSCs can promote T cell survival through apoptosis inhibition. In addition, Li et al. reported that T cell proliferation can be stimulated by MSCs under certain circumstances, such as low availability of proinflammatory factors, e.g. TNFα or IFNγ, and therefore low NO or IDO production by murine or human MSCs, respectively [9]. In fact, the idea of a plasticity of MSC-mediated immunomodulation depending on the inflammatory scenario is becoming relevant [2]. In the context of the IS drugs used by Erkers et al. [1], CsA impairs the transcription of IL-2 as well as IFNγ by inhibiting of NFAT activation. SRL-induced disruption of mTOR signaling also leads to decreased IFNγ production by T cells through poorly defined mechanisms apparently independent of TCR-activated NFAT (Fig. 1 B). Both pathways to control the levels of IFNγ could modify the inflammatory setting and promote a proinflammatory activity by MSC.

Thus, in our opinion, the hypothesis of different action of MSCs according to the inflammatory condition, which can include inhibition versus stimulation of immunity and different availability of factors such as IDO or NO, could explain the interactions with IS drugs found for MSC derived from different sources, such as heart tissue [5], bone marrow [6] and decidua [1]. This point of view could also account for the fact that MSCs do not hamper the action of other IS drugs, such as mycophenolic acid, whose mechanism of action is direct inhibition of DNA replication. In addition, this hypothesis is not in contradiction with results showing a potentiation of the IS effect of MSCs after preincubation with the same drugs [5; 7]. On the other hand, the notion that MSC-mediated immunosuppression is subjected to the particular inflammatory setting, is congruent with the loss of anti-inflammatory effect of MSC in the presence of the steroid immunosuppressant dexamethasone [10]. In this case, Chen et al., showed that dexamethasone inhibited the expression of iNOS in mouse MSCs, and IDO in human MSCs, without effect on the expression of chemokines able to attract T lymphocytes, which could exacerbate the immune response.

A number of pilot clinical trials in which administration of MSCs to patients treated with CsA, tacrolimus o SRL, particularly in steroid-resistant acute GVHD, have shown promising results that encourage the use of MSCs. In addition, a recent randomized phase II trial, in which CsA was included in the GVHD prophylactic treatment, showed that repeated infusion of umbilical cord-derived MSCs resulted in diminished incidence of GVHD in patients after hematopoietic stem cell transplantation in comparison with patients without MSC infusion [11]. These studies suggest that MSCs might, not only alleviate, but also prevent GVHD. However, the abovementioned data on antagonism between MSCs and some IS drugs serve to underscore the need, not only for caution in the use of MSCs in combination with determined IS drugs, but also for

news studies on animal models to clarify the mechanisms of interaction between MSCs and IS drugs. The results of such research might help to elucidate the most suitable combination of IS drugs with MSC or even whether MSCs could be used in the absence of IS drugs with sufficient benefit.

