MAC attack: MSCs and macrophages join forces against chronic lung infection

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Mesenchymal stromal cells (MSCs) possess several characteristics that make them attractive as a potential adjunct therapy for acute and chronic infectious diseases. MSCs are well known for their impressive immunomodulatory,[1 2] pro-repair effects[1 3] and clinical safety profile,[4 5] however, the efficacy of MSCs in controlling bacterial infections, at least directly, remains unclear.[6]

Mycobacterium avium complex pulmonary disease (MAC-PD) is a chronic condition driven by multi-drug resistant bacteria for which there are no robust efficacious treatment strategies.

MAC-PD is associated with high levels of morbidity, and long regimens of antimicrobial drugs, meaning there is an unmet need for novel therapeutics that can effectively target, modulate and re-educate the weakened immune system to effectively clear this bacterial infection. MAC infection predominantly affects macrophage and dendritic cell populations in the lung. Macrophages can be directly activated through MAC infection or by Th1 cells induced by antigen presenting cells. MAC can resist host defence mechanisms and persist in macrophages, where they act as a replication niche to support MAC dispersal.[7] Thus, macrophages play a key role in MAC-disease pathogenesis.

Recently, interaction and communication between live or apoptotic MSCs and macrophages has been identified as one of the major mechanisms of action associated with MSC therapeutic efficacy.[8–13] Thus, MSC-macrophage education may play a role in shaping functional macrophage bacterial clearance and disease progression.

A single study has shown the capacity for human MSCs to reduce pulmonary MAC burden in a 7-day preclinical cystic fibrosis model.[14] In this issue of *Thorax*, Shaw *et al* build on these findings identifying the ability of hMSCs to indirectly inhibit *M. avium* replication in a clinically relevant model of lung infection.[15] While there are differential findings across these two studies, likely associated with the models used, Shaw *et al* are the first to provide a novel mechanistic insight into how MSCs can modulate macrophages to induce enhanced antimicrobial activity against MAC infection, demonstrating the pivotal role of COX-2-mediated-prostaglandin E2 (PGE2) in the induction of the PI3K pathway. Moreover, the PGE2 receptor, prostanoid receptor EP2 and its associated polymorphisms have been shown to play an important role in host resistance to *Mycobacterium tuberculosis* infection.[16 17]

Importantly, the authors report inter-donor variation in macrophage response to MSC treatment, highlighting the need to better understand the MSC mechanism of action and identify parameters that might help to stratify patients based on responsiveness to MSC therapy. This falls in line with other studies investigating MSC responsiveness.[8] An alternative approach is to activate or license MSCs before administration. Indeed, MSCs require threshold levels of inflammatory milieu to become activated or 'licensed'.[1] Shaw *et al* show that MSCs in direct coculture or transwells, but not naive MSC conditioned media, reduced MAC proliferation in monocyte-derived macrophages (MDMs) in vitro, suggesting that MSCs need to receive licensing signals from

