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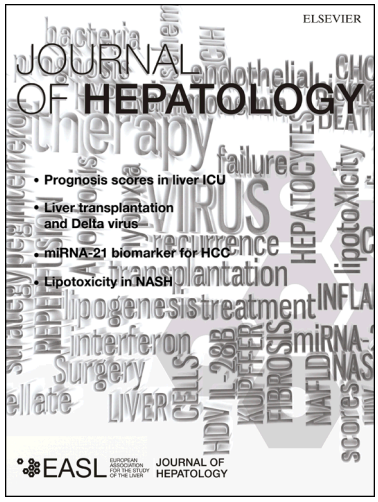

Editorial

Mesenchymal stromal cells – where art thou?

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Title Page:

Mesenchymal stromal cells – where art thou?

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Mesenchymal stromal cells (MSC) have potent immunomodulatory properties which have led to them being considered as a novel therapeutic modality across a wide range of clinical areas including conditions such as steroid refractory paediatric graft versus host disease (GVHD) where they are licensed in New Zealand and Canada. Indeed, more recently adipose derived MSC have also been shown to be clinically effective in a phase 3 clinical trial in fistulising Crohn's disease (http://www.tigenix.com/en/pages/11/news). The pleiotropic actions of MSC in modulating the immune system are often presented as a strength although from a clinical and regulatory perspective a more defined mechanism of action may be preferable. The study by Lee et al presents data on the efficacy of murine MSC in a model of concanavalin A (Con A) induced hepatitis as well as novel insights into their mechanism of action. Early assumptions around cell therapies included their homing to the injured organ whereupon correction of tissue damage would occur either directly by reconstitution of missing cells or indirectly by amelioration of immune-mediated damage.

Homing of MSC to sites of inflammation

This study is notable in that there does not appear to be increased homing to the injured liver, and indeed most of the MSC appear to lodge in the lung from where they are reported as exerting their beneficial effects. Lodging of MSC in the lung is well recognised and is best described as a passive process in contrast to the active homing reported to inflamed organs[1]. Groups have shown previously that either blockade or knock-out of integrin receptors reduces engraftment and vascular rolling of MSC in the setting of injured myocardium[2] suggesting that the engraftment of MSC within target tissues is an active process. Aldridge et al reported that there was increased MSC binding both *ex vivo* and *in vivo* to injured liver which was mediated by a highly ordered interaction between their cell surface receptors CD44 and β_1–integrin and cognate receptors such as VCAM on hepatic sinusoidal endothelium[3].

Thus, this lack of increased hepatic homing is surprising, although this may relate to their usage of Carboxyfluorescein succinimidyl ester (CFSE) as a fluorescent label to perform cellular quantification which has relatively limited resolution. Other methods for quantifying the homing efficiency of MSC such as measurement of the relative level of radioactivity in excised tissues and organs[4] or the number of fluorescently labelled cells in a defined number of microscopic fields[5] are more accurate, with even more detailed quantification of MSC achieved by labelling with super paramagnetic iron oxide nanoparticles or quantum dots. Lee et al quantified distribution of MSC at 12 and 24 hours after intravenous administration, which is relevant as MSC are known to redistribute after their initial localisation to the lungs to injured organs such as the heart[6] and liver, spleen, kidney, and bone marrow[7]. This observation has also been reported after clinical administration to MSC to patients with liver cirrhosis in which cells re-localised to the liver and spleen[8].

An additional consideration when examining MSC localisation within the first 24 hours is their positioning in relation to tissue blood vessels as there is an important distinction between cells passively entrapped within blood vessels and those that have trans-migrated into the tissue.

That this homing to sites of tissue damage has been reported in a range of inflammatory conditions also raises the possibility that mobilisation of endogenous MSC may serve as a response to reduce aberrant tissue inflammation. However, whilst some groups report increased MSC mobilisation in the setting of injury such as intimal hyperplasia[9] or cytokine stimulation[10] this is not a universal finding and thus there is uncertainty regarding its biological relevance.

Relationship between homing of MSC to site of injury and efficacy

Increased homing and engraftment of MSC to the injured myocardium has been reported to enhance their reparative contribution[2], in keeping with the findings of other studies, although this is not a universal finding as other groups report a systemic or distant effect of MSC on the desired

target organ without any significant homing to it[11]. These observations are not mutually exclusive and raise questions regarding the mechanism(s) of action of MSC and also about practical issues such as the optimal route of delivery/administration.