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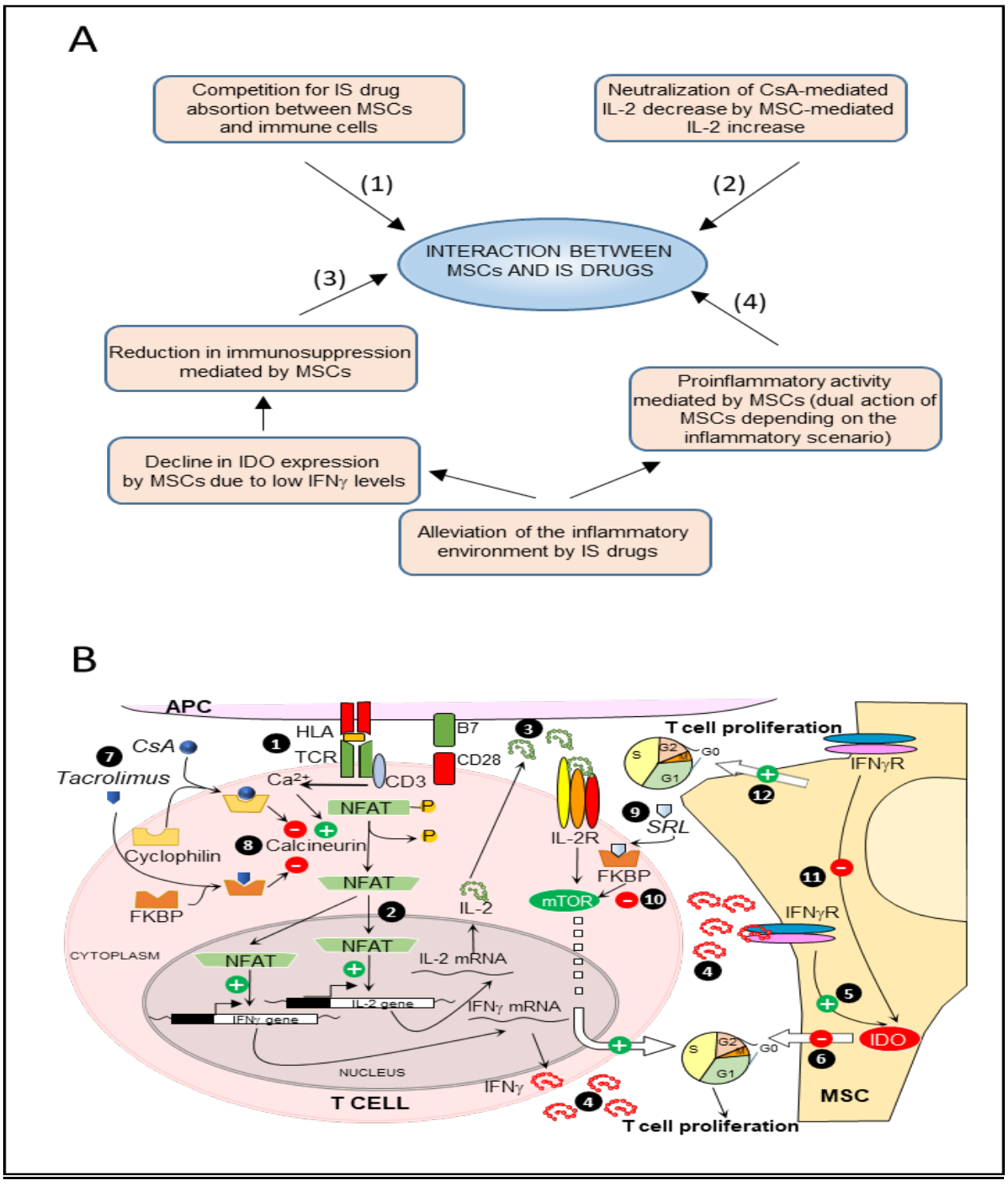

Figure 1. Speculation about the mechanism for antagonism between MSC and IS drug actions based on a dual role for MSCs on immunomodulation, depending on the inflammatory environment. (A): Different possibilities to explain the antagonism between MSCs and IS drugs (for details, see text). **(B):** Antigen recognition by T cell in the context of antigen presenting cell (APC) (1) triggers NFAT activation (2) involved in the gene transcription of numerous participants in cell activity and proliferation, such as IL-2 (3) or IFNγ (4). IFNγ released by T cells is involved in the induction of MSCs to produce IDO (5), which in turn leads to inhibition of T cell proliferation (6). Calcineurin inhibitors CsA and tacrolimus bind to cyclophilin and FKBP (7), respectively. These complexes prevent NFAT signal transduction through impairment of calcineurin action (8). SRL also binds to FKBP (9) and blocks the intracellular pathways in which mTOR is involved (10), such as the one activated by IL-2 binding to IL-2R and long-lasting production of IFNγ. Both mechanisms result in depletion of inflammatory factors. The absence of IFNγ impairs IDO production (11). It is thought that under conditions of insufficient proinflammatory mediators, MSC can stimulate immune cell proliferation (12) [2]. Green and red symbols respectively indicate promotion and inhibition of the depicted pathways.