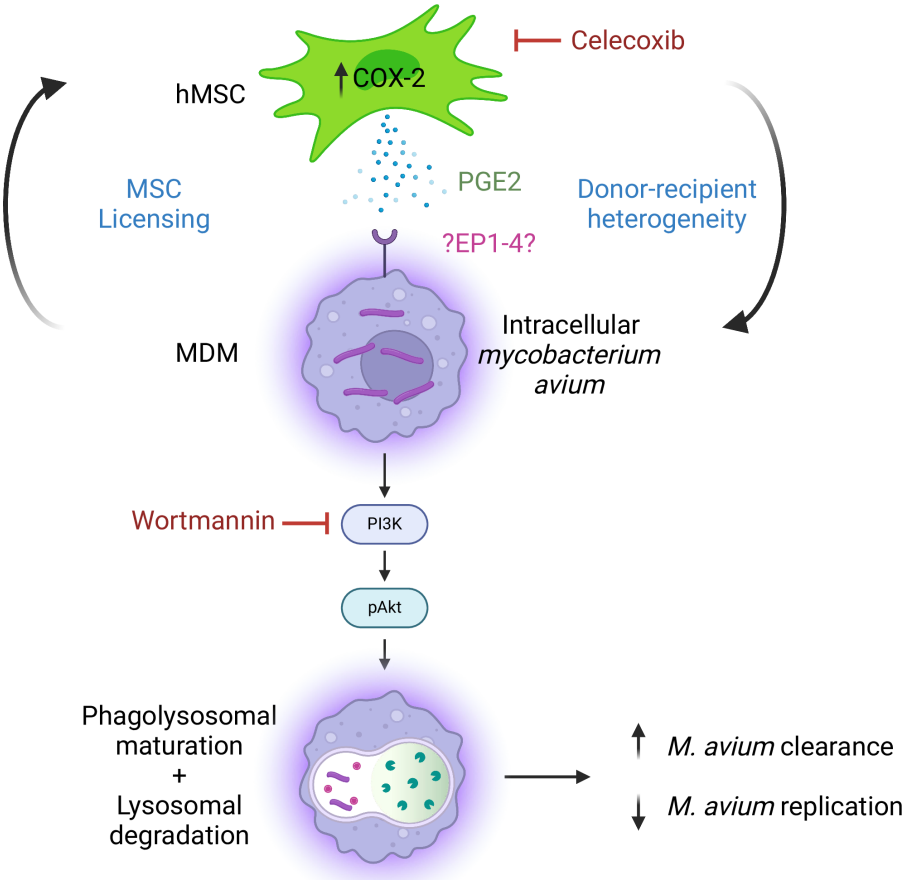

Figure 1 Schematic depicting mechanism of action for MSC-derived PGE2 to inhibit intracellular *Mycobacterium avium* in MDMs. Signals provided by human monocyte-derived macrophages (MDMs) infected with *M. avium* complex (MAC) license human MSCs (hMSCs) to upregulate COX-2 and release PGE2. Addition of the specific COX-2 inhibitor celecoxib prevents MSC upregulation of PGE2 in response to MAC-infected MDM licensing. MSC-derived PGE2 leads to the activation of PI3K and pAKT in MAC-infected MDMs potentially leading to phagolysosomal maturation and lysosomal degradation, clearing MAC and decreasing MAC replication. Blockade of PI3K signalling using wortmannin blocks PGE2-induced MAC clearance in MDMs. Figure created using biorender.com. COX-2, cyclooxygenase-2; EP1-4, E-prostanoid receptors 1–4; hMSCs, human mesenchymal stromal cells; pAKT, phosphorylated AKT; PGE2, prostaglandin E2; PI3K, phosphoinositide 3-kinase.

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MAC infected/activated MDMs in order to secrete factors that impact MDM ability to control MAC. Interestingly, MSCs in a transwell were more effective than MSCs directly cocultured with MDMs and *M. avium* bacteria, as cocultured MSCs no longer produced increased levels of PGE2, perhaps due to a negative impact of MAC being in direct contact with the MSCs. Thus, the elevated PGE2 response by MSCs was associated with a licensing effect mediated by MAC-infected MDMs and is required to initiate MSCs indirect antimicrobial efficacy in the context of MAC-PD.

Shaw *et al* identify a signalling pathway involved in MSC-derived PGE2 mediated inhibition of intracellular bacterial growth in infected MDMs. Addition of the COX-2-specific inhibitor celecoxib abrogated the associated antimicrobial effects of MSCs on MAC-infected MDMs, along with MDM production of TNFα. Importantly, Shaw *et al* saw no effect of celecoxib when it was applied directly to MDMs in the absence of MSCs, illustrating that production of PGE2 specifically from MSCs is critical for the activation of MDMs. Moreover, pretreatment of MAC-infected MDMs with wortmannin (an irreversible inhibitor of PI3K) inhibited the antimicrobial effect of MSCs, demonstrating that MSC-derived PGE2 signals through PI3K to initiate the clearance of intracellular *M. avium* in MDMs (figure 1).

To translate these findings in vivo, Shaw *et al* used a murine model of chronic pulmonary MAC infection by nebulising MAC (10^9 CFU/mL) to establish a proliferative pulmonary infection with extrapulmonary dissemination over 42 days. Systemic administration of 1×10^6 hBM-MSC on days 21 and 28 modestly but significantly reduced pulmonary bacteria, but failed to reduce the bacterial burden in distal organs (spleen and liver). Interestingly, however, MSC administration did not have any effect on the inflammatory cytokine milieu measured from lung homogenates. Timing of MSC administration on days 21 and 28 in the chronic phase of infection correlates with the applicable dosing regimen in a clinical scenario, however, perhaps the inflammatory milieu present in this model at this time is not suitable to adequately license MSCs following in vivo administration. Perhaps a licensing strategy would enhance MSC therapeutic efficacy as previously described.[1 18 19]

To date, this novel research has provided convincing data illustrating the role of COX-2-dependent PGE2 production in MSCs' ability to inhibit intracellular MAC replication, possibly through phagolysosomal maturation and lysosomal degradation in infected macrophages.[20] Although more work emphasising the longevity of the MSCs' therapeutic effects perhaps involving a licensing strategy and better understanding of patient heterogeneity and donor responsiveness to MSC therapy would be beneficial moving forward, these data provide us with a stepping stone to further understand MSC efficacy in the context of bacterial pulmonary infection. Notably, the authors point out that MSCs may be valuable as an adjunct therapy in conjunction to classic antimicrobials; thus, it would be beneficial to investigate the impact of MSCs in combination to antibiotics to demonstrate a possible additive or synergistic response, using clinically relevant preclinical models of MAC-PD in vitro and in vivo.

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