Most notably Zanotti et al reported that alginate-encapsulated MSC injected subcutaneously, which were not able to migrate, exerted a potent immunosuppressive effect *in vivo* in models of cutaneous antigen-specific immune-mediated model of injury and acute GVHD, which was superior to that seen with systemically administered MSC[11]. Whilst this may reflect prolongation of *in vivo* viability of MSC by alginate encapsulation it also demonstrates that MSC homing to specific organs is not always needed to control inflammation and that, in certain settings their immunosuppressive effects may be mediated by the release of systemically acting soluble factors. One possible candidate is TSG-6 which has been previously reported to mediate the therapeutic effects of MSC in models of encephalitis and colitis where cells have not been able to cross the blood-brain barrier[12].

In this paper MSC are hypothesised to mediate their beneficial effects on the liver by polarising macrophages to an anti-inflammatory M2 phenotype, which is achieved by the release of MSC-derived IL1 receptor antagonist (IL1Ra). Ortiz et al previously determined that IL1Ra was present in a sub-population of MSC and that MSC administration was more effective than recombinant IL1Ra delivered via adenoviral infection or osmotic pumps in a model of bleomycin (BLM)-induced lung injury[13]. MSC administration protected lung tissue from BLM-induced injury by interfering with the action/production of two key proinflammatory cytokines in lung, IL-1α and TNF-α. These factors may also be of relevance in liver disease as they drive the expression of endothelial adhesion molecules thus accentuating the early inflammatory response. It would also be interesting to understand the factors regulating the release of IL1Ra as the immunosuppressive effects of MSC are critically influenced by their sensing of the environment; in the presence of inflammation with high levels of cytokines such as TNF-a and IFN-γ MSC can become activated and adopt an immune-suppressive phenotype (MSC2)[14]. Thus, it would be informative to establish the constitutive

production of IL1-Ra by MSC and/or whether cells need to be primed/infused into an inflammatory environment for this release to occur.

Indeed, although Lee et al report co-localisation of macrophages and MSC in the lungs, this does not preclude interactions elsewhere such as the liver and the circulation as evidenced by the efficacy of alginate-encapsulated MSC placed subcutaneously. The influence of MSC on macrophage polarity is well-established, with factors such as MSC-derived IL6 polarising monocytes to anti-inflammatory M2 macrophages that secrete IL10[15]. However, in the absence of IL6 and a pro-inflammatory milieu MSC can induce polarisation of monocytes toward pro-inflammatory M1 macrophages, thus it would be important to establish the role of IL1-Ra release alongside other known factors in this finely calibrated system. There was no effect of MSC on induction of regulatory T cells in this study as determined by hepatic expression of FoxP3, which is a surprise given that this effect is closely linked to their polarisation of M2 macrophages. It would perhaps have been informative to look for the presence of CD4+CD25+FoxP3+ regulatory T cells in digested livers to provide a more detailed quantitative analysis rather than relying on gene expression alone.

Relevance to clinical practice

MSC used for these studies were heterogeneous bone marrow derived plastic adherent cells isolated from BALBc mice, and it would be important therefore to establish to what extent these findings would hold true for human MSC and also MSC isolated from Bl/6 mice for which mechanisms of immunosuppression are different. Specifically, production of nitric oxide (NO) is restricted to BALBc derived MSC whereas indoleamine 2,3 dioxygenase (IDO) is required for the action of human, and also Bl/6, derived MSC[14]. It would be important to establish whether human MSC therefore need to be primed prior to infusion to ensure an anti-inflammatory effect, or is a pro-inflammatory environment provide the necessary stimulus?

In this study 10^4 infused MSC were not found to be effective, with a dose of 10^5 being needed – this equates to a dose of 2.5 x 10^6 cells/kg, which is in keeping with the doses being contemplated for systemic administration in patients. The likeliest route of administration in patients will be intravenous, which would appear to be effective, as well as the most expedient route. The finding that large numbers of MSC aggregate in the lungs with no adverse effects is reassuring and in keeping with prior studies in patients with cirrhosis. Ultimately, the question around site of action of MSC will be answered by clinical studies utilising state of the art non-invasive imaging to correlate the extent of hepatic homing with clinical efficacy. In the meantime the first challenge is to demonstrate efficacy with MSC in inflammatory liver disease, and hopefully upcoming studies in patients with primary sclerosing cholangitis will start to shed light on this (http://fp7merlin.eu/project/the-research-plan/).

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